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XDE-729 Methyl
(Halauxifen-methyl)
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B.6 TOXICOLOGY AND METABOLISM

B.6.1 Absorption, distribution, metabolism and excretion (toxicokinetics) (IIA 5.1)

The toxicokinetics of XDE-729 Acid or the Na salt have been investigated in a preliminary study in rats and mice, a definitive study in rats which included groups exposed to low (10 mg/kg) and high (750 mg/kg) single doses and repeated low doses by the oral route and single low dose exposure by the intravenous route, and in a single dose (50 mg/kg) study in dogs. Also, the comparative toxicokinetics of XDE-729 Methyl following a low single oral dose were investigated in the definitive study. Additionally, a number of the repeated dose toxicity and mechanistic studies evaluated in Sections B.6.3 and B.6.5.3 and the reproductive studies evaluated in Section B6.6 include investigations into toxicokinetic aspects.

B.6.1.1 Absorption, distribution and excretion

XDE-729 Acid	
Study	IIA 5.1.1/01 Probe study to determine absorption, distribution, metabolism and elimination in F344/DUCRL rats and CRL:CD1(ICR) mice
Reference	[redacted] (2009)
Date performed	2008
Test facility	[redacted]
Report reference	Laboratory study ID 081108
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Acid [Phenyl-U-^{14}C], lot INV 030887-0001, specific activity 33.69 mCi/mmol, radiochemical purity 96.6%. Non-radiolabelled XDE-729 Acid, lot E2350-93, TSN030751-0002, 99.0% purity
Study acceptable	Yes

METHODS

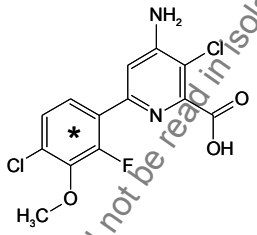
This preliminary study was conducted to investigate absorption, distribution, metabolism and elimination (ADME) of ^{14}C-XDE-729 Acid by rats and mice following exposure to a single oral dose of 100 mg/kg of ^{14}C-XDE-729.

A summary of the investigations conducted is presented in Table B.6.1.1-1

Table B.6.1.1–1: Study design

Group	Single dose of XDE-729 Acid (mg/kg)	Number, sex, species	Investigations
1	100	Rat, 1M, 1F	Time course blood, urine, faeces collected up to 72 h post-dosing. Expired volatiles and CO_2 collected up to 24 h post dosing. Radioactivity in collected samples measured. Urine (0-12 h collection) analysed for parent and metabolites.
2	100	Rat, 1M, 1F	Killed at C_{max} (i.e. 0.5 h post dose) and blood collected for analysis for parent and metabolites.
3	100	Mouse, 2M, 2F	Time course urine, faeces collected up to 72 h post-dosing. Expired volatiles and CO_2 collected up to 24 h post dosing. Radioactivity in collected samples measured. Urine (0-12 h collection) analysed for parent and metabolites.
4	100	Mouse, 2M, 2F	Killed at assumed C_{max} (i.e. 0.5 h post dose) and blood collected for analysis for parent and metabolites.

The test substance was suspended in 0.5% methylcellulose and administered orally, by gavage. The targeted radioactivity was 80 µCi/rat and 25 µCi/mouse. The test substance was labelled on the phenyl ring, as indicated by an asterisk below.



Group 1 animals were fitted with indwelling jugular vein cannulae from which time course blood samples were collected 15, 30 minutes and 1, 2, 3, 6, 12, 24, 48 and 72 hours post-dosing. Urine was collected in dry-ice cooled traps which were changed at 12, 24, 48 and 72 hour post-dosing. Faeces were collected in dry-ice chilled containers at 24-hour intervals. Expired air was passed through charcoal (trap changed at 24 h intervals) to trap expired volatiles and then through a solution of monoethanolamine:1-methoxy-2-propanol (3:7 v/v) (changed at 12 h intervals) to trap expired CO2.

Radioactivity in the collected samples was quantified using a liquid scintillation spectrometer. Parent and metabolites were analysed using high performance liquid chromatography/radioactivity monitor/mass spectrometry (HPLC/RAM/MS). Certain pharmacokinetic parameters were estimated for blood and urine data, including AUC (area-under-the-curve), Cmax, ½Cmax, and elimination rate constants, using a two compartment pharmacokinetic computer modelling program.

RESULTS

The plasma C_{max} in male and female rats was 220 and 254 µg/g, respectively, which was reached 0.5 hours after the administration of the dose. The C_{max} for RBC was 28 and 45 µg/g in the male and female rat, which was reached in 1 and 0.5 hours, respectively. These findings indicate rapid absorption from the gastrointestinal tract.

Elimination of radioactivity from blood was bi-exponential with plasma $t_{½\alpha}$ of 0.14 hours and $t_{½\beta}$ ranging from 3.37 (male) to 3.67 (female) hours. Elimination from RBC was mono-exponential with $t_{½}$ of 0.84 (male) and 0.77 (female) hours. In male and female rats, respectively, the AUC was 529 and 428 µg h g^{-1}, mean residence time was 1.8 and 1.6 h and clearance was 201 µg/h and 244 µg/h.

The AUC value for females was lower than males, despite the females having a higher C_{max} and longer plasma $t_{½\alpha}$. This is because females had higher plasma levels at the first two time points (0.25 and 0.5 hours) than males, with C_{max} occurring at 0.5 hours for both sexes. However, at later time points the males had higher µg-eq./g values than females. These higher values in the male coupled with the longer time intervals contributed substantially more to the overall male AUC than just two relatively short-time intervals of higher concentration for the female. Therefore, in spite of females having the higher C_{max}, males had a greater overall AUC than females. Females had a longer half life since they had detectable values through 48 hours, while the male was NQ at 48 hours. The contributing AUC value for female during the 24-48 hour time interval was very low due to the low levels detected in the plasma; thus, it contributed very little to the overall female AUC.

Urinary elimination was 63-73% (rat) and 56-60% (mouse) of administered radioactivity within 12 hours after dosing. Urinary elimination increased to 65-75% (rat) and 62-64% (mouse) of the administered dose within 24 hours. A total of 65-75% and 65-66% of the administered radioactivity was recovered in the urine of rats and mice, respectively, within 72 hours post-dosing. A total of about 12-21% of the orally administered dose was recovered in faeces of the rats, most (~11-18%) within the first 24 hours post-dosing. Negligible radioactivity was detected in expired air. The radioactivity in the faecal samples could represent unabsorbed dose eliminated within the GI transit time of 21 hours, if it is the case that biliary elimination of parent compound and/or metabolites is negligible. With this assumption, the faecal elimination data suggests that absorption from the gastrointestinal tract of rats could be as high as around 80-90% of the dose. Overall, these results also show that elimination of parent compound and/or metabolites is rapid and extensive.

The major radiolabelled component present in the urine was unchanged XDE-729 Acid, as shown in Table B.6.1.1–2. Four metabolites were tentatively identified: the glucuronide conjugate of O-demethyl XDE-729, the sulfate conjugate of O-demethyl XDE-729, O-demethyl XDE-729, and acyl-glucuronide conjugate of parent XDE-729 Acid. The metabolic profile was very similar between sexes and species with only slight changes in the percentages of the peaks. Unchanged XDE-729 Acid was found in all C_{max} plasma extracts. The acyl glucuronide conjugate of XDE-729 was also found in the male mouse plasma extract, representing approximately 7.5% of the administered dose.

Table B.6.1.1–2: Metabolite profile in urine of rats and mice dosed with ^{14}C-XDE-729 Acid (single dose, 100 mg/kg)

Compound	% of administered radioactivity in urine (0-72 h collection)			
	Rat		Mice	
	Male	Female	Male	Female
XDE-729 Acid (parent)	57.41	71.00	57.73	55.59
Sulfate conjugate of O-demethyl-XDE-729[a]	4.01	1.77	1.27	2.25
Acyl-glucuronide conjugate of XDE-729[a]	1.93	0.64	3.32	4.11
O-demethyl-XDE-729[a]	0.97	1.01	0.64	0.51
Glucuronide conjugate of O-demethyl-XDE-729[a]	0.36	0.44	1.44	1.49
Total identified	64.68	74.86	64.40	63.95
Unidentified metabolite X	0.27	0.38	0.28	0.64
Unidentified metabolite Y	ND	ND	0.52	ND
Unidentified metabolite Z	ND	ND	0.44	0.53
Total unidentified	0.27	0.38	1.24	1.17
Total accounted for (i.e. totals indentified + unidentified)	64.94	75.24	65.63	65.10
Lost/unaccounted for	35.06	24.76	34.37	34.90
Total	100	100	100	100

[a] tentatively identified

CONCLUSION

Absorption of XDE-729 Acid following a single dose at 100 mg/kg by the oral route is extensive (~80-90% of administered dose) and rapid in rats and mice. Elimination is also rapid, with the urine being the main route of elimination. The majority of the radioactivity in the urine and plasma of rats and mice is present as the parent compound. The toxicokinetic behaviour of XDE-729 Acid is similar in males and females.

[redacted] (2009)

XDE-729 Acid & XDE-729 Methyl	
Study	IIA 5.1.2/01 Pharmacokinetics and metabolism in F344/DUCRL rats
Reference	[redacted] (2010)
Date performed	2008
Test facility	[redacted]
Report reference	Laboratory study ID 091098
Guideline(s)	OECD 417 (1984)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 sodium salt [Phenyl-U-^{14}C], lot XS9-100040-68, INV031412-0001, specific activity 45.3 mCi/mmol, purity 97.2 XDE-729 sodium salt [2,6-Pyridine-^{14}C], lot XS9-100040-69, INV027480-0002, specific activity 30.6 mCi/mmol, purity 97.3% XDE-729 Methyl [Phenyl-U-^{14}C], lot INV031089-003; GA&PC G-09-23, specific activity 45.3 mCi/mmol, purity 97.4% Non-radiolabelled XDE-729 sodium salt, lot # E2978-05-01, TSN030751-0005, purity 96.5% Non-radiolabelled XDE-729 Methyl, lot # 2622-83, TSN031117-0003, purity 96.1%
Study acceptable	Yes

METHODS

This study was conducted to determine absorption, distribution, metabolism, and excretion (ADME) of ^{14}C-XDE-729, as the sodium salt, following oral (single low and high doses, multiple low doses) and intravenous (single low dose) exposure to male and female F344/DuCrl rats. In addition, ADME of ^{14}C-XDE-729 Methyl was investigated to determine the bioequivalence between the acid and methyl ester forms of XDE-729.

A summary of the investigations conducted is presented in Table B.6.1.1-3.

Table B.6.1.1–3: Study design

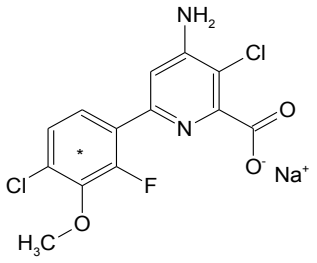
Group	Treatment	No./sex	Investigations
1	10 mg/kg, single dose, oral, XDE-729 sodium salt [phenyl label]	4M, 4F	Time course blood, urine, faeces collected up to 168 h post-dosing. Tissues sampled at termination at 168 h post-dosing.
2	750 mg/kg, single dose, oral, XDE-729 sodium salt [phenyl label]	4M, 4F	Radioactivity in collected blood (plasma & RBC) and excreta samples measured. Selected pooled urine and faeces samples analysed for parent and metabolites.
3	10 mg/kg, repeat dose, oral, unlabelled XDE-729 sodium salt for 14 d; XDE-729 sodium salt [phenyl label] on d 15	4M, 4F	Sampling post-last dose and analyses were as for groups 1 & 2, except that blood was not sampled and analysed.
4	10 mg/kg, single dose, intravenous, XDE-729 sodium salt [phenyl label]	4M, 4F	As for groups 1 & 2.
5	10 mg/kg, single dose, oral, XDE-729 sodium salt [pyridine label]	4M, 4F	As for groups 1 & 2.
6	10 mg/kg, single dose, oral, XDE-729 Methyl [phenyl label]	4M, 4F	As for groups 1 & 2. Additionally, blood samples taken at C_{max}, ½C_{max} and ¼C_{max}were analysed for parent and metabolites

For oral administration, the test substances were suspended in 0.5% methylcellulose and administered by gavage; the targeted radioactivity was 500 µCi/kg bodyweight. For intravenous

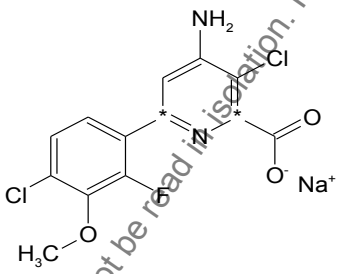
dosing the test substance was suspended in ~10% Intralipid (a fat emulsion); the targeted radioactivity was 500 µCi/kg bodyweight.

The test substances were labelled on the phenyl ring or pyridine structure, as indicated by the asterisks below:

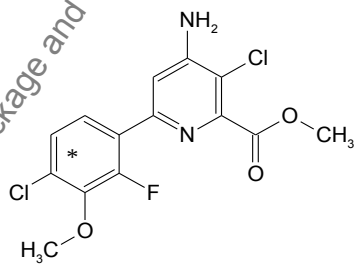
^{14}C-(UL-phenyl)-XDE-729 sodium salt:



^{14}C-(2,6-pyridine)-XDE-729 sodium salt:



^{14}C-(UL-phenyl)-XDE-729 Methyl:



Groups 1-2 and 4-6 animals were fitted with indwelling jugular vein cannulae from which time course blood samples were collected 0.08, 0.17, 0.25, 0.5, 1, 2, 3, 6, 12, 24 h post dosing and every 24 h thereafter up to 168 h post-dosing. Urine was collected in dry-ice cooled traps which were changed at 12, 24h post-dosing and every 24 hours post-dosing thereafter to 168 h post-dosing. Faeces were collected in dry-ice chilled containers at 24 h intervals to 168 h post dosing. Expired air was not sampled because the probe study (IIA 5.1.1/01, Saghir et al, 2009) showed that negligible XDE-729 is excreted via this route. At study termination (168 h post-dosing) adrenals, GI tract, perirenal fat, liver, testis, uterus, ovary, skin, kidney, spleen and residual carcass were sampled.

Radioactivity in the collected samples was quantified using a liquid scintillation spectrometer. Parent and metabolites were analysed using HPLC/RAM/MS. Certain pharmacokinetic parameters were estimated for blood and urine data, including AUC (area-under-the-curve), C_{max},

½C_{max}, and elimination rate constants, using a two compartment pharmacokinetic computer modeling program. Estimations of systemic bioavailability (F) values used the equation:

$$F = (AUC_{oral} / AUC_{IV}) \times (Dose_{IV} / Dose_{oral})$$

In the metabolite characterisation investigation, pooled urine and faeces samples for selected intervals were analysed for parent and metabolite(s) (1 analysis per sample) via eluent HPLC with in-line radiochemical detection, or via fraction collection with LSS analysis.

RESULTS

The radioactivity detected in the faeces and urine over time, and the distribution of the radioactivity recovered from the tissues at termination, are presented in Tables B.6.1.1-4 (males) and B.6.1.1-5 (females). The kinetic parameters measured in the plasma and red blood cells are presented in Tables B.6.1.1-6 (males) and B.6.1.1-7 (females).

The metabolite profiles found in the excreta are presented in Tables B.6.1.1-8 (males) and B.6.1.1-9 (females). The metabolite profiles measured the whole blood of group 6 (XDE-729 Methyl single oral dose, 10 mg/kg) only are presented in Tables B.6.1.1-10 (males) and B.6.1.1-11 (females). A proposed metabolic pathway for XDE-729 Acid in rats is presented in Figure 6.1.1-1.

Total recovery of radioactivity from all animals was high, averaging 102 and 99% of administered dose in the oral and IV dose groups, respectively.

The results for the animals dosed with pyridine labeled XDE-729 Na salt (group 5, single oral dose 10 mg/kg) were very similar to those of the equivalent pyridine labeled XDE-729 Na salt group (group 1).

Kinetic parameters (absorption, elimination $t_{½}$, AUC, clearance) were reported for radioactivity measurement made in plasma and RBC. For RBC, the values of these parameters were substantially lower than for plasma.

Absorption

Plasma T_{max} values were less than 0.2 hours for the XDE-729 Na salt following single doses of 10 mg/kg, demonstrating that the substance was rapidly absorbed by the oral route at the low dose, without any apparent lag time. T_{max} values for the single dose 750 mg/kg were similar to the low dose groups for males, but longer at 1.8 hours for females. A single dose of XDE-729 Methyl was also rapidly absorbed, with T_{max} values of 0.5 h or less.

In the XDE-729 Na salt groups, the recovery of radioactivity from urine and non-GI tract tissues ranged from about 68% for 750 mg/kg males to about 92% in repeat dose females. Thus, a conservative estimate of the extent of oral absorption, excluding the possibility of biliary excretion, is also 68 – 92%. Systemic bioavailability of XDE-729 Na salt for the oral route can also be estimated by comparing the dose-corrected AUC ratio of plasma radioactivity from the low dose oral (group 1) and IV (group 4) dose groups; this comparison indicates that systemic bioavailability of XDE-729 Na salt is about 100%. For the XDE-729 Methyl group the extent of oral absorption was similar to the XDE-729 Na salt groups.

Distribution

The presence of a high proportion of administered radioactivity in the blood plasma indicates widespread systemic circulation of both XDE-729 Na salt and XDE-729 Methyl. At 168 hours post-dosing, radioactivity levels in the tissues ranged from non-quantifiable to 0.3% of the orally administered dose from either XDE-729 Na salt (low single, high single, multiple dose, and pyridine-ring label) or XDE-729 Methyl. Less than 0.03% of the IV administered XDE-729 Na salt remained in the tissues at 168 hours post-dosing. There was no evidence of preferential distribution to any particular tissue, or of accumulation.

Metabolism

The major radiolabelled component present in the urine and faeces from the oral XDE-729 Na salt groups in both genders was XDE-729 Acid, accounting for between 82% and 98% of administered dose. This indicates that metabolism of the parent compound is limited. Minor metabolites of XDE-729 Na salt consisted of the acyl-glucuronide conjugate of XDE-729 (accounting for up to about 7% of radioactivity dose) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (accounting for up to about 6% of dose).

The major radiolabelled component present in urine and faeces of animals dosed with XDE-729 Methyl was also XDE-729 Acid, the hydrolysis product of the parent. XDE-729 Acid accounted for 78% and 81% of administered radioactivity, in males and females, respectively. As is the case for XDE-729 Na groups, minor metabolites of XDE-729 Methyl were the acyl-glucuronide conjugate of XDE-729 (accounting for 6.2% and 3.5% of dose in males and females, respectively) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (accounting for 10.9% and 8.3% of dose in males and females, respectively). The slightly higher proportion of administered radioactivity present as the O-demethylated XDE-729 or corresponding conjugates, when compared to the XDE-729 Na groups, is not considered to be of toxicological significance. Analysis of blood samples from the XDE-729 Methyl group detected primarily XDE-729 and no parent ester, indicating rapid hydrolysis of the ester *in vivo*.

Elimination

In the XDE-729-Na salt oral groups, the absorbed dose was rapidly and extensively excreted, mainly via urine (68-92% of the administered dose) in both genders, with the proportion in urine being highest among females. The majority (90-99%) of the urinary elimination occurred within the first 24 hours post-dosing. A smaller percent (11-29%) of the oral dose was eliminated in faeces. Also, the majority (78-93%) of faecal elimination occurred within 24 h of dosing. The extent and rate of urinary and faecal elimination of radioactivity for the XDE-729 Methyl group was similar to the XDE-729 Na salt groups.

Table B.6.1.1–4 Distribution of radioactivity, % of dose administered, group mean values: males

Group		1	2	3	4	5	6
Treatment		10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]	10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]
Tissues (168 h)	Adrenals	NQ	NQ	NQ	NQ	NQ	NQ
	Blood	NQ	NQ	NQ	NQ	NQ	NQ
	Carcass	NQ	NQ	NQ	NQ	NQ	NQ
	Fat	NQ	NQ	NQ	NQ	NQ	NQ
	GI tract	0.01	0.01	NQ	NQ	NQ	NQ
	Kidneys	0.00	NQ	NQ	NQ	0.00	0.00
	Liver	0.00	NQ	NQ	NQ	NQ	0.00
	Plasma	-	-	NQ	-	-	-
	RBC	-	-	NQ	-	-	-
	Skin	0.01	0.29	NQ	0.03	NQ	0.02
	Spleen	NQ	0.00	NQ	NQ	NQ	NQ
	Testes	NQ	NQ	NQ	NQ	NQ	NQ
	Tissues total	0.02	0.29	NQ	0.03	0.01	0.02
Final cage wash		0.29	3.43	0.16	0.20	1.76	0.43
Faeces	24 h	23.58	22.87	13.67	12.91	9.44	16.17
	48 h	1.83	5.02	0.75	1.48	2.25	1.23
	72 h	0.17	0.72	0.13	0.11	0.12	0.15
	96 h	0.19	0.34	0.05	NQ	0.04	0.08
	120 h	0.05	0.06	0.03	NQ	0.03	0.03
	144 h	0.02	0.03	0.01	NQ	0.02	0.02
	168 h	0.15	0.25	0.01	NQ	0.03	0.02
	Faeces total	25.99	29.29	14.66	14.52	11.92	18.24
Urine/rinse	12 h	4.93/68.10	2.63/50.14	4.25/83.40	4.06/78.60	3.20/82.38	2.93/75.02
	24 h	0.60/1.67	0.65/7.68	0.46/1.76	0.30/1.01	0.55/1.24	0.34/2.16
	48 h	0.15/0.55	0.25/5.78	0.06/0.38	0.09/0.31	0.22/0.40	0.12/0.38
	72 h	0.11/0.16	0.15/0.42	0.06/0.08	0.06/0.12	0.08/0.17	0.07/0.12
	96 h	0.11/0.13	0.05/0.18	0.03/0.08	0.03/0.08	0.05/0.10	0.04/0.07
	120 h	0.03/0.06	0.05/0.10	0.01/0.03	0.03/0.04	0.03/0.04	0.03/0.04
	144 h	0.02/0.02	0.04/0.05	0.01/0.01	0.01/0.02	0.02/0.03	0.03/0.05
	168 h	0.01/0.03	0.00/0.00	0.00/0.01	0.02/0.02	0.01/0.02	0.02/0.04
	U+rinse total	76.69	68.15	90.63	84.81	88.54	81.45
Grand total		102.99	101.16	105.44	99.56	102.23	100.15

NQ = less than the limit of quantitation

Table B.6.1.1–5 Distribution of radioactivity, % of dose administered, group mean values: females

Group		1	2	3	4	5	6
Treatment		10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]	10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]
Tissues (168 h)	Adrenals	NQ	NQ	NQ	NQ	NQ	NQ
	Blood	NQ	NQ	NQ	NQ	NQ	NQ
	Carcass	NQ	NQ	NQ	NQ	NQ	NQ
	Fat	NQ	NQ	0.00	NQ	NQ	NQ
	GI tract	NQ	0.01	NQ	NQ	0.00	0.02
	Kidneys	0.00	NQ	NQ	NQ	0.00	0.00
	Liver	NQ	NQ	NQ	NQ	NQ	0.00
	Ovaries	NQ	NQ	NQ	NQ	NQ	NQ
	Plasma	-	-	NQ	-	-	-
	RBC	-	-	NQ	-	-	-
	Skin	0.01	0.13	NQ	NQ	0.02	0.01
	Spleen	NQ	0.00	NQ	NQ	NQ	NQ
	Uterus	NQ	0.00	NQ	NQ	NQ	NQ
	Tissues total	0.01	0.14	NQ	NQ	0.05	0.02
Final cage wash		0.15	1.95	0.35	0.25	0.32	0.41
Faeces	24 h	10.51	19.81	11.15	9.03	10.28	16.52
	48 h	0.64	4.11	0.67	1.41	0.97	1.40
	72 h	0.11	0.51	0.15	0.17	0.19	0.18
	96 h	0.10	0.15	0.07	0.08	0.11	0.06
	120 h	0.06	0.04	0.03	NQ	0.07	0.08
	144 h	0.04	0.03	0.01	NQ	0.07	0.02
	168 h	0.01	0.02	0.01	NQ	0.03	0.04
	Faeces total	11.47	24.67	12.09	10.69	11.72	18.30
Urine/rinse	12 h	4.49/79.00	4.01/58.43	3.54/86.45	5.12/78.28	10.67/73.05	3.81/73.27
	24 h	1.04/1.64	1.13/5.98	0.32/1.26	0.73/2.30	1.42/2.12	0.57/2.23
	48 h	0.22/0.66	0.20/2.31	0.07/0.44	0.19/0.45	0.28/0.49	0.19/0.52
	72 h	0.15/0.21	0.12/0.26	0.06/0.09	0.14/0.21	0.17/0.31	0.12/0.20
	96 h	0.06/0.14	0.09/0.11	0.04/0.07	0.11/0.15	0.27/0.12	0.06/0.12
	120 h	0.05/0.06	0.05/0.08	0.02/0.03	0.09/0.10	0.36/0.15	0.05/0.08
	144 h	0.03/0.05	0.04/0.07	0.01/0.03	0.04/0.06	0.07/0.06	0.07/0.13
	168 h	0.02/0.02	0.00/0.00	0.01/0.02	0.39/0.04	0.02/0.05	0.02/0.04
	U+rinse total	88.14	72.88	92.45	88.39	89.62	81.49
Grand total		99.76	99.63	105.17	99.34	101.71	100.23

NQ = less than the limit of quantitation

Table B.6.1.1–6 Kinetic parameters in plasma and red blood cells, group mean values: males

Group	1	2	3	4	5	6
Treatment	10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]	10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]
Kinetic parameters in plasma						
t_{max} (h)	0.190	0.250	-	-	0.190	0.500
C_{max} (µg/g)	21.05	510.45	-	-	36.425	12.500
Absorption $t_{½}$ (h)	a	0.182	-	-	0.086	0.162
Elimination $t_{½\alpha}$ (h)	0.880	4.320	-	0.119	0.495	0.614
Elimination $t_{½\beta}$ (h)	6.043	5.574	-	3.285	6.765	4.824
AUC_{0-t} (µg h g^{-1})	23.82	4345	-	24.350	30.275	22.425
Clearance (ml kg^{-1} h^{-1})	-	-	-	0.500	-	-
Kinetic parameters in red blood cells						
t_{max} (h)	0.335	0.250	-	-	0.168	0.438
C_{max} (µg/g)	2.625	158.9	-	-	1.125	0.450
Absorption $t_{½}$ (h)	0.250	0.056	-	-	0.123	0.269
Elimination $t_{½\alpha}$ (h)	0.291	1.435	-	0.081	0.300	0.437
Elimination $t_{½\beta}$ (h)	1.328	15.21	-	0.873	2.106	0.657
AUC_{0-t} (µg h g^{-1})	1.850	499.5	-	10.275	1.000	0.550
Clearance (ml kg^{-1} h^{-1})	-	-	-	3.892	-	-

Table B.6.1.1–7 Kinetic parameters in plasma and red blood cells, group mean values: females

Group	1	2	3	4	5	6
Treatment	10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]	10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]	10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]
Kinetic parameters in plasma						
t_{max} (h)	0.148	1.81	-	-	0.103	0.438
C_{max} (µg/g)	32.22	636.3	-	-	45.200	13.275
Absorption $t_{½}$ (h)	a	0.196	-	-	0.356	0.103
Elimination $t_{½\alpha}$ (h)	0.67	2.65	-	0.274	0.806	0.734
Elimination $t_{½\beta}$ (h)	9.12	8.66	-	5.518	15.711	7.886
AUC_{0-t} (µg h g^{-1})	25.38	4497	-	23.025	31.100	22.575
Clearance (ml kg^{-1} h^{-1})	-	-	-	0.552	-	-
Kinetic parameters in red blood cells						
t_{max} (h)	0.333	1.438	-	-	0.080	0.375
C_{max} (µg/g)	1.625	310.15	-	-	1.375	0.475
Absorption $t_{½}$ (h)	0.088	0.287	-	-	a	0.132
Elimination $t_{½\alpha}$ (h)	0.206	0.961	-	0.036	0.287	0.692
Elimination $t_{½\beta}$ (h)	1.492	38.51	-	0.660	3.759	0.836
AUC_{0-t} (µg h g^{-1})	1.100	1126	-	5.375	1.025	0.575
Clearance (ml kg^{-1} h^{-1})	-	-	-	2.820	-	-

a = insufficient data points to model the parameter

Table B.6.1.1-8 Metabolite profile in excreta, as % of dose administered, males

Group	1		2		3		4		5		6	
Treatment	10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]		750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]		10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]		10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]		10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]		10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]	
Urine or faeces:	urine	fecal	urine	fecal	urine	fecal	urine	fecal	urine	fecal	urine	fecal
Unknown (peak A)	ND	ND	0.200	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronide conjugate of O-demethyl-XDE-729 (peak B)	0.27	ND	1.32	ND	0.19	ND	ND	ND	0.25	ND	0.74	ND
Sulphate conjugate of O-demethyl-XDE-729 (peak C)	2.48	ND	2.45	ND	2.31	ND	1.94	ND	2.58	ND	4.43	ND
Unknown (peak D)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-demethyl-XDE-729 (peak E)	0.66	2.30	0.10	2.58	0.58	1.46	0.71	1.33	1.28	2.07	0.31	5.42
XDE-729 (peak F)	80.0	10.4	61.6	20.8	84.6	10.3	78.8	8.93	81.0	6.00	69.8	7.79
Unknown (peak G)	ND	ND	ND	ND	0.32	ND	ND	ND	0.26	ND	1.03	ND
Acyl glucuronide of XDE-729 (peak H)	4.32	1.01	1.63	5.35	2.33	1.42	3.31	0.60	3.13	2.20	4.90	1.31
Unknown (peak I)	0.14	0.20	ND	0.52	0.24	ND	ND	0.89	ND	0.32	0.24	1.68
Unknown (peak J)	ND	ND	ND	ND	ND	ND	ND	0.49	ND	0.20	ND	1.34
Total identified	101.5		96.7		103.3		95.6		98.6		94.7	
Total unidentified	0.3		0.7		0.2		1.4		0.8		4.3	

Table B.6.1.1–9 Metabolite profile in excreta, as % of dose administered, females

Group	1		2		3		4		5		6	
Treatment	10 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]		750 mg/kg, single dose, oral, XDE-729 Na salt [ph. label]		10 mg/kg, repeat dose, oral, XDE-729 Na salt [ph. label]		10 mg/kg, single dose, iv, XDE-729 Na salt [ph. label]		10 mg/kg, single dose, oral, XDE-729 Na salt [pyr. label]		10 mg/kg, single dose, oral, XDE-729 Methyl [ph. label]	
Urine or faeces:	urine	fecal	urine	fecal	urine	fecal	urine	fecal	urine	fecal	urine	fecal
Unknown (peak A)	ND	ND	0.42	ND	ND	ND	ND	ND	ND	ND	ND	ND
Glucuronide conjugate of O-demethyl-XDE-729 (peak B)	0.51	ND	1.22	ND	0.41	ND	ND	ND	0.39	ND	1.58	ND
Sulphate conjugate of O-demethyl-XDE-729 (peak C)	1.14	ND	1.56	ND	1.02	ND	1.43	ND	1.16	ND	2.56	ND
Unknown (peak D)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
O-demethyl-XDE-729 (peak E)	0.67	2.56	1.18	1.95	0.82	1.11	1.08	1.33	1.23	1.72	0.70	3.46
XDE-729 (peak F)	83.3	6.66	66.8	18.0	88.6	9.02	84.6	7.34	85.0	7.46	71.6	9.21
Unknown (peak G)	ND	ND	ND	ND	0.23	ND	ND	ND	0.23	ND	1.77	0.00
Acyl glucuronide of XDE-729 (peak H)	2.35	1.02	1.16	2.78	1.11	1.28	1.28	0.91	1.61	1.11	2.47	1.06
Unknown (peak I)	0.17	0.27	ND	0.61	0.27	0.28	ND	0.62	ND	0.28	0.83	1.63
Unknown (peak J)	ND	ND	ND	0.51	ND	0.39	ND	ND	ND	0.30	ND	1.11
Total identified	98.2		94.6		103.4		98.0		99.7		92.6	
Total unidentified	0.4		1.5		1.2		0.6		0.8		5.3	

ND = not detected

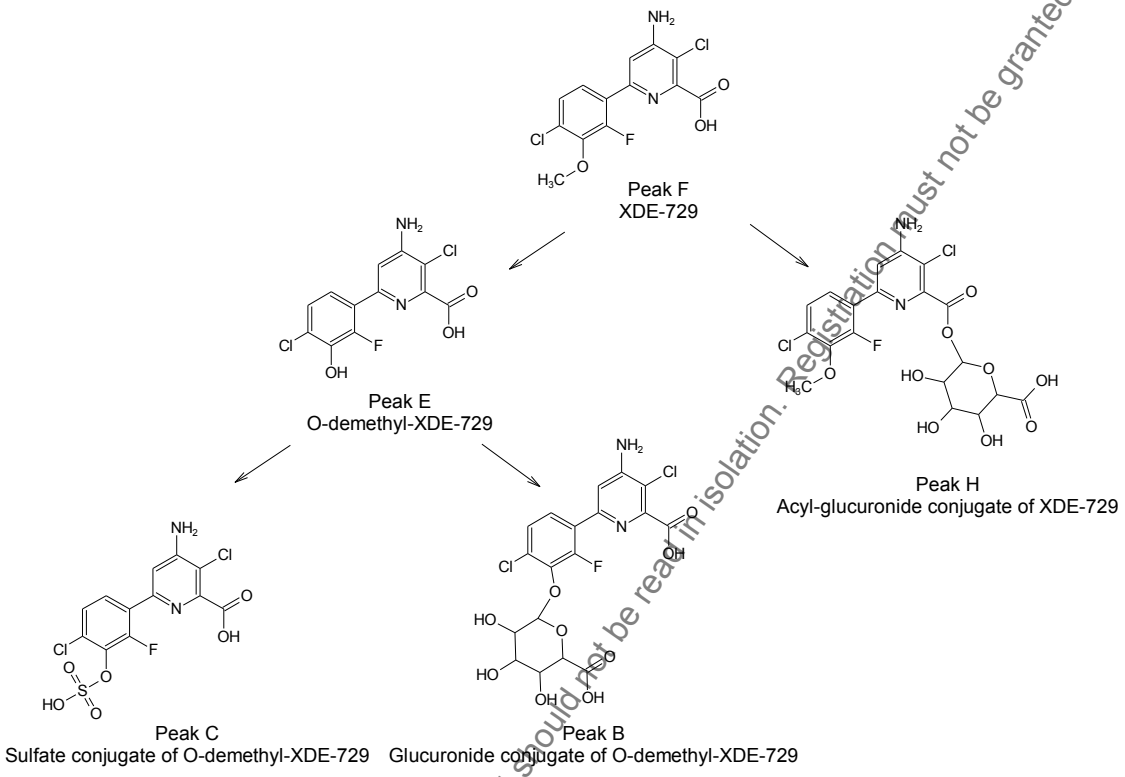
Table B.6.1.1–10 Metabolite profile in whole blood, group 6 (10 mg/kg, single dose, oral, XDE-729 Methyl), as µg equivalents/g whole blood, males

Metabolite	Sampling time			Total
	0.5 h, C_{max}	1 h, ½C_{max}	2 h, ¼C_{max}	
Unknown (peak A)	0	0	0	0
Glucuronide conjugate of O-demethyl-XDE-729 (peak B)	0	0	0	0
Sulphate conjugate of O-demethyl-XDE-729 (peak C)	0.359	0.478	0.266	1.10
Unknown (peak D)	0.049	0.148	0.077	0.275
O-demethyl-XDE-729 (peak E)	0	0	0	0
XDE-729 (peak F)	13.0	9.74	3.38	26.1
Unknown (peak G)	0	0	0	0
Acyl glucuronide of XDE-729 (peak H)	0.136	0.084	0.045	0.265
Unknown (peak I)	0	0	0.026	0.026
Unknown (peak J)	0.085	0.054	0.009	0.148

Table B.6.1.1–11 Metabolite profile in whole blood, group 6 (10 mg/kg, single dose, oral, XDE-729 Methyl), as µg equivalents/g whole blood, females

Metabolite	Sampling time			Total
	0.5 h, C_{max}	1 h, ½C_{max}	2 h, ¼C_{max}	
Unknown (peak A)	0.044	0.048	0.033	0.0125
Glucuronide conjugate of O-demethyl-XDE-729 (peak B)	0	0	0	0
Sulphate conjugate of O-demethyl-XDE-729 (peak C)	0.258	0.295	0.193	0.745
Unknown (peak D)	0.060	0.062	0.048	0.170
O-demethyl-XDE-729 (peak E)	0	0	0	0
XDE-729 (peak F)	12.4	9.40	3.19	25.0
Unknown (peak G)	0	0	0	0
Acyl glucuronide of XDE-729 (peak H)	0.212	0.141	0.070	0.423
Unknown (peak I)	0.020	0	0.020	0.040
Unknown (peak J)	0.078	0.055	0.019	0.152

Figure 6.1.1-1 Proposed metabolic pathway for XDE-729 Acid in Rats



CONCLUSION

Plasma T_{max} values of less than 0.2 hours for the XDE-729 Na salt following single doses of 10 mg/kg demonstrate that the substance is rapidly absorbed by the oral route. Absorption is also relatively rapid following a single dose of the Na salt at 750 mg/kg, with T_{max} values of 0.25 h in males and 1.8 h in females. A single dose of XDE-729 Methyl at 10 mg/kg is also rapidly absorbed, with T_{max} values of 0.5 h or less. A comparison of the AUC ratio of plasma radioactivity in groups receiving a single dose of 10 mg/kg XDE-729 Na salt by the oral and intravenous routes indicates that the extent of oral absorption is about 100% of administered dose. The extent of oral absorption of XDE-729 Methyl is likely to be similar to the XDE-729 Na salt.

To consider distribution, the presence of a high proportion of administered radioactivity in the blood plasma indicates widespread systemic circulation of both XDE-729 Na salt and XDE-729 Methyl. At 168 hours post-dosing radioactivity levels in the tissues ranged from non-quantifiable to 0.3% of the orally administered dose for both XDE-729 Na salt or XDE-729 Methyl. There was no evidence of preferential distribution to any particular tissue, or of accumulation.

The major radiolabelled component present in the urine and faeces for the oral XDE-729 Na salt groups in both genders is XDE-729 Acid, accounting for between 82% and 98% of administered dose. This indicates that metabolism of XDE-729 Acid is limited. Minor metabolites of XDE-729 Na salt consisted of the acyl-glucuronide conjugate of XDE-729 (accounting for up to about 7% of radioactivity dose) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (accounting for up to about 6% of dose). The metabolic profile of XDE-729 Methyl is very similar, with XDE-729 Acid being the major radiolabelled component the urine and faeces and minor acyl-glucuronide (up ~6% of dose) and O-demethyl (up to ~11% of dose) metabolites being present. Analysis of blood samples from the XDE-729 Methyl group detected primarily XDE-729 and no parent ester, confirming rapid hydrolysis of the ester *in vivo*.

Absorbed XDE-729-Na salt is rapidly and extensively excreted in both genders mainly via urine (68-92% of the administered dose), with the proportion in urine being highest among females. The majority of the urinary elimination (90-99%) occurred within the first 24 hours post-dosing. A smaller percent (11-29%) of the oral dose was eliminated in faeces, the majority of which (78-93%) occurred within 24 h of dosing. The extent and rates of urinary and faecal elimination of XDE-729 Methyl is similar to the XDE-729 Na salts.

In summary, in the rat XDE-729 Na salt is rapidly and extensively absorbed by the oral route, subject to limited metabolism but widespread distribution in blood, and is rapidly and extensively eliminated mainly in the urine leaving very low tissue residues. There are no major sex differences in the toxicokinetic behaviour of XDE-729 Na salt. The absorption, elimination kinetics and metabolite profiles between XDE-729 Na salt and XDE-729 Methyl are very similar, demonstrating that the methyl ester of XDE-729 is rapidly hydrolyzed *in vivo* at doses of 10 mg/kg to XDE-729 Acid, thereby resulting in bioequivalence between these two test materials at low doses.

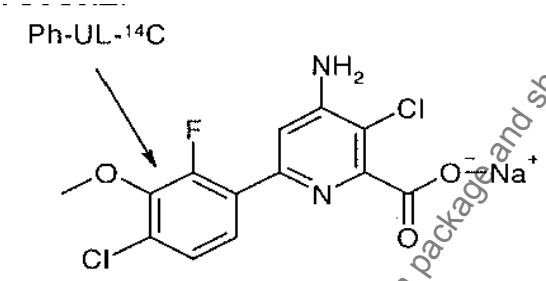
[redacted] (2010)

XDE-729 Acid	
Study	IIA 5.1.1/02 Investigation of [14C]-labelled XDE-729 metabolism and excretion balance in beagle dogs following a single oral (gavage) administration
Reference	[redacted] (2012)
Date performed	April – September 2010
Test facility	[redacted]
Report reference	Dow study ID 101055, [redacted] study number [redacted]-410030
Guideline(s)	No
Deviations from the guideline	N/A
GLP	Yes
Test material	^{14}C-(UL-phenyl)-XDE-729 sodium salt, lot DE3-102869-30, INV-031412-0003, specific activity 40.9 mCi/mmol, radiochemical purity 96.8% XDE-729 Acid, Lot E2978-05-01, TSN030751-0005, purity 96.5%
Study acceptable	Yes

METHODS

The primary objective of this study was to provide information on the rate and extent of absorption, elimination (including the route of elimination) and metabolism of [^{14}C]-labelled XDE-729 sodium salt in beagle dogs following a single oral (gavage) dose of XDE-729 sodium salt. This study was also conducted to evaluate the potential for renal saturation of elimination.

The test substance was labelled on the phenyl ring, as indicated below:



Three male and three female beagle dogs, 6-7 months of age, received a single oral (gavage) dose of 50 mg/kg ^{14}C-XDE-729 sodium salt. The concentration and homogeneity of the formulation was confirmed by HPLC-UV analysis. Time-course plasma and excreta were collected up to 168 hours post-dosing. Samples were analysed for ^{14}C concentration using liquid scintillation counting (LSC) techniques. Selected urine, faecal and plasma samples were analysed for the presence of XDE-729 using an LC/MS-MS method and for metabolites using HPLC with in-line radiochemical (RAM) detection.

RESULTS

Plasma radioactivity was quantifiable for all animals up to 24 hours post-dosing and for one male at 48 hours. Plasma radioactivity concentrations were generally similar between genders. Concentrations of non-labelled XDE-729 were also analysed, and a comparison with the radioactivity measurements suggested that XDE-729 was the major radioactive component in the plasma. These findings are consistent with metabolite profile results obtained by HPLC-RAM

analysis (see Tables B.6.1.1-14 and B.6.1.1-15 below) and indicate that little metabolism of XDE-729 sodium salt occurs in the dog.

Kinetic parameters calculated from the individual plasma radioactivity data are presented in Table B.6.1.1-12. Plasma C_{max} was achieved slightly earlier (0.7 vs. 1.3 h) and was slightly higher in female than male dogs (143 vs. 118 µg-eq./g). The vast majority of the ^{14}C-radioactivity cleared from plasma by 6 hours post-dosing. Elimination of radioactivity from the blood was biphasic, with initial (α) half-life of 1 h and terminal (β) half-life of 9-12 h. Comparison of kinetic parameters with rats showed that the dose corrected plasma AUC_{0-24h} in dogs was 2 to 4-fold higher than rats administered 10 and 100 mg/kg of ^{14}C-XDE-729 sodium salt. These slower clearance data are consistent with saturation of renal elimination in the dog, a phenomenon reported previously for other phenoxyacids.

Table B.6.1.1–12 Kinetic parameters for [^{14}C]-XDE-729 sodium salt equivalents in plasma

Parameter	Males (50 mg/kg)	Females (50 mg/kg)
t_{max} (h)	1.3	0.7
C_{max} (µg-eq./g)	118	143
Absorption $t_{½}$ (h)	0.4	0.2
Elimination $t_{½\alpha}$ (h)	1.2	1.0
Elimination $t_{½\beta}$ (h)	11.6	8.6
AUC_{0-24} (µg-eq. h g^{-1})	509	460

The group mean excretion data are summerised in Table B.6.1.1-13. For both genders, approximately 80% of the administered radioactivity was excreted via the urine, with the largest fraction recovered in the first 12 hours post-dosing. Approximately 10% (males) or 14% (females) of the administered radioactivity was excreted via the faeces, predominantly in the first 24 to 48 hours post-dosing. When combined with cage rinse/wash findings, about 95% and 98% of the administered radioactivity was recovered from males and females, respectively, within 168 hours post-dosing.

Table B.6.1.1–13 Mean % of administered radioactivity recovered in urine, faeces and cage rinse

Time post dose	Males (50 mg/kg)			Females (50 mg/kg)		
	Urine	Faeces	Cage rinse	Urine	Faeces	Cage rinse
0-12	65.11	-	5.91	73.38	-	2.45
12-24	7.07	5.66	1.43	4.65	11.42	0.74
24-48	2.55	4.20	0.62	1.23	2.54	0.22
48-72	0.52	0.34	0.22	0.29	0.27	0.08
72-96	0.31	0.09	0.07	0.21	0.04	0.06
96-120	0.20	0.04	0.08	0.12	0.02	0.04
120-144	0.21	0.05	0.13	0.07	0.01	0.04
144-168	0.12	0.02	0.08	0.08	0.01	0.04
Total	76.1	10.4	8.54	80.5	14.3	3.7
Total excreted incl. cage washings	95.3			98.1		

Metabolite profiles in plasma, urine and faeces are presented in Tables B.6.1.1-14 and B.6.1.1-15. XDE-729 was the major radiolabelled component in in plasma and urine samples and was

present in faeces at levels higher than or similar to those of other identified metabolites. In plasma, at sampling times estimated to coincide with the C_{max} and $½C_{max}$, the identified metabolites included XDE-729, a sulphate conjugate of O-demethyl XDE-729, and an acyl glucuronide conjugate of XDE-729, with the parent being the major radioactive component. In urine, XDE-729, O-demethyl XDE-729 and its glucose and sulphate conjugates thereof, hydroxy XDE-729 and des-chloro XDE-729 were indentified (des-chloro XDE-729 is an impurity of test substance). In faeces, recovered radioactivity was generally evenly distributed between unchanged XDE-729, O-demethyl XDE-729, and an acyl glucuronide conjugate of XDE-729.

Table B.6.1.1–14 Metabolite profile in plasma, urine and faeces: males

Metabolite	Conc. in plasma (µg-equiv./g)		% of administered dose found in		
	Sampling time		Urine/cage rinse	Faeces	Total urine/cage rinse, faeces
	C_{max} (1-2h)	$½C_{max}$ (3-6 h)			
Glucose cong. of O-demethyl-XDE-729	ND	ND	3.61	ND	3.61
Sulphate cong. of O-demethyl-XDE-729	8.4	11.7	1.94	ND	1.94
O-demethyl-XDE-729	ND	ND	1.63	3.91	5.54
Parent XDE-729	106.6	34.5	77.1	2.10	79.2
Des-chloro-XDE-729 (an impurity in test material)	ND	ND	0.45	ND	0.45
Hydroxy XDE-729	ND	ND	0.22	ND	0.22
Acyl glucuronide of XDE-729	3.05	1.98	ND	1.53	1.53
Unknown	ND	ND	0.29	0.35	0.64
Total activity	118.1	48.2	85.3	10.4	95.7

Table B.6.1.1–15 Metabolite profile in plasma, urine and faeces: females

Metabolite	Conc. in plasma (µg-equiv./g)		% of administered dose found in		
	Sampling time		Urine/cage rinse	Faeces	Total urine/cage rinse, faeces
	C_{max} (0.5-1h)	$½C_{max}$ (2-3h)			
Glucose cong. of O-demethyl-XDE-729	ND	ND	2.05	ND	2.05
Sulphate cong. of O-demethyl-XDE-729	4.14	8.33	1.11	ND	1.11
O-demethyl-XDE-729	ND	ND	0.42	ND	0.42
Parent XDE-729	137	51.9	79.4	4.5	83.9
Des-chloro-XDE-729 (an impurity in test material)	ND	ND	0.42	ND	0.42
Hydroxy XDE-729	ND	ND	ND	0.21	0.21
Acyl glucuronide of XDE-729	1.97	1.79	ND	1.62	1.62
Unknown	ND	ND	ND	0.42	0.42
Total activity	143.1	62.0	83.7	14.3	98.0

CONCLUSION

Dogs administered a single oral gavage dose of 50 mg/kg XDE-729 sodium salt are systemically exposed to XDE-729 and associated metabolites, as assessed by the radioactivity in plasma samples. Absorption of the dose appears to be fairly rapid and extensive, based on a plasma t_{max}

of about 1 h post-dosing and the fact that about 80% of the administered dose was excreted in urine. Parent XDE-729 represented the major radioactive component in plasma and urine, which indicates that metabolism of XDE-729 sodium salt in dogs is limited. Indentified metabolites of XDE-729 are O-demethyl XDE-729, its sulphate and glucose conjugates, hydroxyl XDE-729 and an acyl glucuronide conjugate of XDE-729. Excretion of the administered dose is fairly rapid, evidenced by most of the urinary excretion occurring within 12 h of dosing and most of the faecal excretion occurring within 48 h; furthermore, by 168 h at least 95% of administered radioactivity was recovered in the excreta.

[redacted] (2012)

Toxicokinetic investigations conducted as part of studies evaluated elsewhere in Volume 3, Annex B.6.

Table B.6.1.1–16: Summaries of toxicokinetic investigations conducted as part of other studies

Study	Toxicokinetic parameters investigated	Summary of results	Reference (study ID)
Rat, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000/500 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured towards end of study	Significant amounts of parent were systemically absorbed. Elimination was rapid, with urine being a significant route of elimination.	[redacted] 2009 (081115)
Rat, 28 day oral dietary XDE-729 Methyl 0-10-52-261-782 mg/kg/day target	Plasma and urinary XDE-729 Methyl and XDE-729 Acid levels measured towards end of study	Almost all systemically absorbed XDE-729 Methyl was present as the XDE-729 Acid metabolite.	[redacted], 2011 111005
Rat, 90 day oral dietary XDE-729 Acid 0-10-50-250-750 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured towards end of study	Significant amounts of XDE-729 Acid were systemically absorbed. A significant route of elimination is as the parent via the urine.	[redacted] 2010 (091016)
Rat, 90 day oral dietary XDE-729 Methyl 0-3-10-52-261-500 mg/kg/day target	Levels of XDE-729 Methyl, XDE-729 Acid and five additional known metabolites measured in blood and urine towards end of study and in liver samples taken at necropsy.	Post-hepatic exposure to parent XDE-729 Methyl was negligible. In blood the administered dose was present mainly as the XDE-729 Acid metabolite and, to a lesser extent, to demethylated and conjugated XDE-729 Methyl and Acid metabolites. Most of the administered dose of XDE-729 Methyl was excreted as the Acid metabolite or as the demethylated and conjugated metabolites.	S[redacted] 2012 (111082)
Mouse, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured towards end of study	Systemic bioavailability and urinary excretion of XDE-729 Acid was dose-proportional.	[redacted] 2009 (081116)
Mouse, 90 day oral dietary XDE-729 Acid 0-50-250-500-1000 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured towards end of study	Systemic bioavailability and urinary excretion of XDE-729 Acid was dose-proportional.	[redacted] 2010 (091056)
Dog, 28 day oral dietary XDE-729 Acid 0-300-3000-15000(F)-30000(M) ppm	Plasma and urinary XDE-729 Acid levels measured towards end of study	Systemic bioavailability of XDE-729 Acid was dose-proportional at dietary levels of up to 3000 ppm in males and up to 15000 ppm in females.	[redacted] 2010 (081127)
Dog, 90 day oral dietary XDE-729 Acid 0-500-2500-12500 ppm	Plasma and urinary XDE-729 Acid levels measured towards end of study	Systemic bioavailability of parent was dose-proportional at the low and mid doses, but much greater than dose proportional at the highest dose. Urinary elimination processes occurring at 500 ppm appeared to be saturated at the mid and high doses.	[redacted] 2011 (091070)
Dog, 1 year day oral dietary XDE-729 Acid	Plasma and urinary XDE-729 Acid levels measured at 3, 6 and 12 months	Systemic bioavailability of XDE-729 Acid was approximately dose-proportional at all dose levels in females and at the low and mid dose levels in males. Respectively, males and females excreted on	[redacted] 2012 (101163)

0-500-2500-10000/5000 ppm		average 40% and 55% of the dose as intact XDE-729 Acid in the urine.	
Rat, 12 mo. chronic/2 year carcinogenicity, oral dietary XDE-729 Acid 0-20-100-400-625/750 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured at 6 and 12 months	Systemic bioavailability of XDE-729 Acid was approximately dose-proportional (except for males at the highest dose level at 6 months). A mean of 72% of the administered dose of XDE-729 Acid was excreted intact in the urine at 6 months, but at 12 months the proportion of dose excreted in the urine had declined to a mean of 41%, which may be due to an age-related decline in renal clearance.	[redacted] 2012 (091121)
Mouse, 18 mo. carcinogenicity, oral dietary XDE-729 Acid 0-50-250-750/1000 mg/kg/day target	Plasma and urinary XDE-729 Acid levels measured at 6 and 12 months	Systemic bioavailability and urinary excretion of XDE-729 Acid was generally dose-proportional	[redacted] (101021)
Rat, 7 day hepatic gene expression and biomarker analyses, oral dietary XDE-729 Methyl 787 mg/kg/day or XDE-729 Acid 750 mg/kg/day	Levels of XDE-729 Methyl, XDE-729 Acid and five additional known metabolites measured in blood and urine towards end of study and in liver sampled at necropsy.	Parent XDE-729 Methyl was extensively converted to XDE-729 Acid and excreted mainly as either XDE-729 Acid or as O-demethyl XDE-729 Acid and its sulphate, glucuronide or acyl glucuronide conjugates. O-demethylation of the parent followed by glucuronide conjugation constituted a minor metabolic pathway for XDE-729 Methyl. Extent of O-demethylation and subsequent sulphate, glucuronide or acyl glucuronide conjugation for parent XDE-729 Acid was lower than for XDE-729 Methyl.	[redacted] 2012 (111088)
Mice, 7 day probe, oral dietary XDE-729 Methyl 0-261-782 mg/kg/day target	Levels of XDE-729 Methyl, XDE-729 Acid and five additional known metabolites measured in blood and urine towards end of study and in liver sampled at necropsy.	Most of the parent XDE-729 Methyl was rapidly converted to XDE-729 Acid	[redacted] 2012 (110177)
Rat, 4 w exposure, evaluation of molecular & cellular changes in liver XDE-729 Methyl 0-3-10-52-261 mg/kg/day target	Levels of XDE-729 Methyl, XDE-729 Acid and five additional known metabolites measured in blood and urine towards end of study and in liver sampled at necropsy.	XDE-729 Methyl was extensively converted to XDE-729 Acid. About 50% of the administered dose was excreted in the urine as the XDE-729 Acid metabolite. The glucuronide conjugate of O-demethyl XDE-729 Methyl and O-demethyl XDE-729 Acid and its sulphate, glucuronide and acyl glucuronide conjugate were confirmed as metabolites of XDE-729 Methyl. XDE-729 Methyl or its metabolites were not detected in the blood, liver or urine of 4 and 28 day recovery groups.	[redacted] 2012 (120037)
In vitro metabolism study	Assessment of hydrolysis rates of XD-729 Methyl in liver S9, blood and synthetic gastric fluid of mouse, rat and human	Liver S9: rapid hydrolysis to XDE-729 Acid with the relative rates human >> rat > mouse. Hydrolysis saturable, following Michaelis-Menten kinetics. Blood: hydrolysis to XDE-729 Acid follows 1st order kinetics, with the relative rates rat>mouse>>human. Gastric fluid: hydrolysis to the Acid follows 1st order kinetics, but very much slower than liver or blood; fastest rate at pH of 1.2, representing human GIT	[redacted] 2012 110199
PBPK simulations	Predictions of systemic exposure in rats and humans following XDE-729 Methyl exposure	Hydrolysis to XDE-729 Acid is most dependent on the rates of metabolism in liver, accounting for ~60% of total hydrolysis in rat and >95% in human. Human liver/systemic exposure to XDE-729 Methyl is predicted to be negligible at 0.0001 and 0.1 mg/kg/day, exposure levels relevant to predicted human exposure and a proposed ADI respectively.	
Rat reproductive probe, oral dietary XDE-729 Acid 0-50-250-750/500 mg/kg/day target	Plasma XDE-729 Acid levels measured in parents on days 15 and 28 and in offspring on	Systemic exposure to XDE-729 Acid (as indicated by plasma concentrations) was approximately dose-proportional (linear) at all dose levels for parents and offspring.	[redacted] 2010 (091061)

	PND 4.		
Rat developmental probe Dietary XDE-729 Methyl 0-521-2083-4167-8333 ppm	Blood XDE-729 Methyl and XDE-729 Acid levels measured in samples from maternal jugular vein taken towards end of study and in samples from umbilical cord at termination.	Maternal systemic exposure to the XDE-729 Acid metabolite was much greater (~2 orders of magnitude) than to the parent. Foetal exposure to the parent is negligible. Systemic exposure to the Acid metabolite in mothers and foetuses is dose-proportional. Acid metabolite levels in the foetus are about 70% of those found in the mother.	[redacted] 2012 (111070)
Rat developmental Dietary XDE-729 Methyl 0-521-2083-4167 ppm	Blood XDE-729 Methyl and XDE-729 Acid levels measured at termination in samples from maternal jugular vein and umbilical cords.	Negligible maternal and foetal systemic exposure to parent. Systemic exposure to the Acid metabolite occurred in both mothers and foetuses, which was dose-proportional. Acid metabolite levels in the foetus were about 65% of those found in the mother.	[redacted] 2012 (111071)
Rabbit developmental probe Dietary XDE-729 Acid 0-5000-10000-15000-23000 ppm	Plasma XDE-729 Acid levels measured at termination in samples from maternal jugular vein and umbilical cords.	Systemic bioavailability of XDE-729 Acid following dietary administration was approximately dose-proportional in both the mothers and foetuses. In the foetus, plasma concentrations of XDE-729 Acid were about 40% of maternal concentrations.	[redacted] 2011 (091142)
Rabbit developmental Dietary XDE-729 Acid 0-4000-10000-28292 ppm	Plasma XDE-729 Acid levels measured at termination in samples from maternal jugular vein and umbilical cords.	Maternal and foetal systemic exposure to parent occurred in all treated groups. Foetal plasma concentrations of XDE-729 Acid were about 35% of maternal concentrations.	[redacted] 2011 (091143)
Rabbit developmental probe Dietary XDE-729 Methyl 0-521-2083-4167-8333-10417-15625-24500 ppm	Maternal blood XDE-729 Methyl and XDE-729 Acid levels measured towards end of study	Negligible maternal systemic exposure to parent. Maternal systemic exposure to Acid metabolite occurred, which was dose-proportional and showing little diurnal variation. A comparison with maternal systemic exposure to the Acid in the XDE-729 Acid rabbit study (0911043) showed equivalence for maternal systemic exposure to the Acid following dietary exposure to XDE-729-Methyl and to XDE-729-Acid in the rabbit.	[redacted] 2012 (111045)
Rabbit developmental Dietary XDE-729 Methyl 0-122-391-1539 ppm	Blood XDE-729 Methyl and XDE-729 Acid levels measured at termination in samples from maternal jugular vein and umbilical cords.	Negligible systemic maternal or foetal exposure to XDE-729 Methyl. Maternal systemic exposure to XDE-729 Acid occurs at all exposure levels and was approximately dose-proportional. Foetal exposure to XDE-729 Acid also occurs, but was only demonstrated at 391 and 1539 ppm, and dose-proportionality was not observed.	[redacted] 2012 (111137)
In vivo percutaneous absorption of XDE-729 Methyl (formulated as GF-2573), rats, 7.5 g/L	Plasma XDE-729 Methyl and XDE-729 Acid levels, 4 & 10 h after a 10 h application	XDE-729 Methyl was not detected in pooled plasma samples. XDE-729 Acid was present in plasma.	[redacted] 2011 (V9029)

The additional toxicokinetic investigation confirmed the metabolic characteristics identified in the specialised *in vivo* toxicokinetic studies, which is that XDE-729 Methyl is rapidly and extensively hydrolysed to XDE-729 Acid such that post-hepatic systemic exposure to parent XDE-729 Methyl is very low for the oral route. A XDE-729 Methyl percutaneous absorption study in rats demonstrated the absence of systemic exposure to XDE-729 Methyl following exposure by this route.

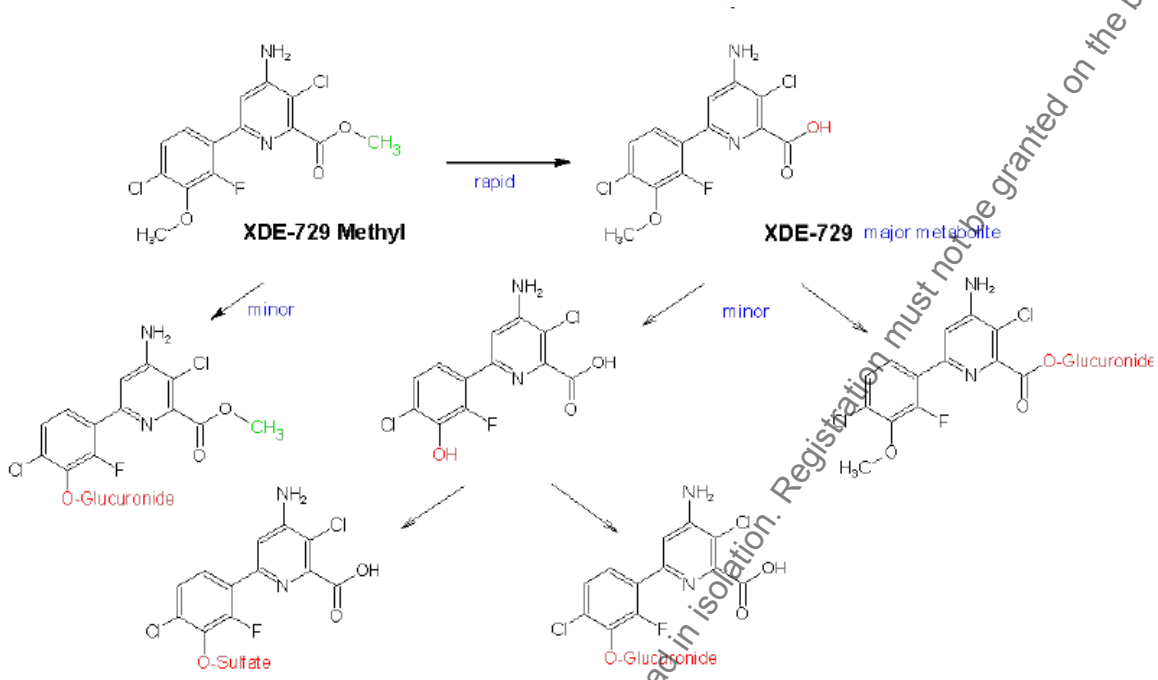
However, the toxicokinetic investigations in the XDE-729 Methyl 90-day oral rat study [redacted]. 2012, IIA 5.3.2/02) revealed the presence an additional metabolite of XDE-729 Methyl, the glucuronide conjugate of O-demethyl XDE-729 Methyl, detected in blood, liver and urine. The amount of this metabolite excreted in urine in females accounted for up to 5.5% of the dose of XDE-729 Methyl (adjusted for equivalence) at a dose level of 3 mg/kg/day and 12.4% at 500 mg/kg/day. This suggests that not all of administered XDE-729 Methyl hydrolyses to XDE-729 Acid, even at dose levels as low as 3 mg/kg/day. This glucuronide conjugate of O-demethyl

XDE-729 Methyl was also detected in two mechanistic studies (IIA 5.5.4/01 and IIA 5.5.4/03) evaluated in Section B.6.5.3. The RMS concludes that the possibility of systemic exposure to direct metabolites of the parent following dosing with XDE-729 Methyl is not of concern for the following reason (a) absolutely no free O-demethyl XDE-729 Methyl has been detected in blood, liver or urine at any exposure level of XDE-729 Methyl; (b) conjugated O-demethyl XDE-729 Methyl was present in blood only at dose levels above the NOAEL of 10 mg/kg/day, implying that post-hepatic exposure to this direct XDE-729 Methyl metabolite will be zero at exposure levels below the key NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity in the rat; and, finally, (c) the presence of only the glucuronide conjugate in liver and urine provides strong support for the notion that O-demethyl XDE-729 Methyl undergoes rapid metabolism and elimination as the glucuronide conjugate, implying that concerns for toxicity of O-demethyl XDE-729 Methyl will be low.

The toxicokinetic investigations in the rat and rabbit developmental toxicity studies demonstrate that following XDE-729 Methyl administration there is both maternal systemic and foetal exposure to the Acid metabolite, but negligible maternal systemic and foetal exposure to the parent compound. In the rabbit it was shown that that maternal systemic exposure to XDE-729 Acid shows dose-equivalence for dietary exposure to XDE-729 Methyl and the Acid (not investigated in the rat). Foetal plasma concentrations of XDE-729 Acid are about 40-70% of maternal levels.

Based on the blood, liver and urine analysis results from the XDE-729 Methyl 90-day study metabolic pathways for XDE-729 Methyl in the rat are proposed in Figure 6.1.1-2.

Figure 6.1.1-2 Proposed metabolic pathway for XDE-729 Methyl in rats (from the 90-day oral dietary study in the rat, IIA 5.3.2/02)



B.6.1.2 Metabolism

See Section B.6.1.1.

B.6.1.3 Summary of toxicokinetics studies

Absorption

XDE-729 Acid and XDE-729 Methyl are rapidly and extensively absorbed following oral administration in rats, with plasma C_{max} occurring in the rat within 30 minutes post-dosing. Absolute oral bioavailability, calculated from the dose-corrected plasma AUC data for the low oral and IV dose groups is close to 100% for both female and male rats. In dogs, absorption XDE-729 Acid is fairly rapid with t_{max} at 0.5 to 1 hour post-dosing for both sexes. Oral bioavailability in the dog is also high with about 80% of the administered dose of XDE-729 Acid eliminated in the urine.

Distribution

The presence of a high proportion of administered radioactivity in the blood plasma indicates widespread systemic circulation of both XDE-729 Na salt and XDE-729 Methyl metabolites. There is no evidence of preferential distribution to any particular tissue, or of accumulation. Foetal exposure to XDE-729 Acid in systemic circulation is demonstrated.

Metabolism

Metabolism of XDE-729 Acid is limited in rats, mice and dogs evidenced by the parent compound being the major radiolabelled component in urine and faeces, accounting for 82-98% of administered dose in rats. Minor metabolites of XDE-729 Acid are the acyl-glucuronide conjugate of XDE-729 (accounting for up to ~7% of radioactivity dose in rats) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (~6% of dose).

The major radiolabelled component present in urine and faeces following oral administration of XDE-729 Methyl is also XDE-729 Acid (DAS metabolite code X11449757), demonstrating that XDE-729 Methyl is rapidly and extensively hydrolysed to XDE-729 Acid. The Acid accounts for about 80% of administered radioactivity, in males and females, respectively. As is the case for XDE-729 Acid, minor metabolites of XDE-729 Methyl are the acyl-glucuronide conjugate of XDE-729 (accounting for up to ~6% of radioactive dose) and O-demethyl-XDE-729 (X11449757) and the corresponding sulphate and glucuronide conjugates (up to ~11%). The primary analyte in blood samples is XDE-729 Acid, with no parent compound being detected, confirming the rapid hydrolysis of XDE-729 Methyl in vivo and providing evidence of post-hepatic systemic bioequivalence between XDE-729 Acid and XDE-729 Methyl. Toxicokinetic investigations in the 90-day oral dietary study in the rat (IIA 5.3.2/02) identified an additional metabolite of XDE-729 Methyl, the glucuronide conjugate of demethyl XDE-729 Methyl (metabolite code for demethyl XDE-729 Methyl is X11406790), detected in blood, liver and urine. The amount of this metabolite excreted in urine in females accounts for up to 5.5% of the dose of XDE-729 Methyl (adjusted for equivalence) at a dose level of 3 mg/kg/day and 12.4% at 500 mg/kg/day. This indicates that not all administered XDE-729 Methyl undergoes hydrolysis to XDE-729 Acid, even at dose levels as low as 3 mg/kg/day.

For XDE-729 Methyl administration by the dermal route, systemic exposure is shown also to be primarily to XDE-729 Acid following application of concentrations up to 7.5 g/L for 10 hours.

Excretion

Absorbed XDE-729 Acid is rapidly and extensively excreted mainly via urine (68-92% of the administered dose), with the proportion in urine being highest among females. The majority of the urinary elimination occurs within the first 24 hours post-dosing. A smaller percent of the oral dose is eliminated in faeces, the majority of which (78-93%) occurred within 24 h of dosing. The extent and rates of urinary and faecal elimination of absorbed XDE-729 Methyl is similar to the XDE-729 Acid salts.

B.6.1.4 Comparison of rat metabolism with animal, plant and environmental metabolism

In lactating goats and laying hens the metabolism XDE-729 Methyl (see Volume 3, B.7.2) is very similar to rats. In the two domestic livestock species studied, XDE-729 Methyl either hydrolyses to XDE-729 Acid (X11393729) which can subsequently be demethylated to o-demethyl XDE-729 Acid (X11449757), or is demethylated to o-demethyl XDE-729 Methyl (X11406790); these pathways also occur in rats. In the goat, glucuronide conjugates of XDE-729 Acid and o-demethyl XDE-729 Methyl and sulphate conjugates of o-demethyl XDE-729 Acid and o-demethyl XDE-729 Methyl are detected, as is the case for rats. In hens, the only conjugates found are the sulphate conjugates of o-demethyl XDE-729 Acid and o-demethyl XDE-729, which are found in rats.

In plants the metabolism of XDE-729 Methyl (see Volume 3, B.7.1) is similar to rats. In both wheat and turnips, XDE-729 Methyl either hydrolyses to XDE-729 Acid (X11393729) or is demethylated to form o-demethyl XDE-729 Methyl (X11406790); both pathways occur in rats. In wheat, XDE-729 Acid is demethylated to o-demethyl XDE-729 Acid (X11449757) and o-demethyl XDE-729 Methyl is rapidly conjugated with glucose followed by malonylation. In turnips, XDE-729 Acid is conjugated with glucose, either through the nitrogen or oxygen; o-demethyl XDE-729 Methyl is also conjugated with glucose followed by malonylation. In rats, different conjugates of o-demethyl XDE-729 Acid (sulphate and glucuronide conjugates) and o-demethyl XDE-729 Methyl (a glucuronide conjugate) are found, but this is considered not to be of toxicological concern because a conjugated metabolite is likely to be less toxic than its unconjugated form and deconjugation will give rise to aglycones that are also formed in rats. Furthermore, an evaluation presented in Volume 3, B.7.1, which includes a TTC analysis, concludes that the metabolites of XDE-729 Methyl, including their conjugates, are not of concern in the human health assessment, supporting their non-inclusion in the residue definition for crops.

In the environment, the main residues found are XDE-729 Methyl parent, XDE-729 Acid (X11393729), o-demethyl XDE-729 Methyl (X11406790) and o-demethyl XDE-729 Acid (X11449757) (see Volume 3, B.8.), which are also found in rats. However, in surface water and sediment, three transient aqueous photolysis metabolites Deg 10, Deg 11 and Deg 14 can be found at >10% AR. Structures for these metabolites are proposed in Volume 3, B.8.4.6. Deg 10 maintains the structure of XDE-729 Methyl, with the 3-chlorine on the pyridine ring replaced by a hydroxyl group; in both Deg 11 and Deg 14 the pyridine ring is opened to give structures with no identified correlate in rats. Deg 10, Deg 11 and Deg 14 are included in the residues definitions with respect to surface water and sediment.

B.6.2 Acute toxicity, irritancy and skin sensitisation studies (IIA 5.2)

Acute toxicity, irritancy and skin sensitisation testing has been conducted with both XDE-729 Acid and XDE-729 Methyl.

B.6.2.1 Acute oral toxicity (IIA 5.2.1)

XDE-729 Acid	
Study	IIA 5.2.1/01 Acute oral toxicity up and down procedure in rats
Reference	[redacted] (2010a)
Date performed	October 2009
Test facility	[redacted]
Report reference	[redacted] study no. 28268, Dow study no. 090506
Guideline(s)	OECD 425
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 11 weeks old, were used. Dosing was by gavage. The animals were fasted prior to dosing. The vehicle was distilled water. The stability of the test substance in the vehicle was not determined.

One group of 4 females received a single dose of 5000 mg/kg bodyweight, administered as 13.5% mixture. As three animals survived at this dose level, which is the limit dose for this type of study, no further testing was necessary.

Animals were examined for mortality and clinical signs frequently on the day of dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

RESULTS

One animal died, two days after dosing. Toxic signs noted in the decedent prior to death included facial and ano-genital staining and hypoactivity. Apart from soft faeces noted in two surviving rats one day post-dosing, all survivors appeared active and healthy over the observation period, gaining body weight over the course of the study. The necropsy findings for the decedent were discoloration of the lungs, pale liver and distended stomach. No gross abnormalities were noted at necropsy in any of the animals killed at the conclusion of the 14-day observation period.

CONCLUSION

The acute oral LD_{50} of XDE-729 Acid in female rats is >5000 mg/kg. Accordingly, XDE-729 Acid is not classified for acute oral toxicity.

([redacted] 2010a)

XDE-729 Methyl	
Study	IIA 5.2.1/02 Acute oral toxicity up and down procedure in rats
Reference	[redacted] (2011a)
Date performed	June – July 2011
Test facility	[redacted]
Report reference	[redacted] study no. 32454, Dow study no. 110543
Guideline(s)	OECD 425
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 9 weeks old, were used. Dosing was by gavage. The animals were fasted prior to dosing. The vehicle was distilled water. The stability of the test substance in the vehicle was not determined.

One group of 3 females received a single dose of 5000 mg/kg bodyweight, administered as 20% mixture. As three animals survived at this dose level, which is the limit dose for this type of study, no further testing was necessary.

Animals were examined for mortality and clinical signs frequently on the day of dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

The current study was conducted to confirm results of a previous study which investigated the acute oral toxicity of the test substance in a corn oil vehicle (EPSL Study no. 31305, Dow study no. 102068). In this study, at the limit dose of 5000 mg/kg the animals were administered approximately 35 mL/kg of a corn oil suspension, and one of a group of five females died. The sponsor was concerned that excess corn oil might adversely affect the absorption of the test substance. Therefore, the Sponsor requested that the study be repeated using a different vehicle.

The Sponsor requested that the current study be conducted using PEG400 as the vehicle. Two animals were administered 5000 mg/kg body weight doses of a 30% w/w mixture of the test substance in PEG400. Both animals died due to technical error and complications during administration with the chosen vehicle (i.e., punctured trachea and/or test substance evident in the thoracic cavity). Due to these unforeseen circumstances, dosing of the PEG400 preparation was terminated and additional solubility work was conducted using distilled water as the vehicle. After several attempts, it was determined that the test substance could be administered as a 20% mixture in distilled water. The data from the two animals treated with the test substance in PEG400 were not considered in the interpretation of the current study.

RESULTS

Following administration of the test substance using a water vehicle, one animal exhibited reduced faecal volume, but recovered from this clinical sign by day 2. All three animals gained body weight and appeared active and healthy for the remainder of the 14-day observation period.

No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

CONCLUSION

The acute oral LD_{50} of XDE-729 Methyl in female rats is >5000 mg/kg. Accordingly, XDE-729 Methyl is not classified for acute oral toxicity.

([redacted] 2011a)

B.6.2.2 Acute dermal toxicity (IIA 5.2.2)

XDE-729 Acid	
Study	IIA 5.2.2/01 Acute dermal toxicity study in rats
Reference	[redacted] (2009a)
Date performed	October 2009
Test facility	[redacted]
Report reference	[redacted] study no. 28269, Dow study no. 090507
Guideline(s)	OECD 402
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 9 weeks old, were used. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk. The test material was moistened with water and applied to a 5 x 7.5 cm, 4-ply, gauze pad and placed on an area of clipped skin, measuring 5 x 7.5 cm (approximately 10% of the body surface) on each animal. The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. After an exposure period of 24 hours, the dressings were removed and dosing site was rinsed with a 3% soap solution and then with tap water.

A group of 5 males and 5 females received a single dose of 5000 mg/kg bodyweight.

Animals were examined for mortality and clinical signs frequently on the day of dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

RESULTS

All animals survived exposure to the test substance, appeared active and healthy and gained body weight throughout the study. There were no signs of gross toxicity, dermal irritation, adverse clinical signs, or abnormal behaviour. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

CONCLUSION

The acute dermal LD_{50} of XDE-729 Acid in rats is >5000 mg/kg. Accordingly, XDE-729 Acid is not classified for acute dermal toxicity.

([redacted] 2009a)

XDE-729 Methyl	
Study	IIA 5.2.2/02 Acute dermal toxicity study in rats
Reference	[redacted] 2011b)
Date performed	December 2010 – January 2011
Test facility	[redacted], Dayton, NJ, USA
Report reference	[redacted] study no. 31306, Dow study no. 102069
Guideline(s)	OECD 402
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 10 weeks old, were used. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk. The test material was moistened with water and applied to a 5 x 7.5 cm, 4-ply, gauze pad and placed on an area of clipped skin, measuring 5 x 7.5 cm (approximately 10% of the body surface) on each animal. The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. After an exposure period of 24 hours, the dressings were removed and dosing site was rinsed with a 3% soap solution and then with tap water.

A group of 5 males and 5 females received a single dose of 5000 mg/kg bodyweight.

Animals were examined for mortality and clinical signs frequently on the day of dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

RESULTS

All animals survived exposure to the test substance. Dermal irritation (erythema and oedema) was noted at the dose site of three animals between days 1 and 4. There were no other clinical observations recorded for any animal over the course of the study. Although two males and one female lost or failed to gain body weight through to day 7, all animals gained weight by the end of the study. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

CONCLUSION

The acute dermal LD_{50} of XDE-729 Methyl in rats is >5000 mg/kg. Accordingly, XDE-729 Methyl is not classified for acute dermal toxicity.

([redacted] 2011b)

B.6.2.3 Acute inhalation toxicity (IIA 5.2.3)

XDE-729 Acid & XDE-729 Methyl	
Study	IIA 5.2.3/01 Acute dust aerosol inhalation toxicity studies in F344/DuCrl rats
Reference	[redacted] (2011)
Date performed	Completed April 2011
Test facility	[redacted]
Report reference	Laboratory study ID 091096
Guideline(s)	Study not conducted because respirable dry powder aerosols of the test substances could not be generated
Deviations from the guideline	N/A
GLP	No
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Justification for non-conduct of study is acceptable

METHODS

Attempts were made to determine the acute inhalation toxicological properties of XDE-729 Acid and XDE-729 Methyl. However, significant technical problems were encountered which prevented the generation of a stable, respirable, dry-powder aerosol necessary to conduct guideline-compliant acute inhalation toxicity studies.

Repeated attempts to generate a stable aerosol of XDE-729 Acid with a mass median aerodynamic diameter (MMAD) of 1 to 4 microns, using a various aerosol generation systems, failed due to (1) clogging of the aerosol generator, cyclones, and aerosol delivery lines; (2) large particle size (MMAD > 4 microns); and (3) high variability in chamber aerosol concentration.

Repeated attempts to generate a stable aerosol of XDE-729 methyl with a MMAD of 1 to 4 microns, using a various aerosol generation systems, failed due to (1) clogging or stalling of the aerosol generator and (2) large particle size (MMAD > 4 microns).

Based on the inability to generate a guideline-compliant respirable dry powder aerosol of either XDE-729 or XDE-729 methyl dry-powder aerosol (i.e., 1-4 microns MMAD) at any exposure concentration for 4 hours, acute inhalation toxicity studies in rats were not conducted.

It is noted that both XDE-729 Acid and XDE-729 Methyl have low vapour pressures (~10^{-5} Pa and ~10^{-8} Pa respectively) and therefore there will be no significant vapour production under normal conditions.

[redacted] (2011)

B.6.2.4 Skin irritancy (IIA 5.2.4)

XDE-729 Acid	
Study	IIA 5.2.4/01 Primary skin irritation study in rabbits
Reference	[redacted] 2009b)
Date performed	September 2009

Test facility	[redacted]
Report reference	[redacted] study no. 28271, Dow study no. 090508
Guideline(s)	OECD 404
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

XDE-729 Acid was applied by semi-occlusive application of 0.5 g (moistened with water) to a clipped area of intact skin on the dorsal area of the trunk of each of three young adult male New Zealand white rabbits. The duration of treatment was 4 hours, after which the dressings were removed and dosing site was rinsed with a 3% soap solution followed by tap water.

The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study. Any skin reactions were assessed according to the numerical scoring system of OECD test guideline 404, approximately 1, 24, 48 and 72 hours after the removal of the dressings. The study was terminated at 72 hours.

RESULTS

No deaths occurred. No systemic signs of toxicity were noted during the study. The body weights of the rabbits were considered to be within the normal range of variability.

Slight erythema (grade 1) was observed in two animals 1 hour after removal of the patch, which persisted in one rabbit to the 24 hour observation. No skin reactions were observed at the 48 and 72 hour observations. The mean irritation scores for the 24, 48 and 72 hours observations are shown in the following table, demonstrating that the criteria for classification as a skin irritant are not met.

Table B.6.2.4-1: Mean skin irritation scores at 24, 48 and 72 h

Number tested	Score for each animal: mean of 24, 48 & 72 h observations		Reversibility (Yes/No)	Result
	Erythema	Oedema		
3 NZW rabbits	0.3, 0, 0	0, 0, 0	Yes	Not irritating

CONCLUSION

The application of XDE-729 Acid to the skin did not elicit any significant skin reactions. Accordingly, this substance is not classified for skin irritancy.

([redacted] 2009b)

XDE-729 Methyl	
Study	IIA 5.2.4/02 Primary skin irritation study in rabbits
Reference	[redacted] 2011c)
Date performed	January 2011
Test facility	[redacted]
Report reference	[redacted] study no. 31308, Dow study no. 102070
Guideline(s)	OECD 404
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl was applied by semi-occlusive application of 0.5 g (moistened with water) to a clipped area of intact skin on the dorsal area of the trunk of each of three young adult male New Zealand white rabbits. The duration of treatment was 4 hours, after which the dressings were removed and dosing site was rinsed with a 3% soap solution followed by tap water.

The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study. Any skin reactions were assessed according to the numerical scoring system of OECD test guideline 404, approximately 1, 24, 48 and 72 hours after the removal of the dressings. The study was terminated at 72 hours.

RESULTS

No deaths occurred. No systemic signs of toxicity were noted during the study. The body weights of the rabbits were considered to be within the normal range of variability.

Slight erythema (grade 1) was observed in all three animals 1 hour after removal of the patches. However, no skin reactions were observed at the 24, 48 and 72 hour observations, indicating that the criteria for classification as a skin irritant are not met.

CONCLUSION

The application of XDE-729 Methyl to the skin did not elicit any significant skin reactions. Accordingly, this substance is not classified for skin irritancy.

([redacted] 2011c)

B.6.2.5 Eye irritancy (IIA 5.2.5)

XDE-729 Acid	
Study	IIA 5.2.5/01 Primary eye irritation study in rabbits
Reference	[redacted] (2010b)
Date performed	September – October 2009
Test facility	[redacted]
Report reference	[redacted] study no. 28270, Dow study no. 090509
Guideline(s)	OECD 405
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

A 0.1 ml aliquot XDE-729 Acid was installed into one eye of each of three young adult male New Zealand white rabbits. The other eye, which was untreated, served as a control. Scoring of eye reactions, according to the numerical system of OECD test guideline 405, was conducted at 1, 24, 48 and 72 hours. The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study

RESULTS

No deaths occurred. No systemic signs of toxicity were reported.

The mean irritation scores for the 24, 48 and 72 h observations are presented in the table below, showing than XDE-729 Acid elicited only very mild eye reactions. All reactions had resolved by 72 h. Thus, the criteria for classification as an eye irritant are not met.

Table B.6.2.5–1: Mean irritation scores at 24, 48 and 72 h

Number tested	Score for each animal: mean of 24, 48 & 72 h observations				Reversibility Yes/no	Result
	Cornea	Iris	Conjunctiva			
			Redness	Chemosis		
3 NZW rabbits	0, 0, 0	0, 0, 0	0.3, 0.3, 0.7	0, 0, 0	Yes	Not irritating

CONCLUSION

XDE-729 Acid did not induce significant or irreversible damage to the eye and therefore this substance is not classified for eye irritancy.

[redacted] (2010b)

XDE-729 Methyl	
Study	IIA 5.2.5/02 Primary eye irritation study in rabbit
Reference	[redacted] (2011d)
Date performed	January 2011
Test facility	[redacted]
Report reference	[redacted] study no. 31307, Dow study no.102071
Guideline(s)	OECD 405
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

A 0.1 ml aliquot XDE-729 Methyl was installed into one eye of each of three young adult female New Zealand white rabbits. The other eye, which was untreated, served as a control. Scoring of eye reactions, according to the numerical system of OECD test guideline 405, was conducted at 1, 24, 48 and 72 hours. The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study

RESULTS

No deaths occurred. No systemic signs of toxicity were reported.

One hour after instillation, minimal conjunctival discharge (grade 1) was observed in all three animals. However, no eye reactions were observed at the 24, 48 and 72 hour observations, indicating that the criteria for classification as a skin irritant are not met.

CONCLUSION

XDE-729 Methyl did not induce significant or irreversible damage to the eye and therefore this substance is not classified for eye irritancy.

[redacted] (2011d)

B.6.2.6 Skin sensitisation (IIA 5.2.6)

XDE-729 Acid	
Study	IIA 5.2.6/01 Local lymph node assay in CBA/J mice
Reference	[redacted] (2011a)
Date performed	August 2009
Test facility	[redacted]
Report reference	Laboratory study ID 091092
Guideline(s)	OECD 429 (2002)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Female mice of the CBA/J strain, aged 9-12 weeks, were used.

In a screening study, 3 daily topical applications (25 µl to dorsal surface of each ear) of 1%, 5%, 10%, 20%, 40%, or 80% XDE-729 Acid in DMSO were given to one animal at each dose level. Erythema was absent and bodyweights were unaffected in all dose groups. Results from this study were used to select concentrations for the main LLNA.

In the LLNA, six female mice/group received 3 daily topical applications (25 µl to dorsal surface of each ear) of 5%, 20%, or 80% of XDE-729 Acid. A vehicle and positive control groups were similarly treated with DMSO or 30% α-hexylcinnamaldehyde (HCA), respectively. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µl of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were killed and the draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS. A single cell suspension of the auricular lymph nodes from each mouse was prepared by gentle mechanical disaggregation using a tissue homogenizer. The cells were washed two times and were suspended in 3 ml of 5% trichloroacetic acid (TCA) for approximately 18 hours. The suspended precipitates were centrifuged (200 x g for 10 minutes) and the supernatant removed. The pellet from each mouse was reconstituted in 1 ml of 5% TCA and subsequently transferred to a scintillation vial containing 10 ml of Aquasol-2 scintillation cocktail. Two additional 2 ml aliquots of water were used to rinse the tubes and the rinses were added to the scintillation vials containing the 1 ml of pellet in TCA and cocktail. The radioactivity in each precipitate was measured using a β-scintillation counter and reported as disintegrations per minute (dpm) per mouse. The mean activity of each test group is then divided by the mean activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration. The criterion for a positive response is that the SI for one or more concentrations is ≥3.

During the study any clinical signs were recorded, the ears were examined for erythema, and bodyweights were recorded.

RESULTS

There were no clinical signs of toxicity, adverse effects on bodyweights or evidence of irritation of the ears.

As shown in Table B.6.2.6-1, the SI values for all XDE-treated groups were <3, indicating a negative response. For the positive control group, the SI was 5.4 (i.e. ≥3), demonstrating the validity of test system.

Table B.6.2.6–1: Group mean disintegrations/min and stimulation indices

Dose group	Mean disintegrations/min	SI
0 (DMSO, vehicle control)	1000	1.0
XDE-729 Acid 5%	675	0.7
XDE-729 Acid 20%	690	0.7
XDE-729 Acid 80%	1037	1.1
HCA 30% (positive control)	5370	5.4

There was a large variation between vehicle control responses reported in this study (mean of 1000 dpm) and in the LLNAs for the Methyl ester (2377 dpm, see study IIA 5.2.6/02) and the representative product (1954 dpm, see study IIIA1 6.11.6/01). These studies were conducted in the same laboratory. The vehicles used were DMSO for the Acid and Methyl ester tests and Pluronic L92 surfactant for the test on the product. Consideration of the laboratory historical control data for LLNAs (7 studies conducted 2008-2011 using DMSO vehicle) indicates the observed variation is normal for this type of study. Control responses ranged from 546 dpm to the 2377 dpm observed in study IIA 5.2.6/02. Also, published reports show large variations in dpm values for DMSO vehicle control groups from independent experiments (Anzai *et al*, 2010, Exp Anim, 59, 245-249; Ryan *et al* 2002, Food Chem Toxicol, 40, 1719-1725). Additionally, another published study shows dpm variations as high as 4-fold within in a single experiment associated with the use of different vehicles (Warbrick et al 1999 Contact Dermatitis **41**, 325-329).

CONCLUSION

XDE-729 Acid does not show skin sensitising potential in the LLNA.

([redacted] 2011a)

XDE-729 Methyl	
Study	IIA 5.2.6/02 Local lymph node assay in CBA/J mice
Reference	[redacted] (2011b)
Date performed	December 2010
Test facility	[redacted]
Report reference	Laboratory study ID 101177
Guideline(s)	OECD 429 (2010)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Female mice of the CBA/J strain, aged 8-12 weeks, were used.

In a screening study, 3 daily topical applications (25 µl to dorsal surface of each ear) of 5%, 10%, 25%, 50%, or 75% XDE-729 Acid in DMSO were given to one animal at each dose level. Erythema was absent, and bodyweights and ear thickness were unaffected in all dose groups. Results from this study were used to select concentrations for the main LLNA.

In the LLNA, five female mice/group received 3 daily topical applications (25 µl to dorsal surface of each ear) of 5%, 25%, or 75% of XDE-729 Acid. A vehicle and positive control groups were similarly treated with DMSO or 25% α-hexylcinnamaldehyde (HCA), respectively. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µl of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were killed and the draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS. A single cell suspension

of the auricular lymph nodes from each mouse was prepared by gentle mechanical disaggregation using a tissue homogenizer. The cells were washed two times and were suspended in 3 ml of 5% trichloroacetic acid (TCA) for approximately 18 hours. The suspended precipitates were centrifuged (200 x g for 10 minutes) and the supernatant removed. The pellet from each mouse was reconstituted in 1 ml of 5% TCA and subsequently transferred to a scintillation vial containing 10 ml of Aquasol-2 scintillation cocktail. Two additional 2 ml aliquots of water were used to rinse the tubes and the rinses were added to the scintillation vials containing the 1 ml of pellet in TCA and cocktail. The radioactivity in each precipitate was measured using a β-scintillation counter and reported as disintegrations per minute (dpm) per mouse. The stimulation index (SI) was calculated using the absolute dpm value for each mouse as the numerator and the mean dpm value from the vehicle control mice as the denominator; the mean SI was calculated for each experimental group. The criterion for a positive response is that the SI for one or more concentrations is ≥3.

The criterion for a positive response is that the SI for one or more concentrations is ≥3.

During the study any clinical signs were recorded, the ears were examined for erythema, and bodyweights were recorded.

RESULTS

There were no clinical signs of toxicity, adverse effects on bodyweights or evidence of irritation of the ears.

As shown in Table B.6.2.6-2, the SI values for all XDE-treated groups were <3, indicating a negative response. For the positive control group, the SI was 3.5 (i.e. ≥3), demonstrating the validity of test system.

Table B.6.2.6–2: Group mean disintegrations/min and stimulation indices

Dose group	Mean disintegrations/min	SI
0 (DMSO, vehicle control)	2377	1.0
XDE-729 Methyl 5%	1996	0.8
XDE-729 Methyl 25%	2091	0.9
XDE-729 Methyl 75%	3116	1.3
HCA 25% (positive control)	8455	3.5

CONCLUSION

XDE-729 Methyl does not show skin sensitising potential in the LLNA.

([redacted] 2011b)

B.6.2.7 Summary of acute toxicity, irritancy and sensitisation studies

Table B.6.7.2-1 Summary of acute toxicity studies

Test	Species	Result	Classification		Reference (study ID)
			67/548/EEC	EC1272/2008	
Acute oral XDE-729 Acid	Rat	LD_{50} >5000 mg/kg	-	-	[redacted] 2010a (090506)
Acute oral XDE-729 Methyl		LD_{50} >5000 mg/kg	-	-	[redacted] 2011a (110543)
Acute dermal XDE-729 Acid	Rat	LD_{50} >5000 mg/kg	-	-	[redacted] 2009a (090507)
Acute dermal XDE-729 Methyl		LD_{50} >5000 mg/kg	-	-	[redacted] 2011b (102069)
Acute inhalation XDE-729 Acid	–	Studies not conducted: not technically possible to generate respirable test atmosphere	-	-	[redacted] 2011 (091096)
Acute inhalation XDE-729 Methyl			-	-	
Skin irritation XDE-729 Acid	Rabbit	Negative	-	-	[redacted] 2009b (090508)
Skin irritation XDE-729 Methyl		Negative		-	[redacted] 2011c (102070)
Eye irritation XDE-729 Acid	Rabbit	Negative	-	-	[redacted] 2010b (090509)
Eye irritation XDE-729 Methyl		Negative	-	-	[redacted] 2011d (102071)
Skin sensitisation XDE-729 Acid	Mouse (LLNA)	Negative	-	-	[redacted], [redacted] 2011a (091092)
Skin sensitisation XDE-729 Methyl		Negative	-	-	[redacted] [redacted] 2011b (101177)

The acute toxicity, irritancy and skin sensitisation potential of XDE-729 Acid and XDE-729 Methyl have been investigated in standard GLP and OECD guideline compliant studies. These studies showed that both XDE-729 Acid and XDE-729 Methyl are of low acute oral and dermal toxicity, with LD_{50} values above those required for classification, and that neither is irritating to the skin and eye or show skin sensitising potential. Acute inhalation studies were not conducted because it is not technically possible to generate respirable test atmospheres; furthermore, human inhalation exposure is unlikely as both XDE-729 Acid and XDE-729 Methyl are classed as very slightly volatile (see B.2.1.5).

B.6.3 Short-term toxicity studies (IIA 5.3)

The short-term toxicity of XDE-729 Acid has been investigated in 28 and 90 day oral feeding studies in rats, mice and dogs, and in a 1-year feeding study in dogs. Additionally, the toxicity of XDE-729 Methyl has been investigated in 28 and 90 day oral feeding studies in rats. Also, a 28 day repeated dose dermal exposure study in rats for XDE-729 Acid has been conducted.

B.6.3.1 Oral short-term toxicity in the rat (IIA 5.3.1, 5.3.2)

XDE-729 Acid	
Study	IIA 5.3.1/01 28-day dietary toxicity study in F344/DuCrl rats
Reference	[redacted] (2009)
Date performed	August – September 2008
Test facility	[redacted]
Report reference	Laboratory study ID 081115
Guideline(s)	OECD 407 (1995)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2350-93 TSN030751-0002, 99.00% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.1–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	5	5
2	10	5	5
3	50	5	5
4	250	5	5
5	1000/500*	5	5

On study day 11, the dose level for group 5 males was reduced from 1000 mg/kg/day to 500 mg/kg/day following observation of marked reductions in bodyweight gain and food consumption for this group.

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 13% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. On day 23, blood samples were taken from the jugular vein of all animals (non-fasted) at 06.00, 09.00 and 17.00 hours, for determination of plasma XDE-729 Acid levels. Additional blood samples were taken at study termination, after fasting, for determination of plasma XDE-729 Acid levels. On day 26, 24 hour urine samples were collected from all animals for determination of XDE-729 Acid levels. Additionally, selected urine samples were analysed by LC/MC analysis for tentative identification of XDE-729 metabolites.

At the end of the study, and after an overnight fast, blood samples were taken from the retroorbital venous plexus and a standard range of haematology and clinical chemistry parameters were measured. Additionally, towards the end of the study, overnight urine samples were collected and a standard range of urinalyses parameters were assessed.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The liver, kidneys, spleen and bone marrow (sternum) and macroscopic abnormalities from all animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.1–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	10	50	250	500/1000
Males	10.5	52.7	270	734
Females	10	50.3	250	982

There were no treatment related deaths. The only treatment related clinical sign was thin appearance of one male at 500/1000 mg/kg/day, which reflected the lower bodyweight of this animal.

There was a reduction in bodyweight gain and food consumption at the highest dose level tested in both sexes, as shown in Tables 6.3.1-3 and 6.3.1-4. For males, bodyweights and food consumption at 1000 mg/kg/day were markedly reduced up to day 11. After day 11, when the target dose level for males was reduced to 500 mg/kg/day, food consumption was similar to controls although bodyweights remained lower. For females, bodyweights and food consumption were less than controls throughout the study, although the effect was less severe that for males at 1000 mg/kg/day.

Table B.6.3.1–3 Group mean bodyweights, selected values (g)

Day	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
1	144	144	143	146	144	109	109	109	106	107
8	171	173	170	176	144*	122	124	123	120	117*
15	199	203	201	204	161*	136	138	138	133	127*
22	223	226	227	231	190*	147	152	149	144	141*
29	229	233	229	242	206	151	156	155	147	142
Gain days 1-29[a]	85	89	87	96	62	42	47	46	41	35

* significantly different from control, p≤0.05 [a]bodyweight gain was not subjected to statistical analyses

Table B.6.3.1–4 Group mean food consumption (g/animal/day)

Day	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
1-3	14.6	14.6	14.3	14.8	11.3	11.6	11.5	11.8	11.6	10.0*
3-5	15.7	15.5	15.4	15.7	9.9	11.9	12.1	12.0	12.0	10.5*
5-8	14.9	15.3	14.6	14.8	9.7*	11.7	11.9	11.8	11.7	11.3
8-15	15.3	15.9	15.5	15.7	8.2[a] 15.1[b]	12.4	12.1	12.6	12.2	11.4*
15-22	16.2	16.7	16.3	16.7	14.8	12.6	12.9	12.8	12.7	12.2
22-29	15.9	15.6	15.0	17.2	14.4	11.1	11.2	11.2	10.9	10.4

* significantly different from control, p≤0.05
[a] food consumption for days 8-11 [b] food consumption for days 11-15

There were no treatment-related ophthalmoscopy findings.

Several differences, possibly treatment-related, were observed in some haematology parameters for high dose males and females, as shown in Table B.6.3.1-5. Males at 1000/500 mg/kg/day had a lower red blood cell (RBC) count and higher mean corpuscular volume (MCV), reticulocyte count and platelet count, in comparison with controls, although the differences were not statistically significant. The RBC alterations were associated with a very slight splenic extramedullary haematopoiesis (see Table B6.3.1-9), and may represent a mild regenerative anemia. One female at 1000 mg/kg/day showed a number of haematological differences that may represent a treatment related effect. This female had lower RBC, haemoglobin and haematocrit values, and higher MCV, MCH, and reticulocyte and platelet counts than control females; the female also had several possibly related microscopic pathology findings, namely a slight increase in splenic erythrocytic extramedullary haematopoiesis, very slight erythrocytic extramedullary haematopoiesis in the liver and very slight erythroid hyperplasia of the bone marrow.

Table B.6.3.1–5 Selected group mean haematology findings

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
RBC (10^6/µL)	9.17	9.03	8.93	9.14	8.77	8.69	8.45	8.49	8.94	8.22
MCV (fL)[a]	54.6	54.4	54.9	54.7	56.1	56.5	57.1	56.7	56.3	58.4
Reticulocytes (10^9/L)	175.6	169.8	211.4	185.4	233.9	170.6	183.7	146.1	116.2	163.1
Platelets (10^3/ µL)	780	818	837	831	938	945	943	862	794	943

[a]parameter not subjected to statistical analyses

Selected clinical chemistry parameters are presented in Table B.6.3.1-6. Males at 1000/500 mg/kg/day had significantly higher alkaline phosphatase activity and higher chloride levels than controls. The toxicological significance of these marginal changes is uncertain. Females at 1000 mg/kg/day had a significantly higher urea nitrogen levels; this was not accompanied by elevated creatinine levels, and is therefore unlikely to represent kidney toxicity.

Table B.6.3.1–6 Selected group mean clinical chemistry parameters

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
Alkaline phos. (U/L)	145	145	151	158	172*	136	128	125	128	130
Chloride (mmol/l)	97	97	99	98	99*	99	99	100	99	100
Urea nitrogen (mg/dl)	15	14	15	16	16	14	15	15	14	17*

* significantly different from control, p≤0.05

Treatment-related changes in some urinalysis parameters were present in the high dose group in both sexes, as shown in Table B.6.3.1-7. Urine volume was higher and specific gravity was lower than controls; the differences were statistically significant only for males. Possibly these findings are related to the microscopic changes observed in the kidney in high dose males and females. Also, all high dose females had cloudy urine. On centrifugation, the urine was clear and crystalline phosphates (not amorphous or triple phosphate crystals) were noted in the pooled urine sediment.

Table B.6.3.1–7 Selected group mean urinalysis parameters

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
Volume (ml)	2.5	3.1	2.5	3.3	4.6*	3.2	2.9	2.5	2.5	4.5
Specific gravity	1.085	1.079	1.087	1.076	1.057*	1.063	1068	1.072	1.074	1.049
Appearance- cloudy	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5
Sediment- crystalline phosphates	-	-	-	-	-	-	-	-	-	Present

* significantly different from control, p≤0.05

The organ weight analysis revealed, at the highest dose level, significantly higher relative kidney weights in males and significantly higher relative brain weights in males and females (see Table B.6.3.1-8). The increased relative kidney weight may be a result of the microscopic kidney changes (Table B.6.3.1-9), but the increased brain weight can be attributed to the lower terminal bodyweights of the high dose group.

Table B.6.3.1–8 Selected group mean absolute and bodyweight related organ weights

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
Terminal bodywt. (g)	207	213	208	218	186	136	141	141	133	127
Kidney (g)	1.55	1.57	1.50	1.67	1.47	1.10	1.09	1.11	1.10	1.06
Kidney (% of bodywt.)	0.75	0.74	0.72	0.77	0.80*	0.80	0.78	0.78	0.83	0.83
Brain (g)	1.84	1.84	1.85	1.85	1.83	1.72	1.73	1.74	1.70	1.69
Brain (% of bodywt.)	0.89	0.86	0.89	0.85	1.00*	1.26	1.23	1.23	1.27	1.32*

* significantly different from control, $p \leq 0.05$

There were no treatment related macroscopic necropsy findings.

Treatment-related microscopic pathology findings, seen only at the high dose level, are summarised in Table B.6.3.1-9. The primary target organ was the kidneys. The changes observed were multifocal tubular degenerative changes, multifocal hypertrophy of the lining epithelial cells of the collecting ducts and multifocal vacuolization of the epithelium lining the collecting ducts located in the distal third of the papilla. The tubular degenerative changes were characterized by variable combinations of tubular basophilia with slightly enlarged nuclei, nuclear crowding and thickened tubular basement membrane, small numbers of mononuclear cell infiltrates around the degenerated tubules, presence of small amounts of pyknotic debris within the affected epithelium in some cases and tubular dilatation with or without cellular debris in the lumens. Dilated tubules were located in the cortex and/or the medulla. In some animals, tubular dilatation in the medulla was accompanied by very slight peritubular fibrosis and inflammation. Glomerular changes were occasionally present in the areas of tubular degeneration and consisted of thickened Bowman's capsule. In general, hypertrophy of the epithelium was noted in the collecting ducts located at the base and within the renal papilla (inner medulla), and in some cases the collecting ducts located within the inner stripe of the outer zone of the medulla were also affected. In addition, collecting ducts located within the distal third of the papilla of high-dose males and females had epithelial cell vacuolization with swelling. Small numbers of exfoliated cells were present within the lumens of the distal collecting ducts in some cases. Furthermore, in some animals, there was slight expansion of the renal papillary interstitium with oedema. However, there were no indications of renal papillary necrosis in these animals.
In the spleen of most high dose males, there was a very slight or slight, treatment-related increase in extramedullary erythrocytic haematopoiesis. The increased erythrocytic haematopoiesis was associated with decreased RBC count and increased MCV and reticulocytosis in the high dose males. Increased bone marrow erythroid haematopoiesis was noted in one high dose female; this female also has slight changes in a number of haematology parameters (as discussed above).

Table B.6.3.1–9 Selected microscopic pathology findings

Finding	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/ 1000	0	10	50	250	1000
No. of animals examined	5	5	5	5	5	5	5	5	5	5
Kidney										
Degeneration, tubule multifocal: v slight	0	0	0	0	2	1	0	1	0	4
slight	0	0	0	0	3	0	0	0	0	0
Hypertrophy, epithelium, collecting duct, multifocal v slight	0	0	0	0	0	0	0	0	0	2
slight	0	0	0	0	5	0	0	0	0	3
Vacuolisation, collecting ducts, papilla, mulifocal: v slight	0	0	0	0	1	0	0	0	0	4
slight	0	0	0	0	0	0	0	0	0	1
Spleen										
Extramedullary haematopoiesis, increased, multifocal v slight	1	1	1	1	3	2	2	0	0	0
slight	0	0	0	0	1	0	1	2	0	1
Bone marrow										
Haematopoiesis – Increased, erythroid cell v slight	0	0	0	0	0	0	0	0	0	1

The quantitative toxicokinetic findings are presented in Tables B.6.3.1-10 and B.6.3.1–11. Systemic absorption of XDE-729 Acid was approximately proportional to dose, based on the calculated AUC_{24h} values, although the extent of absorption appeared to be slightly lower at the higher dose levels. Systemically absorbed XDE-729 Acid was rapidly eliminated from plasma, demonstrated by the absence (or very low levels) of XDE-729 Acid in the plasma at study termination, 16 h after the removal of the test diets. Urinary elimination was approximately proportional to dose, with males eliminating 53-63% and females eliminating 48-69% of the daily dose XDE-729 Acid in a 24 h period. The LC/MC analysis of the urine identified the presence of parent XDE-729 and four metabolites (glucuronide conjugate of O-demethyl XDE-729, sulphate conjugate of O-demethyl XDE-729, O-demethyl XDE-729, and acyl-glucuronide conjugate of parent XDE-729). This metabolic profile was comparable to that observed following oral gavage administration of XDE-729 Acid and Na salt in the toxicokinetic studies (IIA 5.1.1/01, Saghir et al 2009; IIA 5.1.2/01, Hansen et al 2010). Overall, these data show that significant amounts of XDE-729 Acid are systemically absorbed following dietary administration and that elimination is rapid, with the urine being a significant route of elimination.

Table B.6.3.1–10 Mean plasma XDE-729 Acid concentration and toxicokinetic parameters

Parameter		Target dose level of XDE-729 Acid (mg/kg/day)									
		Males					Females				
		0	10	50	250	500/ 1000	0	10	50	250	1000
Plasma conc.	Day 23 06.00 h (µg/g)	-	1.65	6.86	29.98	63.92	-	1.26	6.05	32.86	103.8
	Day 23 09.00 h (µg/g)	-	1.07	4.36	24.38	47.56	-	1.18	3.26	16.27	72.82
	Day 23 17.00 h (µg/g)	-	0.76	2.58	11.96	36.57	-	0.71	2.10	6.45	44.48
	At termination (µg/g)	-	NQ	NQ	NQ	0.46	-	NQ	NQ	0.73	0.78
AUC_{24h}	(µg h/ml)	-	27.14	108.0	499.5	1157	-	23.91	88.29	420.1	1698
$t_{½}$	(h)	-	ND	ND	ND	ND	-	ND	ND	ND	ND

ND = not determined because plasma concentration was below the limit of detection in all/most animals after 16 h fasting
NQ = below limit of quantification (~0.4 µg/g)

Table B.6.3.1–11 Mean amount of XDE-729 Acid eliminated in urine in 24 h, on study day 26

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	500/1000	0	10	50	250	1000
Actual intake of XDE-729 Acid (µg/rat/day)	0	2430	11930	67788	111322	0	1478	7126	33946	130479
Amount of XDE-729 Acid in 24 h urine (µg/rat)	-	1326	6874	35794	69275	-	1002	4910	18237	62304
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	54.6	56.6	52.6	63.0	-	67.9	68.9	53.7	47.5

NQ = below limit of quantification (1.18 µg/g urine)

CONCLUSION

Dietary administration of XDE-729 Acid for 28 days to the rat at target dose levels of 500/1000 mg/kg/day for males and 1000 mg/kg/day for females caused adverse effects in the kidney (tubular degenerative changes, hypertrophy and vacuolation of the collecting duct epithelium), spleen (extramedullary haematopoiesis), blood (mild regenerative anemia only in males), accompanied by reduced bodyweight gain and food consumption and minor clinical chemistry and urinalysis changes. Toxicokinetic investigations showed that significant amounts of XDE-729 Acid are systemically absorbed following dietary administration and that elimination is rapid, with the urine being a significant route of elimination. The study NOAEL is 250 mg/kg/day.

[redacted] (2009)

XDE-729 Methyl	
Study	IIA 5.3.1/02 28-day dietary toxicity study in F344/DuCrl rats
Reference	[redacted] (2011)
Date performed	January – March 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111005
Guideline(s)	OECD 407 (2008)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031147-0004, 99.1% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.1–12: Study design

Test group	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of animals	
		Males	Females
1	0	5	5
2	10	5	5
3	52	5	5
4	261	5	5
5	782	5	5

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 8% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. On one day during the last week of the study, blood samples were taken from the jugular vein of all animals (non-fasted) at 06.00, 09.00 and 17.00 hours, for determination of plasma XDE-729 Methyl and XDE-729 Acid. Additional blood samples were taken at study termination, after fasting, for determination of plasma XDE-729 (Methyl and Acid) levels. Towards the end of the study, 24 hour urine samples were collected from all animals for determination of XDE-729 (Methyl and Acid).

At the end of the study, and after an overnight fast, blood samples were taken from the retroorbital venous plexus and a standard range of haematology and clinical chemistry parameters were measured. Additionally, towards the end of the study, overnight urine samples were collected and a standard range of urinalyses parameters were assessed.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The liver, kidneys, spleen, bone marrow, cervix, salivary gland, thyroids, thymus, uterus, vagina and macroscopic abnormalities from all animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination. Also, peripheral blood smears from control and high dose animals were examined.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.3.1–13 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Methyl (mg/kg/day)	10	52	261	782
Males	10.8	55.6	296	822
Females	10.7	55.5	277	825

There were no treatment related deaths or clinical signs of toxicity.

There were no treatment-related effects on bodyweights and food consumption among males (see Tables B.6.3.1–14 and B.6.3.1–15). However, among females, bodyweight gain at 261 and 782 mg/kg/day was reduced, by 11% and 17%, respectively, over the study period. These treatment-related effects on bodyweight correlated with a slight reduction in food consumption at 261 mg/kg/day and a significant reduction in food consumption at 782 mg/kg/day.

Table B.6.3.1–14 Group mean bodyweights, selected values (g)

Day	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
1	145	137	139	142	140	93	93	92	93	92
4	160	154	157	160	155	106	104	103	104	99
8	174	170	172	174	170	113	111	111	111	107
15	200	196	197	200	194	129	126	126	124	120
22	223	224	222	227	217	142	141	139	138	132
29	234	236	235	241	229	150	149	146	144	139
Gain days 1-4[a]	14.6	17.7	17.4	17.3	14.7	12.5	11.3	11.1	10.9	7.3
Gain days 1-29[a]	88.9	100	95.8	98.8	88.7	57.0	55.8	53.8	50.7	47.2

[a]bodyweight gain was not subjected to statistical analyses

Table B.6.3.1–15 Group mean food consumption (g/animal/day)

Day	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
1-4	14.1	13.8	14.3	14.3	13.1	11.4	10.8	10.7	10.5	9.1*
4-8	15.1	14.5	14.9	14.6	14.5	11.5	11.1	11.3	10.9	9.5*
8-15	15.9	15.5	16.4	15.8	15.1	11.7	11.5	11.5	11.2	10.3*
15-22	15.6	15.8	15.6	15.6	14.8	11.9	11.5	11.1	11.0	9.8*
22-29	15.0	15.5	15.4	16.0	14.9	10.7	10.8	10.3	10.3	10.0

* significantly different from control, $p \leq 0.05$

There were no treatment-related ophthalmoscopy findings.

At the highest dose level, a slightly lower haemoglobin concentration and haematocrit (both genders) and red blood cell count (RBC) (males only) was observed (see Table B.6.3.1–16). Although the values were within the laboratory historical control ranges, these differences were regarded as treatment related because the microscopic pathology examination revealed very slight erythoid cell hyperplasia of the bone marrow and an increased incidence of very slight extramedullary haematopoiesis of erythroid cells in the spleen. These findings are consistent with those of the XDE-728 Acid 28-day study in rats.

Table B.6.3.1–16 Selected group mean haematology findings

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
RBC (10^6/µL)	9.13	9.13	9.45	9.39	8.89	8.73	8.49	8.56	8.77	8.60
Historical control	8.14 – 9.57					8.09 – 9.15				
Haemoglobin conc. (g/dL)	16.7	16.5	16.9	16.7	15.9	16.5	16.2	16.2	16.4	16.0
Historical control	14.8 - 17.0					14.3 – 16.7				
Haematocrit (%)	50.0	49.9	51.0	50.0	47.2*	48.5	48.4	48.0	48.5	47.4*
Historical control	43.5 – 56.2					42.1 – 53.3				

* significantly different from control, $p \leq 0.05$
Historical control data obtained from five 28-day dietary studies conducted in this laboratory in 2009 and 2010.

Selected clinical chemistry findings are presented in Table B.6.3.1–17. Males and females at 261 and 782 mg/kg/day had significantly higher cholesterol concentrations, which were considered to be treatment-related. Mean ASAT activities for males and females at 782 mg/kg/day were greater than controls, although statistical significance was not achieved; nevertheless, these differences were considered to be treatment-related because two individual males and two individual females at this dose level had much higher values than any of control animals. The higher cholesterol and AST activities may be indicative of liver toxicity. Males and females at 782 mg/kg/day had significantly lower ALP activities; for males this may be treatment related because the value was lower than the historical control range, but for the females this was considered to be unrelated to treatment because the value was within the historical control range. Males at 782 mg/kg/day and females at 261 and 782 mg/kg/day had lower triglyceride concentrations, though statistical significant was not achieved; for males this may be related to treatment because the value was lower than the historical control range, but for females these differences were considered unlikely to be treatment-related because of the lack of a dose-response relationship and because the values were near the historical control range. The lower ALP activities and triglyceride concentrations

were considered to have no toxicological significance. Males and females at 261 and 782 mg/kg/day had significantly higher total protein and albumin concentrations, which were interpreted to be unrelated to treatment because the total protein values were within the historical control ranges, and the albumin values lacked a clear dose-response relationship.

Table B.6.3.1–17 Selected group mean clinical chemistry findings

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
Alkaline phosph. (u/L)	162	154	156	157	130*	143	136	136	128	122*
Historical control	145 - 170					120 - 144				
ASAT (u/L)	91	83	85	89	113	89	86	80	87	110
Historical control	87 - 103					80 - 108				
Total protein (g/dl)	7.0	6.9	7.1	7.3*	7.6*	6.4	6..4	6.4	6.7*	6.6*
Historical control	6.8 - 7.9					6.2 - 6.9				
Albumin (g/dl)	4.6	4.6	4.8	4.8*	5.0*	4.5	4.5	4.5	4.7*	4.7*
Historical control	4.0 - 4.6					3.8 - 4.1				
Cholesterol (mg/dl)	52	51	58	78*	112*	76	82	95	148*	139*
Historical control	51 - 71					74 – 97				
Triglycerides (mg/dl)	81	77	82	72	53	40	34	37	31	31
Historical control	70 - 137					33 - 47				

* significantly different from control, p≤0.05
Historical control data obtained from five 28-day dietary studies conducted in the testing laboratory in 2009 and 2010.

There were no treatment-related differences in the urinalysis parameters.

The organ weight analysis revealed a number of statistically significant differences (see Table B.6.3.1–18). Relative liver weight was significantly increased in both males and females at 52, 261 and 782 mg/kg/day, in comparison with controls. The higher liver weights were consistent with the observation of hepatocyte hypertrophy in the microscopic pathology examination among males at 52, 261 and 782 mg/kg/day and females at the two highest doses levels only (see Table B.6.3.1–19). Absolute and relative thyroid weight was significantly increased in males at the top two dose levels, which corresponded with the presence of slight follicular cell hypertrophy in these groups. Thyroid weight increases also occurred in among females, at 52, 261 and 782 mg/kg/day, but in the absence of a dose-related response and the observation of hypertrophy in the microscopic examination these differences were considered not to be treatment-related. Thymus weights were lower in males and females at the highest dose level which, being consistent with the observation of lymphoid tissue atrophy in the microscopic examination, were considered to be a treatment-related effect. The observation of significantly higher kidney weights (only when adjusted for bodyweight) in males and females at the highest dose group was considered unlikely to be treatment-related as there were no correlating microscopic pathology findings.

Table B.6.3.1–18 Selected group mean absolute and bodyweight related organ weights

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
Final bodyweight (g)	211	211	209	216	205	135	133	130	129	124
Liver (g)	6.320	6.671	6.735	7.879*	8.926*	4.116	4.127	4.273	4.664*	4.849*
Historical control	6.435 – 6.888					3.793 – 4.199				
Liver (% bodyweight)	2.984	3.148	3.229*	3.653*	4.348*	3.047	3.099	3.277*	3.618*	3.918*
Historical control	3.106 - 3.492					2.847 - 3.120				
Thymus (g)	0.329	0.319	0.307	0.312	0.274*	0.350	0.334	0.354	0.302	0.278*
Historical control	0.282 – 0.314					0.278 - 0.306				
Thymus (% bodyweight)	0.156	0.151	0.148	0.145	0.133*	0.259	0.252	0.271	0.234	0.224*
Historical control	0.130 - 0.168					0.203 - 0.235				
Thyroid (g)	0.0102	0.0104	0.0107	.0118*	.0132*	0.0071	0.0075	.0079*	.0086*	0.0078
Historical control	-					-				
Thyroid (% bodyweight)	0.0048	0.0049	0.0051	.0055*	.0064*	0.0053	0.0056	.0061*	.0066*	.0063*
Historical control	-					-				
Kidneys (% bodyweight)	0.740	0.734	0.752	0.762	0.801*	0.843	0.831	0.841	0.872	0.869*
Historical control	0.723 – 0.790					0.770 - 0.825				

* significantly different from control, $p \leq 0.05$
Historical control data obtained from five 28-day dietary studies conducted in the testing laboratory in 2009 and 2010.

There were no treatment-related macroscopic necropsy findings. The microscopic pathology examination, summarised in Table B.6.3.1–19, identified the liver and kidneys as primary targets. Hepatocyte hypertrophy was reported in males at 52, 261 and 782 mg/kg/day and females at the two highest doses levels. The severity (very slight to slight) increased with the dose in both sexes and the distribution of the hypertrophy expanded from centrilobular/midzonal in males at 52 or 261 mg/kg/day to panlobular in males at 782 mg/kg/day. Other treatment-related changes in liver consisted of very slightly increased numbers of mitotic figures (hepatocytes in mitosis) in males at 52, 261 and 782 mg/kg/day and females at 782 mg/kg/day, and slight hepatocellular vacuolisation (consistent with fatty change) in males given 52, 261 and 782 mg/kg/day. In the kidneys, slight multifocal hypertrophy of collecting duct epithelial cells was present in two males at 782 mg/kg/day. The hypertrophy was present in a few principal and intercalated epithelial cells of collecting ducts at the tip of the renal papilla in both affected males; the hypertrophic cells had increased cytoplasmic volume and enlarged nuclei.

Other treatment-related microscopic effects were diffuse hypertrophy of thyroid gland follicular cells in males at 52, 261 and 782 mg/kg/day. Also, there was a slight diffuse acinar cell hypertrophy of the submandibular salivary gland and very slight lymphoid atrophy of the thymus in males and females at 782 mg/kg/day, possibly secondary stress-related changes.

Males and females at 782 mg/kg/day had treatment-related very slight hyperplasia of erythroid cells in the bone marrow, and a treatment-related increase in the incidence of very slight erythroid cell extramedullary haematopoiesis in the spleen. These changes may be reflective of a regenerative response to the reduced red blood cell counts, haemoglobin concentrations and haematocrit seen in these groups. However, reticulocyte counts were not significantly increased, and there was no evidence of polychromasia in the peripheral blood smears of high dose males and females.

Some females at 782 mg/kg/day had decreased size of the cervix, uterus and vagina. These changes are of uncertain toxicological significance; possibly these are secondary to reduced bodyweight gain and food consumption.

Table B.6.3.1–19 Selected microscopic pathology findings

Finding	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
No. of animals examined	5	5	5	5	5	5	5	5	5	5
Liver										
Hypertrophy, centrilob/midzone: v slight	0	0	3	0	0	0	0	0	5	1
slight	0	0	0	5	0	0	0	0	0	4
Hypertrophy, panlobular: v slight	0	0	0	0	5	0	0	0	0	0
Increased no. mitotic figures v slight	0	0	1	2	4	0	0	0	0	1
Hepatocyte vacuolisation, cons. with fatty change, multifocal: v slight	0	0	3	4	2	0	0	0	0	0
slight	0	0	0	0	3	0	0	0	0	0
Kidney										
Hypertrophy, epithelium, collecting duct, multifocal v slight	0	0	0	0	2	0	0	0	0	0
Thyroid gland										
Hypertrophy, follic. cell, diffuse: v slight	0	0	2	0	0	0	0	0	0	0
slight	0	0	0	5	5	0	0	0	0	0
Salivary gland										
Hypertrophy, acinus, diffuse: v slight	0	0	0	0	1	0	0	0	0	4
Thymus										
Atrophy, lymphoid tissue: v slight	0	0	0	0	2	0	0	0	0	2
Spleen										
Extramedullary haematopoiesis, increased, multifocal v slight	0	0	0	0	4	1	0	2	1	4
Bone marrow										
Hyperplasia,erythroid cell v slight	0	0	0	0	4	0	0	0	0	2
Cervix										
Decreased size: v slight	-	-	-	-	-	0	0	0	0	2
Uterus										
Decreased size: v slight	-	-	-	-	-	0	0	0	0	1
Vagina										
Decreased size: v slight	-	-	-	-	-	0	0	0	0	2

The toxicokinetic findings are summarised in Tables B.6.3.1-20 and B.6.3.1–21. XDE-729 Methyl was not present in most of the blood samples, with the exception of the high dose group in which Methyl levels were <1% of the corresponding XDE-729 Acid concentrations. In contrast, XDE-729 Acid was present at quantifiable levels in almost all of the blood samples from exposed animals. Daily systemic exposures (as indicated by AUC_{24h}) for XDE-729 Acid were dose-proportional (linear) at all dose levels for males, but only up to 261 mg/kg/day for females; at the highest dose level the AUC_{24h} for females was considerably less than dose-proportional.

In urine, both XDE-729 Methyl and XDE-729 Acid were present in all urine samples from exposed animals, except for the low dose group in which no XDE-729 was detected. On average, 0.03% of the dose was excreted as XDE-729 Methyl and 29% as XDE-729 Acid. For both XDE-729 Acid and Methyl, the total amounts excreted in the urine in 24 h were dose-proportional at all dose levels in males, but only up but only up to 261 mg/kg/day for females.

The sublinear kinetics in blood and urine for high dose females suggests the possible saturation of absorption and/or saturation of the conversion of XDE-729 Methyl to XDE-729 Acid at higher doses. It can be speculated that the saturation of conversion to XDE-729 Acid is likely to be accompanied by conversion to other metabolite(s) via other metabolic pathway(s) since there is no concurrent increase in XDE-729 Methyl concentrations.

Table B.6.3.1–20 Toxicokinetic investigation: systemic AUC_{24h} values

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
Actual dose of XDE-729 Methyl at blood sampling (mg/kg/day)	0	10.5	50.4	271	779	0	9.7	48.6	245	784
AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	5.68	22.37	108.9	275.6	NQ	5.95	26.7	113.6	44.7
AUC_{24h} for XDE-729 Methyl (µg h/mL)	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ

NQ = not quantifiable

Table B.6.3.1–21 Mean amount of XDE-729 Acid and XDE-729 Methyl eliminated in urine in 24 h

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)									
	Males					Females				
	0	10	52	261	782	0	10	52	261	782
Actual dose of XDE-729 Methyl at urine sampling (mg/kg/day)	0	10.5	50.4	271	779	0	9.7	48.6	245	784
Amount of XDE-729 Acid in 24 h urine (mg/kg)	NQ	2.61	16.87	75.87	178.7	0	2.45	21.23	97.78	98.98
Amount of XDE-729 Methyl in 24 h urine (mg/kg)	NQ	NQ	0.009	0.069	0.258	NQ	NQ	0.014	0.110	0.125

CONCLUSION

The liver and kidneys were identified as the main targets of XDE-729 Methyl toxicity in the rat, following 28 days dietary administration. In the liver, at target dose levels of 52, 261 and 782 mg/kg/day, adverse effects were induced in both genders, observed as increased organ weight, usually accompanied by hepatocyte hypertrophy and, mainly in males, by increased numbers of mitotic figures (hepatocytes in mitosis) and slight hepatocellular vacuolisation (consistent with fatty change). In the kidneys, a slight multifocal hypertrophy of collecting duct epithelial cells was induced, but only in males at 782 mg/kg/day. Other targets of XDE-729 Methyl toxicity were the blood and thyroids. A slight reduction in haemoglobin concentration and haematocrit (both genders) and red blood cell count (males only) was present at 782 mg/kg/day, accompanied by slight erythroid cell hyperplasia of the bone marrow and an increased incidence of very slight extramedullary haematopoiesis in the spleen. In the thyroid glands, diffuse hypertrophy of follicular cells was reported in males at 52, 261 and 782 mg/kg/day. Additionally, in females bodyweight gain was reduced at 261 and 782 mg/kg/day and food consumption was reduced at

782 mg/kg/day. Toxicokinetic investigations showed that almost all systemically absorbed XDE-729 Methyl is present as the Acid metabolite. The study NOAEL is 10 mg/kg/day, based on the presence of evidence of liver toxicity at 52 mg/kg/day and above and hypertrophy of thyroid gland follicular cells in males.

[redacted] (2011)

XDE-729 Acid	
Study	IIA 5.3.2/01 90-day dietary toxicity study in F344/DuCrl rats
Reference	[redacted] (2010)
Date performed	June – September 2009
Test facility	[redacted]
Report reference	Laboratory study ID 091016
Guideline(s)	OECD 408 (1998)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.1–22: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	10	10
2	10	10	10
3	50	10	10
4	250	10	10
5	750	10	10

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were all within 12% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. On day 86 or 87, blood samples were taken from the jugular vein of 4 non-fasted animals from each group at 06.00, 09.00 and 17.00 hours, for determination of plasma XDE-729 Acid levels. Additional blood samples were taken at study termination, after fasting, for determination of plasma XDE-729

Acid levels. On days 91 to 92, 24 hour urine samples were collected from 4 non-fasted animals from each group for determination of XDE-729 Acid levels.

At the end of the study, and after an overnight fast, blood samples were taken from the retroorbital venous plexus and a standard range of haematology and clinical chemistry parameters were measured. Additionally, towards the end of the study, overnight urine samples were collected and a standard range of urinalyses parameters were assessed.

A necropsy was conducted on all animals the end of the 90 day treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The kidney and macroscopic abnormalities from all animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination. Additional samples of kidney were stored in RNALater® or liquid nitrogen for possible further investigations.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.1–23 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	10	50	250	750
Males	10.5	52.4	262	782
Females	10.1	50.3	252	758

There were no treatment related deaths or clinical signs of toxicity.

Group mean bodyweights are summarised in Table B.6.3.1-24. XDE-729 Acid treatment at the highest dose level only caused a slight reduction in bodyweight gain throughout the study among males; at termination bodyweight gain of this was 9% lower than controls. Female bodyweights, or the amount of food consumed by either sex, were not affected by treatment.

Table B.6.3.1–24 Group mean bodyweights, selected values (g)

Day	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
1	123	123	122	122	122	100	101	101	101	102
8	156	155	153	154	151	116	117	117	119	119
43	264	264	260	256	251	158	161	160	163	162
92	324	330	325	323	305	183	184	183	187	182
Gain days 1-92[a]	201	208	203	201	183	83	83	81	85	80

[a]bodyweight gain was not subjected to statistical analyses

There were no treatment-related ophthalmoscopy findings.

Selected group haematology findings are presented in Table B.6.3.1-25. The only noteworthy finding was a significant reduction in haematocrit for high dose females. However, this

difference was considered to be a chance finding as the haematocrit value was within the laboratory historical control range, and RBC, haemoglobin concentration and reticulocytes count were not significantly different from the control values.

Table B.6.3.1–25 Selected group mean haematology findings

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
RBC (10^6/µL)	9.21	9.29	9.11	9.25	9.32	8.48	8.44	8.37	8.42	8.08
Historical controls	-					7.42 – 9.01				
Haemoglobin conc. (g/dL)	15.4	15.6	15.3	15.5	15.5	15.4	15.3	15.2	15.3	14.7
Historical controls	-					14.2 – 16.8				
Haematocrit (%)	45.6	46.2	45.7	45.9	46.1	44.5	44.0	43.9	44.2	42.4*
Historical control	-					40.2 – 46.9				
Reticulocyte count (10^9/L)	205	205	238	200	188	192	188	194	193	196

* significantly different from control, p≤0.05
Historical control data obtained from five 28-day dietary studies from the testing laboratory reported 2005-2007 (historical data for males not presented in study report because no inter-group differences were apparent)

There were no treatment-related clinical chemistry findings.

The urinalysis revealed treatment-related changes only at 750 mg/kg/day (see Table B.6.3.1-26). Urine volume was significantly greater and specific gravity was significantly lower than controls in both males and females. In addition, females given 750 mg/kg/day had no amorphous crystals in the urine sediment, which may have been related to the increase in urine volume and dilution of the microsediment.

Table B.6.3.1–26 Selected group mean urinalysis findings

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
Urine volume (ml)	3.7	3.8	3.7	3.7	9.7*	4.7	5.9	4.6	4.4	8.9*
Historical controls	3.2 – 5.7					3.2 – 4.8				
Specific gravity	1.077	1.077	1.079	1.082	1.047*	1.057	1.048	0.055	1.055	1.033*
Historical controls	1.062 – 1.080					1.052 – 1.075				
Microscopic crystals, triple & amorphous phosphates	Present	Present	Present	Present	Present	Present	Present	Present	Present	Not present

* significantly different from control, p≤0.05
Historical control data obtained from five 90-day dietary studies from the testing laboratory reported 2005-2007

Relative testes, thyroid and brain weights for males at 750 mg/kg/day were significantly higher than the controls (see Table B.6.3.1-27). The absolute weights of these organs were similar to the controls. These relative organ weight differences are considered to be secondary to the lower mean final body weight observed at 750 mg/kg/day. Relative kidney weight was significantly higher in both males and females at 750 mg/kg/day, which may be related to the microscopic pathology changes seen in this group. Relative kidney weight was also significantly higher for females at 250 mg/kg/day, but in the absence of correlating histopathological changes this is considered to be a chance finding.

Table B.6.3.1–27 Selected group mean absolute and bodyweight related organ weights

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
Final bodyweight (g)	305	312	306	304	286	172	172	172	174	171
Historical control	313 - 315					172 – 181				
Testes (g)	3.165	3.146	3.169	3.120	3.179					
Historical control	3.002 – 3.186									
Testes (% bodyweight)	1.039	1.009	1.038	1.029	1.114*					
Historical control	0.964 – 1.011									
Thyroid (g)	0.0137	0.0142	0.0142	0.0146	0.0140	0.0093	0.0091	0.0087	0.0098	0.0097
Historical control	0.0134 – 0.0144					-				
Thyroid (% bodyweight)	0.0045	0.0045	0.0046	0.0048	0.0049*	0.0054	0.0053	0.0051	0.0056	0.0057
Historical control	0.0043 – 0.0046					-				
Kidneys (g)	2.004	2.017	1.997	2.018	2.034	1.149	1.175	1.176	1.231*	1.221
Historical control	2.104 – 2.169					1.239 – 1.319				
Kidneys (% bodyweight)	0.657	0.646	0.652	0.665	0.714*	0.669	0.685	0.685	0.706*	0.713*
Historical control	0.668 – 0.690					0.720 – 0.730				
Brain (g)	1.959	1.975	1.972	1.961	1.960	1.813	1.825	1.820	1.842	1.839
Historical control	1.938 – 1.947					-				
Brain (% bodyweight)	0.644	0.633	0.646	0.647	0.688*	1.058	1.065	1.062	1.057	1.075
Historical control	0.619 – 0.621					-				

* significantly different from control, $p \leq 0.05$
Historical control data obtained from five 90-day dietary studies from the testing laboratory reported 2005-2007 (historical data for some female organs not presented in study report because no inter-group differences were apparent)

There were no macroscopic necropsy findings that were considered to be treatment-related.

The microscopic examination identified the kidney as the primary target organ in both males and females (see Table B.6.3.1-28). The changes, occurring only at the highest dose level, consisted of (a) multifocal hypertrophy of the epithelial cells lining the collecting ducts accompanied by cytoplasmic basophilia and nuclear karyomegaly; (b) necrosis of individual collecting duct epithelial cells; (c) increased mitotic figures within the collecting ducts; (d) tubular dilatation; and (e) vacuolization of the epithelium lining the collecting ducts.
The epithelial hypertrophy was most prominent in collecting ducts in the vicinity of the inner and outer medulla. Cellular hypertrophy was accompanied by karyomegaly (nuclear enlargement approximately 2 to 3 times normal size) and cytoplasmic basophilia. Increased mitotic figures (mitotic alteration) were occasionally observed, as were necrotic epithelial cells (very slight or slight) present in affected tubules/ducts adjacent to the hypertrophic epithelial cells. Dilated tubules were infrequently observed in the medulla, and in some instances, were accompanied by peritubular fibrosis and inflammation and contained exfoliated cells within the lumens. Epithelial cells of the collecting ducts in the distal third of the papilla also were hypertrophic and had a vacuolated cytoplasm. The papillary interstitium in this region also appeared oedematous; however, there were no indications of renal papillary necrosis in these animals. Lesions in one high-dose male were more severe than the other males in that dose group due to the occurrence of hypertrophy of the collecting duct epithelial cells (moderate) and necrosis of collecting ducts with inflammation (slight) and degeneration of tubules (moderate) that were unilateral in distribution.

No treatment-related microscopic changes were present in other organs.

Table B.6.3.1–28 Selected microscopic pathology findings: no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
No. of animals examined	10	10	10	10	10	10	10	10	10	10
Kidney										
Hypertrophy, epithelium, collecting duct, uni or bilateral, multifocal, v slight	0	0	0	0	6	0	0	0	0	4
Hypertrophy, epithelium, collecting duct, uni or bilateral, multifocal, slight	0	0	0	0	4	0	0	0	0	1
Hypertrophy, epithelium, collecting duct, unilateral, multifocal, moderate	0	0	0	0	1	0	0	0	0	0
Necrosis, epithelium, collecting tubule, uni or bilateral, multifocal, v slight	0	0	0	0	3	0	0	0	0	0
Necrosis, tubule, medulla, unilateral, focal, v slight	0	0	0	0	0	0	0	0	0	1
Necrosis with inflammation, unilateral, collecting duct, multifocal, slight	0	0	0	0	1	0	0	0	0	0
Mitotic figures ↑, collecting duct, uni or bilateral, focal or multifocal v slight	0	0	0	0	4	0	0	0	0	1
Dilatation, collecting tubule, medulla, uni or bilateral, focal or multifocal, v slight	0	0	0	0	8	0	0	0	0	0
Vacuolization, papilla, collecting duct, uni or bilateral, multifocal, v slight	0	0	0	0	7	0	0	0	0	4
Vacuolization, papilla, collecting duct, unil or bilateral, multifocal, slight	0	0	0	0	3	0	0	0	0	4
Degeneration, tubule, unilateral, multifocal, moderate	0	0	0	0	1	0	0	0	0	0

The results of the toxicokinetics investigation are summarised in Table B.6.3.1-29. Daily systemic exposures to XDE-729 Acid (as indicated by AUC_{24h}) were approximately dose-proportional (linear) at all dose levels for both males and females. In males and females, respectively, 47-62% and 55-89% of the daily intake of XDE-729 Acid was recovered as the unchanged parent in the 24-h urine samples. Urinary elimination was approximately dose proportional for males at all dose levels, but for females elimination did not appear to increase in proportion to the dose. Overall, the toxicokinetic investigations confirm that significant amounts of XR-729 Acid are systemically absorbed following dietary administration and that a significant route of elimination is as the parent via the urine.

Table B.6.3.1–29 Toxicokinetic investigation: systemic AUC_{24h} values and urinary elimination

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	750	0	10	50	250	750
Actual dose of XDE-729 Acid at time of TK investigation (mg/kg/day)	0	9.58	50.78	244.8	696.7	0	7.18	42.67	193.4	653.1
Actual intake of XDE-729 Acid at time of TK investigation (mg/rat/day)	0	3.19	16.0	78.9	211.5	0	1.30	7.63	35.6	119.5
AUC_{24h} for XDE-729 Acid (µg h/mL)	-	32.0	119.8	406.8	1453	-	31.5	120.3	426.8	2092
Plasma elimination $t_{½}$ (h)	-	ND	ND	ND	3.6-4.1	-	ND	ND	ND	ND
Amount XDE-729 Acid in 24 h urine (mg/rat)	0	1.98	8.39	37.4	121.1	0	1.16	4.26	26.4	66.4
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	61.8	52.5	47.4	57.2	-	88.8	54.9	75.1	55.7

ND = not determined because plasma concentrations of XDE-729 at terminal sacrifice were below the limit of quantitation

CONCLUSION

Dietary administration of XDE-729 Acid for 90 days to the rat at a target dose level of 750 mg/kg/day caused adverse effects in the kidney in both genders, observed microscopically as hypertrophy, vacuolation and sometimes necrosis of collecting duct epithelium and tubular dilatation, accompanied by urine changes (increase volume and lower specific gravity) and, among males only, slightly decreased bodyweight gain. Toxicokinetic investigations confirm that significant amounts of XDE-729 Acid are systemically absorbed following dietary administration and that a significant route of elimination is as the parent via the urine. The study NOAEL is the target dose of 250 mg/kg/day (mean actual dose 262 mg/kg/day in males and 252 mg/kg/day in females).

[redacted] (2010)

XDE-729 Methyl	
Study	IIA 5.3.2/02 90-day dietary toxicity study in F344/DuCrl rats
Reference	[redacted] (2012)
Date performed	May – August 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111082
Guideline(s)	OECD 408 (1998)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.1–30: Study design

Test group	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of animals	
		Males	Females
1	0	10	10
2	3	10	10
3	10	10	10
4	52	10	10
5	261	10	10
6	500	10	10

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were all within 14% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Two additional male rats were dosed with radio-labelled ^{14}C-XDE-729 Methyl in 0.5% METHOCEL™ at a target dose level of 100 mg/kg and urine was collected for 12 hours for quantitation of the following XDE-729 Methyl metabolites; glucuronide conjugate of O-demethyl XDE-729 Methyl, sulfate conjugate of O-demethyl XDE-729, O-demethyl XDE-729, glucuronide conjugate of O-demethyl XDE-729 Methyl, and acyl glucuronide conjugate of XDE-729. The concentration of the radio-labelled dose was determined by HPLC/UV and the radioactivity of the dose was determined by LSS (liquid scintillation spectroscopy). These data were used to calculate the specific activity of the dose (µg XDE-729 Methyl/dpm). The pooled 0-12 hour urine was profiled by HPLC with radio-detection to determine '% radioactivity per peak' which was converted to a concentration (µg/mL) of each metabolite. This pooled, characterized urine was used as the analytical 'stock' standard and dilutions of the urine were used for quantitation of metabolites in the TK samples from the main study animals (urine, terminal blood extracts and liver extracts). The metabolites were identified by HPLC/MS/MS and quantified as described above but were not fully characterized as reference substances by GLPs. The metabolites were identified in pooled urines by HPLC/MS/MS and quantified.

During the last week of the study, blood samples were taken from the jugular vein of 5 non-fasted males and females from each group at 06.00, 11.00 and 15.00 hours, for determination of blood levels of XDE-729 Methyl and its metabolites at steady state. Additional non-fasted blood samples were taken from the same animals at study termination for determination of blood levels of XDE-729 Methyl, XDE-729 Acid levels and the five known additional metabolites. From these animals, liver samples were taken at necropsy, and flash frozen in liquid nitrogen, for determination of levels of XDE-729 Methyl, XDE-729 Acid levels and five known metabolites. In the week prior to necropsy, 24 hour urine samples were collected from 5 non-fasted males and females from each group for determination of blood levels of XDE-729 Methyl, XDE-729 Acid levels and five known metabolites. Analysis was conducted using High Performance Liquid. Chromatography/Electrospray Ionization/Mass Spectrometry (HPLC/ESI-MS/MS).

A necropsy was conducted on all animals the end of the 90 day treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The liver, kidney, thyroid gland, thymus and macroscopic abnormalities from all animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination.

Liver tissue from all animals was preserved in *RNA*later and gene expression analysis was conducted. RNA isolated from each individual animal was used in a reverse transcription reaction to create complementary DNA (cDNA) that then served as the template for assessment of gene expression using a quantitative real-time polymerase chain reaction (QRTPCR) approach. The targeted gene expression studies were conducted using an Applied Biosystems (ABI) 7500 real-time Polymerase Chain Reaction system using Applied Biosystems TaqMan Gene Expression Assays. The following genes were selected as biomarkers for nuclear receptor activation and to aid in understanding possible involvement of other metabolic pathways in response to XDE-729 Methyl in F344/DuCrl rats:

1. *Cyp1a1* "AhR response gene" ABI TaqMan ID: Rn00487218_m1
2. *Cyp2b1* "CAR response gene" [*Cyp2b6* in humans, *Cyp2b10* in mice]. ABI TaqMan ID: Rn01457875_m1
3. *Cyp3a23/3a1* "PXR response gene" [(aka, *Cyp3a3*; *Cyp3a4* in humans, *Cyp3a11* in mice]. ABI TaqMan ID: Rn03062228_m1
4. *Cyp4a22* "PPAR-α response gene" ABI TaqMan ID: Rn00598510_ml
5. Ugt1a6 "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00756113_mH

Gene expression was quantified using the comparative Ct method (ΔΔCt). For this method, the amount of target mRNA is expressed relative to an endogenous reference mRNA (i.e. housekeeping gene such as beta-actin) and relative to a calibrator sample (i.e. vehicle control). For each individual sample well, the Ct of the housekeeping gene was subtracted from the Ct of the target gene, and a mean of these values is generated for each treatment group (ΔCt). The ΔCt results from each sample were subtracted from the vehicle control ΔCt values to generate a ΔΔCt value ($\Delta Ct_{treated} - \Delta Ct_{control} = \Delta\Delta Ct$). The expression of the amount of target mRNA, normalized to an endogenous reference, and relative to a calibrator (i.e., vehicle control), was reported as fold change compared to control by the following formula: fold = $2^{-\Delta\Delta Ct}$.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.3.1–31 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Methyl (mg/kg/day)	3	10	52	261	500
Males	3.07	10.3	53.4	270	513
Females	3.03	10.1	52.3	263	504

There were no treatment related deaths or clinical signs.

Bodyweight gain was reduced at a target dose level of 500 mg/kg/day in both males and females, as shown in Table B.6.3.1-32. At termination, mean bodyweight for this group was 5% and 7%, in males and females respectively, lower than controls. However, food consumption was not affected by XDE-729 Methyl treatment.

Table B.6.3.1–32 Group mean bodyweights, selected values (g)

Day	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
1	152	152	153	150	149	150	110	109	109	107	108	107
36	250	245	258	252	250	240	160	162	161	160	159	153
90	315	312	326	315	314	300	184	186	187	182	179	171
Gain days 1-36[a]	98	93	105	102	101	90	50	53	52	53	51	46
Gain days 1-90[a]	163	160	172	165	164	151	75	78	78	75	72	64

[a]bodyweight gain was not subjected to statistical analyses

There were no treatment-related ophthalmoscopy findings.

Selected group haematology findings are presented in Table B.6.3.1-33. Treatment-related changes in some red blood cell parameters were observed at 500 mg/kg/day in both sexes. Haemoglobin concentration and haematocrit were statistically significantly lower than the controls. Additionally, red blood cell count was reduced at 500 mg/kg day in both sexes, a difference considered likely to be treatment-related though statistical significance was not achieved. Females at 261 and 500 mg/kg/day had lower percent neutrophils and higher percent lymphocytes, relative to controls; these differences are thought to be treatment-related, but are unlikely to be of toxicological significance because there were no histopathologic correlates in the bone marrow or lymphoid tissues.

Table B.6.3.1–33 Group mean selected haematology findings

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
RBC (E^6/µl)	9.61	9.40	9.70	9.35	9.55	9.26	8.57	8.46	8.49	8.44	8.47	8.20
Haem conc. (g/dL)	15.7	15.4	15.8	15.2	15.3	14.8*	15.1	14.8	15.0	14.8	14.5	14.0*
Hct (%)	47.2	46.3	47.8	45.8	46.3	45.2*	43.9	43.4	43.6	43.1	42.6	41.1*
Neutrophils (%)	17.6	18.2	16.8	15.8	15.4	15.5	19.6	17.5	17.8	17.3	14.4	13.6
Lymphocytes (%)	78.5	77.5	78.6	80.5	80.9	80.3	76.7	79.2	78.5	78.6	82.2	83.1

* significantly different from control, $p \leq 0.05$

The key clinical chemistry findings are summarised in Table B.6.3.1-34. Males at 261 and 500 mg/kg/day and females at 52 mg/kg/day and above had treatment-related statistically significant higher cholesterol concentrations. The higher cholesterol was likely associated with increased liver weights and histopathologic effects indicative of treatment-related liver effects. Males and females at 261 or 500 mg/kg/day had significantly lower alkaline phosphatase activities (ALP), and males given 500 mg/kg/day had lower aspartate aminotransferase activities (AST) that were interpreted as possibly being treatment-related; however, decreases in these parameters are not typically indicative of toxicologically significant effects. Statistically significant differences in T4 levels in in males and females and in T3 levels in females were reported; however, in the absence of clear dose-response relationships these differences were considered to be due to chance and unrelated to XDE-729 Methyl treatment.

Table B.6.3.1–34 Group mean selected clinical chemistry and thyroid hormone findings

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
ALP (u/L)	178	176	177	170	140*	133*	174	171	178	165	149*	139*
AST (u/L)	78	76	79	71	85	64*	88	84	104	93	77	75
Chol (mg/dL)	70	72	73	75	99*	121*	88	88	93	109*	137*	140*
T4 (µg/dL)	3.70	3.79	3.97	4.48*	3.97	2.86*	1.74	1.94	2.71*	2.09	2.28	1.97
T3 (ng/dL)	90.2	89.4	92.8	77.0	94.0	92.0	86.9	88.8	99.2	101	119*	112*

* significantly different from control, p≤0.05

Treatment-related differences in several urinalysis parameters were apparent in males only, as shown in Table B.6.3.1-35. At 261 and 500 mg/kg/day there were higher incidences of lower urine pH, decreased amount of urine ketones and bilirubin, increased urine urobilinogen, relative to controls. The lower pH is likely due to the presence of the primary metabolite of XDE-729 Methyl (i.e. XDE-729 Acid) in the urine, although the pH of the urine from some animals in these treatment groups was the same as some of the controls. The lower urine ketones and bilirubin may be related to alterations in liver metabolism, but cannot be regarded as an adverse change. The cause of the higher urobilinogen is unknown, as there was no evidence of haemolysis in treated males.

Table B.6.3.1–35 Group mean selected urinalysis findings, males, no. of animals with stated value

Parameter		Target dose level of XDE-729 Methyl (mg/kg/day)					
		0	3	10	52	261	500
Urine pH =	7.0	2	2	6	1	8	10
	7.5	3	4	3	5	2	0
	8.0	4	3	1	4	0	0
	8.5	1	1	0	0	0	0
Ketones:	small amount	10	10	10	10	6	2
	trace	0	0	0	0	4	8
Bilirubin	small amount	5	4	6	5	0	0
	negative	5	6	4	5	10	10
Urobilinogen, EU/dL = 0.2		10	9	7	9	6	2
	1.0	0	1	3	1	4	8

The organ weight analysis revealed a number of statistically significant differences for the liver, thymus and thyroids, as shown Table B.6.3.1-36. Males at 261 and 500 mg/kg/day and females at 52, 261 and 500 mg/kg/day had significantly higher absolute and relative liver weights, which is considered to be treatment-related adverse effect. The higher liver weights corresponded to microscopic hepatocellular hypertrophy in males and females given 261 or 500 mg/kg/day. Males and females at 261 and 500 mg/kg/day had significantly increased and treatment-related higher absolute and relative thyroid weights. The higher thyroid weights corresponded to microscopic thyroid follicular cell hypertrophy in males and females given 261 or 500 mg/kg/day. Females at 500 mg/kg/day had significantly lower absolute and relative thymus weights that were interpreted as treatment-related. The lower thymus weights corresponded to microscopic atrophy of thymic lymphoid tissue in females at this dose level. The significantly lower thymus weights in males at 261 mg/kg/day was thought to be a chance finding as a dose-response relationship was not present.

Table B.6.3.1–36 Group mean selected organ weights

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
Final bwt. (g)	316	313	327	318	317	303*	185	187	189	185	182	172*
Liver (g)	9.897	9.971	10.69	10.35	12.08*	13.25*	5.476	5.702	5.947	6.130*	6.889*	6.893*
Liver (% bwt.)	3.130	3.177	3.269	3.253	3.820*	4.374*	2.949	3.049	3.146	3.308*	3.784*	4.001*
Thymus (g)	0.209	0.204	0.213	0.221	0.177*	0.185	0.205	0.219	0.212	0.194	0.189	0.144*
Thymus (% bwt.)	0.066	0.065	0.065	0.069	0.056*	0.061	0.111	0.117	0.112	0.104	0.104	0.084*
Thyroid (g)	0.0124	0.0126	0.0131	0.0134	0.0141*	0.0152*	0.0085	0.0092	0.0096*	0.0091	0.0106*	0.0101*
Thyroid (% bwt.)	0.0039	0.0040	0.0040	0.0042	0.0044*	0.0050*	0.0046	0.0049	0.0051*	0.0049	0.0058*	0.0059*

* significantly different from control, $p \leq 0.05$

There were no treatment-related macroscopic necropsy findings. Microscopically, the liver, thyroid and thymus were identified as target organs, as shown in Tables B.6.3.1-37 and B.6.3.1-38. Males and females at 261 and 500 mg/kg/day had treatment-related panlobular hypertrophy of hepatocytes with altered tinctorial properties (increased cytoplasmic eosinophilia). The severity (very slight to slight) increased with the dose in both sexes. Other treatment-related effects in the liver consisted of very slight increased numbers of mitotic figures (hepatocytes in mitosis) in females at 261 and 500 mg/kg/day, and hepatocellular vacuolisation (considered by the study pathologist to be consistent with fatty change, though no specific staining was performed to confirm this) among males and females 261 and 500 mg/kg/day and also in males at 52 mg/kg/day. Both males and females at 261 and 500 mg/kg/day had treatment-related very slight or slight diffuse hypertrophy of thyroid gland follicular cells. Females only at 500 mg/kg/day had treatment-related very slight lymphoid atrophy of the thymus. The thymic atrophy was a minimal effect that is of unknown aetiology (the Applicant points out that stress is a known causative factor in thymic lymphoid atrophy in Fischer 344 rats).

Table B.6.3.1–37 Selected microscopic pathology findings, males: no. of affected animals

Finding		Target dose level of XDE-729 Methyl (mg/kg/day)					
		0	3	10	52	261	500
Liver (no. examined)		10	10	10	10	10	10
Hypertrophy, hepatocellular, panlob.	v slight	0	0	0	0	8	0
	slight	0	0	0	0	2	10
Vacuolisation, consistent with fatty change, hepatocellular, multifocal	v slight	0	0	0	5	8	0
	slight	0	0	0	0	2	3
	moderate	0	0	0	0	0	7
Thyroid gland (no. examined)		10	10	10	10	10	10
Hypertrophy, follicular cell, diffuse	v slight	1	0	0	1	2	0
	slight	0	0	0	0	7	9

Table B.6.3.1–38 Selected microscopic pathology findings, females: no. of affected animals

Finding		Target dose level of XDE-729 Methyl (mg/kg/day)					
		0	3	10	52	261	500
Liver (no. examined)		10	10	10	10	10	10
Hypertrophy, hepatocellular, panlob.	v slight	0	0	0	0	3	0
	slight	0	0	0	0	7	10
Increased mitotic figures	v slight	0	0	0	0	2	5
Valucolisation, consistent with fatty change, hepatocellular, multifocal	v slight	0	0	0	0	8	5
Thymus (no. examined)		10	10	10	10	10	9
Atrophy, lymphoid tissue	v slight	0	0	0	1	0	6
Thyroid gland (no. examined)		10	10	10	10	10	10
Hypertrophy, follicular cell, diffuse	v slight	0	0	1	0	5	7
	slight	0	0	0	0	3	3

The results of the toxicokinetic investigations are summarised in Table B.6.3.1–39.

In blood, XDE-729 Methyl was not present in quantifiable amounts in all but a few samples from the treated rats of either sex. In contrast, XDE-729 Acid was present in quantifiable amounts in most blood samples from treated animals; the blood levels of the Acid metabolite increased with XDE-729 Methyl dose, though the increase was less than dose-proportional. Five additional metabolites investigated, which were demethylated and/or conjugated XDE-729 methyl and/or acid, were present at quantifiable levels in the blood of both sexes at 52 mg/kg/day and above; each was were present at relatively low levels (up to 13% of the levels of XDE-729 Acid) and all showed dose-proportionality.

In the liver, XDE-729 Methyl and XDE-729 Acid were present in all treated groups, with the levels of Methyl being about between 10- and 2-fold lower than for the Acid. The amount of parent or Acid metabolite present increased with dose, though the increase was less than dose-proportional for the Acid in males. The levels of parent in the liver were very low at dose levels that did not cause liver toxicity. The five additional metabolites were also present in all groups, except for the sulphate conjugate of O-demethyl XDE-729 Acid which was absent in males at 3 mg/kg/day and females at 3 and 10 mg/kg/day. Except for O-demethyl XDE-729 acid and its glucuronide conjugate at the higher dose groups, the quantities of these metabolites showed dose-proportionality.

The analysis of urine demonstrated that most of the administered dose of XDE-729 Methyl is excreted as metabolites in the urine. XDE-729 Methyl was not present in quantifiable amounts in urine at any dose level (except for one high dose animal). The total amount (as % of administered XDE-729) excreted in the urine increased with increasing dose, from 48 and 57% in males and females, respectively, at the lowest dose to 61 and 76% in males and females, respectively, at 500 mg/kg/day. This indicates that XDE-729 Methyl is extensively absorbed. All six metabolites were detectable in urine at all doses, with the acid metabolite predominating. In both sexes, the proportion of dose excreted in the urine as conjugated metabolites increased with increasing dose. This suggests that induction of liver metabolism may occur at higher dose levels, consistent with the observation of increased liver weight at higher dose levels observed in this study (see Table B.6.3.1-36).

This toxicokinetic investigation demonstrated the presence of an additional metabolite of XDE-729 Methyl that was not identified in the specialised toxicokinetic studies summarised in section B.6.1. This metabolite is the glucuronide conjugate of O-demethyl XDE-729 Methyl, detected in blood, liver and urine. The amount of this metabolite excreted in urine in females accounted for 5.5% of the dose of XDE-729 Methyl (adjusted for equivalence) at a dose level of 3 mg/kg/day and 12.4% at 500 mg/kg/day. The levels of this metabolite were lower in males. This glucuronide conjugate of O-demethyl XDE-729 Methyl was also detected in two mechanistic studies (IIA 5.5.4/01 and IIA 5.5.4/03) evaluated in Section B.6.5.3.

Table B.6.3.1–39 Blood, liver and urine kinetics of XDE-729 Methyl and its major metabolites

Analyte	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
Blood (mean steady state concentration of analyte, µg/g)												
XDE-729 Methyl	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	NQ	NQ	0.021	0.125	0.275	NQ	NQ	NQ	0.069	0.335	0.785
XDE-729 Acid	NQ	0.127	0.416	1.751	5.588	9.139	NQ	0.123	0.405	1.263	7.812	12.83
O-demethyl XDE-729 Acid	NQ	NQ	NQ	0.006	0.042	0.081	NQ	NQ	NQ	0.010	0.048	0.077
Sulphate conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ	0.098	0.516	1.173	NQ	NQ	NQ	0.045	0.265	0.436
Glucuronide conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ	0.007	0.069	0.190	NQ	NQ	NQ	0.017	0.128	0.243
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ	0.015	0.070	0.201	NQ	NQ	NQ	NQ	0.143	0.330
Total of metabolites, excluding XDE-729 Acid	NQ	NQ	NQ	0.147	0.822	1.920	NQ	NQ	NQ	0.141	0.919	1.871
Liver (mean concentration of analyte, µg/g liver tissue)												
XDE-729 Methyl	NQ	0.026	0.132	0.767	2.721	3.893	NQ	0.031	0.138	0.567	3.129	6.527
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	0.017	0.050	0.285	1.133	1.748	NQ	0.036	0.124	0.603	2.501	4.115
XDE-729 Acid	NQ	0.276	1.009	3.707	9.543	12.13	NQ	0.126	0.457	1.342	7.376	12.93
O-demethyl XDE-729 Acid	NQ	0.004	0.012	0.056	0.186	0.293	NQ	0.002	0.009	0.038	0.130	0.164
Sulphate conj. of O-demethyl XDE-729 Acid	NQ	NQ	0.032	0.189	0.848	1.517	NQ	NQ	NQ	0.110	0.500	1.013
Glucuronide conj. of O-demethyl XDE-729 Acid	NQ	0.011	0.031	0.223	1.275	2.237	NQ	0.008	0.023	0.140	0.786	1.210
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	NQ	0.024	0.088	0.448	1.991	3.300	NQ	0.014	0.047	0.162	1.431	2.322
Total of metabolites, excluding XDE-729 Acid	NQ	0.056	0.213	1.201	5.433	9.125	NQ	0.060	0.203	1.053	5.348	8.824
Urine (mean total % of dose excreted in 24 h, adjusted for equivalence)												
XDE-729 Methyl	-	0	0	0	0	0	-	0	0	0	0	0
Glucuronide conj. of O-demethyl XDE-729 Methyl	-	0.91	0.73	1.46	2.70	4.08	-	5.50	4.41	5.71	9.70	12.37
XDE-729 Acid	-	42.41	33.45	28.29	30.47	32.04	-	44.64	38.66	24.79	34.16	36.98
O-demethyl XDE-729 Acid	-	0.55	0.53	0.74	1.09	1.35	-	1.12	0.67	1.42	1.81	2.13
Sulphate conj. of O-demethyl XDE-729 Acid	-	1.87	2.13	7.22	11.05	11.91	-	0	1.80	5.80	6.62	6.52
Glucuronide conj. of O-demethyl XDE-729 Acid	-	0.53	0.56	1.43	4.02	7.29	-	2.88	2.29	5.56	10.39	13.75
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	-	1.78	1.71	5.24	3.37	4.43	-	2.42	2.55	2.14	3.19	3.96
Total of metabolites, including XDE-729 Acid	-	48.06	39.11	44.37	52.70	61.09	-	56.56	50.39	45.41	65.86	75.71
Total of metabolites, excluding XDE-729 Acid	-	5.65	5.66	16.08	22.24	29.05	-	11.92	11.73	20.62	31.71	38.73

NQ = not quantifiable

Based on these blood, liver and urine analysis results, metabolic pathways for XDE-729 Methyl in the rat are proposed in Figure 6.1.1-2 (see section B.6.1)

The hepatic gene expression responses are shown in Table B.6.3.1-40. *Cyp1a1* expression was increased in both sexes at all dose levels, with the increases being dramatic (>1000-fold) at 52

mg/kg/day and above. *Ugt1a6* expression was increased in both sexes at 52 mg/kg/day and above. The gene expression profile is consistent with ligand-induced activation of the AhR signaling pathway. *Cyp2b1*, *Cyp3a23/3a1* and *Cyp4a22* expression were not increased, indicating that the CAR, PXR or PPAR-α signaling pathways were not activated by XDE-729 Methyl.

The marked increases in *Cyp1a1* expression correlated with the increased liver weight and microscopic changes in the liver (hepatocyte hypertrophy, mitotic figures, vacuolization) observed at 52 mg/kg/day and above (see Tables B.6.3.1-36, B.6.3.1-37 and B.6.3.1-38). At 3 and 10 mg/kg/day the increased *Cyp1a1* expression was much less marked, correlating with the presents of a very marginal increase in liver weight and absence of microscopic liver changes. The increased *Ugt1a6* expression at 261 and 500 mg/kg/day correlates with the increased thyroid weight and microscopic observation of thyroid follicular cell hypertrophy, and indicated the possibility of the involvement of the *Ugt1a6* in mediating the thyroid changes.

Table B.6.3.1-40 Gene expression analysis in liver, shown as fold-change compared with controls

Gene	Target dose level of XDE-729 Methyl (mg/kg/day)											
	Males						Females					
	0	3	10	52	261	500	0	3	10	52	261	500
CYP1a1	1	3.6	51.8	1494	6263	12937	1	5.7	148.8	2268	10358	17082
CYP2b1	1	0.4	1.1	1.0	3.9	37.3	1	0.7	0.7	0.6	1.6	14.6
CYP3a23/3a1	1	0.9	0.8	0.9	0.9	1.0	1	0.9	0.9	0.9	1.5	3.0
CYP4a22	1	0.9	0.8	1.1	1.2	1.2	1	1.1	1.1	1.0	1.5	1.7
Ugt1a6	1	1.0	1.2	3.1	9.2	23.7	1	0.9	1.4	4.9	36.0	64.2

CONCLUSION

Dietary administration for 90 days demonstrated that the liver, thyroids, thymus and blood are targets for XDE-729 Methyl in the rat. In the liver, organ weight was increased and microscopic changes (hepatocyte hypertrophy; mitotic figures; vacuolisation consistent with fatty change) were present in both sexes; the LOAEL for liver toxicity was 52 mg/kg/day for both sexes, based on presence of vacuolisation in males and increased liver weight in females at 52 mg/kg/day. The liver changes were associated with increased serum cholesterol concentrations. Hepatic gene expression investigations described below indicate that that the MOA for the liver changes involves Ah receptor activation. Thyroid weight was increased and diffuse hypertrophy of thyroid gland follicular cells was reported in both sexes at 261 and 500 mg/kg/day. Thymus weight was reduced in females at 500 mg/kg/day, which correlated with the microscopic observation of thymic lymphoid tissue atrophy; these thymus changes were possibly stress related. Haemoglobin concentration, haematocrit and red blood cell count were reduced in both sexes at 500 mg/kg day. Additionally, urine pH was decreased in males at 261 and 500 mg/kg/day, probably due to the presence of the metabolite XDE-729 Acid.

The hepatic gene expression investigations demonstrated increased *Cyp1a1* and *Ugt1a6* expression, notably at dose levels of 52 mg/kg/day and above, which correlated with the observed liver and thyroid changes, and provided evidence of activation of the AhR signaling pathway. The hepatic gene expression profile did not indicate activation CAR, PXR or PPAR-α signaling pathways by XDE-729 Methyl. Increased *Ugt1a6* at the higher exposure levels

correlated with the thyroid changes and indicates the possibility of the involvement of the *Ugt1a6* in mediating the thyroid changes.

A study NOAEL of 10.4 mg/kg/day in males and 10.1 mg/kg/day in females (target 10 mg/kg/day) is identified, based on the observation of liver changes at the target dose level of 52 mg/kg/day.

Toxicokinetic investigations showed that post-hepatic exposure to parent XDE-729 Methyl is negligible. In blood, the administered dose is present mainly as the XDE-729 Acid metabolite and, to a lesser extent, to demethylated and conjugated XDE-729 Acid metabolites. The severity of the hepatic changes correlated with the levels of XDE-729 Methyl detected in the liver. This investigation demonstrated the presence of an additional metabolite of XDE-729 Methyl that was not found in the specialised toxicokinetic studies summarised in section B.6.1, the glucuronide conjugate of O-demethyl XDE-729 Methyl, which was found in blood, lever and urine. Most of the administered dose of XDE-729 Methyl is excreted as the Acid metabolite or as the demethylated and conjugated metabolites.

[redacted]

(2012)

B.6.3.2 Oral short-term toxicity in the mouse

XDE-729 Acid	
Study	IIA 5.3.1/03 28-day dietary toxicity study in Crl:CD1(ICR) mice
Reference	[redacted] (2009)
Date performed	October – November 2008
Test facility	[redacted]
Report reference	Laboratory study ID 081116
Guideline(s)	OECD 407 (1995)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2350-93 TSN030751-0002, 99.0% purity
Study acceptable	Yes

METHODS

Crl:CD1(ICR) mice, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.2–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	5	5
2	10	5	5
3	50	5	5
4	250	5	5
5	1000	5	5

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 11.5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from the jugular vein of all animals (non-fasted) at the time of necropsy (day 31) for determination of plasma XDE-729 Acid concentrations. Spot urine samples were collected from all animals on day 29 for determination of XDE-729 Acid concentrations.

Blood samples (non-fasted) were taken from the orbital sinus at the time of scheduled necropsy and a standard range of haematology and clinical chemistry parameters were measured.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The liver, kidneys and spleen from all males, macroscopic abnormalities from all animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.2–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	10	50	250	1000
Males	10.2	54.0	269	1025
Females	9.94	48.7	241	958

There were no treatment related deaths or clinical signs of toxicity.

There were no treatment-related effects on bodyweights or food consumption.

There were no treatment-related ophthalmoscopy findings or effects on the clinical chemistry parameters.

Red blood cell count was significantly reduced in males at 1000 mg/kg/day, as presented in Tables B.6.3.2-3. Although this is regarded as probably treatment-related, the underlying cause of this change is unclear as no gross or histological indications of internal or external blood loss or histological evidence of accelerated erythrocyte destruction or diminished erythropoiesis were apparent.

Table B.6.3.2–3 Selected group mean haematology findings

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	50	250	1000	0	10	50	250	1000
RBC (10^6/µL)	9.70	9.75	9.67	9.18	8.94*	9.49	8.87	9.27	9.38	9.09

* significantly different from control, p≤0.05

Organ weights were not affected by treatment. There were no treatment-related macroscopic or microscopic pathology findings.

The toxicokinetic findings are summarised in Table B.6.3.2-4. The plasma concentration of XDE-729 Acid, measured at the end of the study, was approximately dose-proportional in both genders, indicating that dose level has no influence on systemic bioavailability. Urinary excretion of XDE-729 Acid was also approximately dose-proportional. The extrapolated amount of XDE-729 Acid excreted in 24 h expressed as a percentage of daily intake (23 – 49%) was less than for rats in the 28-day study (Yano et al 2009), but firm conclusions on excretion should not be drawn from these data because of uncertainties relating to the extrapolation to 24 h from spot urine samples.

Table B.6.3.2-4 Mean amount of XDE-729 Acid in plasma (day 31) and urine (29)

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	10	250	500	1000	0	10	250	500	1000
Actual dose of XDE-729 Acid at blood sampling (mg/kg/day)	0	8.58	43.2	221	909	0	8.24	41.2	216	876
Actual intake of XDE-729 Acid (mg/mouse/day)	0	0.366	1.87	9.59	35.3	0	0.253	1.479	6.73	29.1
Plasma conc of XDE-729 Acid (µg/g)	NQ	0.44	1.96	14.53	45.34	NQ	0.63	1.19	13.56	31.89
Amount of XDE-729 Acid in 24 h[a] urine (mg/mouse)	NQ	0.096	0.512	2.31	8.06	NQ	0.125	0.355	1.66	7.74
Amount of XDE-729 Acid in 24 h[a] urine as % of daily intake	-	26.7	27.5	24.0	22.7	-	49.0	23.7	24.5	26.8

NQ = not quantifiable
[a]amount XDE-729 extrapolated to a 24 h value, based on 24 h urine volume established from historical controls

CONCLUSION

Adverse effects of dietary administration of XDE-729 Acid for 28 days to mice were limited to the observation of a slight reduction in blood cell count in males at a target dose level of 1000 mg/kg/day. A study NOAEL of 269 mg/kg/day (target 250 mg/kg/day) is identified. A toxicokinetic investigation showed that systemic bioavailability and urinary excretion of XDE-729 Acid is dose-proportional.

[redacted] (2009)

XDE-729 Acid	
Study	IIA 5.3.2/03 90-day dietary toxicity study in Crl:CD1(ICR) mice
Reference	[redacted] (2010)
Date performed	July – October 2009
Test facility	[redacted]
Report reference	Laboratory study ID 091056
Guideline(s)	OECD 408 (1998)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Crl:CD1(ICR) mice, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.2–5: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	10	10
2	50	10	10
3	250	10	10
4	500	10	10
5	1000	10	10

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 12.5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from the jugular vein of four males and four females (non-fasted) per group on Day 86 for determination of plasma XDE-729 Acid concentrations. Spot urine samples were collected from all animals on day 86 for determination of XDE-729 Acid concentrations.

Blood samples (non-fasted) were taken from the orbital sinus at the time of scheduled necropsy and a standard range of haematology and clinical chemistry parameters were measured.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The urinary bladder and macroscopic abnormalities from all

animals, and other organs from only the control and high dose animals, were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.2–6 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	50	250	500	1000
Males	49.4	247	495	989
Females	50.2	258	512	1008

There were no treatment related deaths or clinical signs of toxicity.

There were no treatment-related effects on bodyweights or food consumption.

There were no treatment-related ophthalmoscopy findings or effects on the clinical chemistry parameters.

There were no treatment-related differences in the haematology or clinical chemistry parameters.

Organ weights were not affected by treatment. There were no treatment-related macroscopic necropsy findings.

Treatment-related microscopic pathology findings were limited to the observation urinary bladder changes in two high dose males (see Table B.6.3.2–7), comprising of a slight, acute inflammation which affected the mucosa and/or wall of the urinary bladder, accompanied by slight or moderate submucosal oedema. The transitional epithelium lining the bladder in one of these males was slightly hyperplastic (thickened) and had multiple foci of ulceration. Small crystals were noted in the urinary bladder of one of these males.

Table B.6.3.2–7 Selected microscopic pathology findings

Finding	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	50	250	500	1000	0	50	250	500	1000
Urinary bladder										
No. of animals examined	10	10	10	10	10	10	0	0	0	10
Inflammation, acute: slight	0	0	0	0	2	-	-	-	-	0
Oedema, submucosa: slight or moderate	0	0	0	0	2	-	-	-	-	0
Hyperplasia, transitional epithelium: slight	0	0	0	0	1	-	-	-	-	0
Ulceration, multifocal	0	0	0	0	1	-	-	-	-	0

The results of the toxicokinetic investigation are summarised in Table B.6.3.2–8. The plasma concentration of XDE-729 Acid, measured on day 86, was approximately dose-proportional in both genders, indicating that dose level has no influence on systemic bioavailability. It can also

be concluded that urinary excretion of XDE-729 Acid was also approximately dose-proportional, based on linear regression analysis.

Table B.6.3.2-8 Mean amount of XDE-729 Acid in plasma and urine on day 86

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	50	250	500	1000	0	50	250	500	1000
Actual dose of XDE-729 Acid at blood sampling (mg/kg/day)	0	43.2	219	461	924	0	43	177	385	1024
Actual intake of XDE-729 Acid (mg/mouse/day)	0	1.66	8.74	17.5	34.9	0	1.42	6.15	12.9	31.3
Plasma conc of XDE-729 Acid (µg/g)	NQ	4.37	18.45	37.8	68.3	NQ	4.08	10.15	22.6	65.4
Amount of XDE-729 acid in 24 h[a] urine (mg/mouse)	NQ	0.752	2.81	5.87	16.5	NQ	0.628	2.51	11.4	10.3
Amount of XDE-729 acid in 24 h[a] urine as % of daily intake	-	45.4	32.4	34.0	47.0	-	44.1	41.6	86.1	32.8

NQ = not quantifiable
[a]amount XDE-729 Acid extrapolated to a 24 h value, based on 24 h urine volume established from historical controls

CONCLUSION

Adverse effects of dietary administration of XDE-729 Acid for 90 days to mice were limited to the observation of microscopic changes in the urinary bladder, characterised as a slight, acute inflammation affecting the mucosa and/or wall accompanied by submucosal oedema, in two males at a target dose level of 1000 mg/kg/day. Study NOAELs of 495 mg/kg/day (target 500 mg/kg/day) in males and 1008 mg/kg/day (target 1000 mg/kg/day) in females are identified. A toxicokinetic investigation showed that systemic bioavailability and urinary excretion of XDE-729 Acid is dose-proportional.

[redacted] (2010)

B.6.3.3 Oral short term toxicity in the dog (IIA 5.3.1, 5.3.3)

XDE-729 Acid	
Study	IIA 5.3.1/04 Palatability probe and 28-day dietary toxicity study in Beagle dogs
Reference	[redacted] (2010)
Date performed	November 2008 – March 2009
Test facility	[redacted]
Report reference	Laboratory study no. 133-119; Dow study ID 081127
Guideline(s)	Investigations as specified in OECD 409
Deviations from the guideline	Duration of exposure was 28 days
GLP	Yes
Test material	XDE-729 Acid, Lot #E2350-93 TSN030751-0002, 99.00% purity
Study acceptable	Yes

METHODS

Beagle dogs, about 6- 7 months old on receipt, were randomly assigned to the test groups.

In a palatability probe study, groups of 2 dogs of each sex were fed diets containing the test substance at concentrations of 7500, 15000 or 30000 ppm (reduced to 22500 ppm for females)

for up to 3 days. Reductions (slight in males and marked in females) in bodyweight and food consumption were observed at 3000 and 22500 ppm. The dose levels for the 28-day study were selected taking account of the results of this probe investigation.

In the 28-day study, the following dose levels/group sizes were used:

Table B.6.3.3–1 28-day study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of animals	
		Males	Females
1	0	2	2
2	300	2	2
3	3000	2	2
4	15000	-	2
5	30000[a]	2	-

[a] on days 25-28 group 5 males were also fed control diet because of poor consumption of the test diet

The test and control diets were prepared using Lab Diet Certified Canine #5007. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the 28-day study were within 5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from all animals via the jugular vein for determination of plasma XDE-729 Acid levels at the following times: 1, 2, 6 and 16 hours postdose on days 27 and 16 hours after the day 28 food removal (Day 29). The animals were not fasted prior to the Day 27 blood collection. Additional blood samples were taken for two males at 30000 ppm at 1, 2, 6, and 16 hours postdose on Day 24. Twenty-four hour urine samples were collected from all animals (non-fasted) just prior to necropsy for determination of XDE-729 Acid levels.

Blood samples were taken from the jugular vein before treatment commenced and at the end of the treatment period, after an overnight fast. A standard range of haematology and clinical chemistry parameters were measured. Urine samples were collected before treatment commenced and towards the end of the treatment period for analysis of the standard parameters.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The fixed organs from all animals were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.3–2 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	300	3000	15000/30000
Males	11	80	635
Females	9	85	323

There were no unscheduled deaths. The only clinical signs that could possibly be due to treatment were observed in high dose males during the last week of treatment. One animal had atypical muscle tone (lower than normal) and one had atypical palpebral closure (eyes closed more than normal).

Adverse effects on bodyweight and food consumption occurred in high dose males and females, as shown in Tables B6.3.3-3 and B6.3.3-4. Group mean bodyweight of males and females at termination was 17% and 5% lower, respectively, than at the start of treatment. The amount of food consumed by high dose males was markedly lower than controls throughout the study. For high dose females, food consumption was slightly reduced.

Table B.6.3.3–3 Group mean bodyweights, selected values (kg)

Week	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	300	3000	30000	0	300	3000	15000
0	9.025	8.000	8.750	7.850	7.350	8.375	9.150	9.500
1	9.075	8.100	8.950	7.225	7.350	8.575	9.345	9.475
2	9.260	8.350	8.975	6.880	7.465	8.500	9.200	9.240
3	9.100	8.565	8.975	6.825	7.525	8.515	9.440	9.450
4	9.070	8.490	8.835	6.475	7.460	8.470	9.355	8.990

Table B.6.3.3–4 Group mean food consumption, selected values (g/animal/day)

Week	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	300	3000	30000	0	300	3000	15000
1	240.6	316.9	232.0	153.2	227.5	247.1	261.8	169.4
2	303.3	313.6	253.4	159.0	252.5	252.7	272.1	212.8
3	217.9	298.6	237.5	155.3	285.3	245.1	274.9	222.1
4	246.9	288.7	233.5	69.3	305.0	260.4	243.3	226.5

There were no treatment-related ophthalmoscopy findings.

There were no differences in haematology, clinical chemistry or urinalysis findings that were attributable to XDE-729 Acid treatment.

Organ weights were not affected by treatment. There were no treatment-related macroscopic or microscopic pathology findings.

The microscopic pathology examination identified the kidneys at the main target organ, as shown in Table B6.3.3-5. Both high dose males had slight, multifocal, tubular degeneration and regeneration, involving approximately 5 to 10% of the renal parenchyma. Both high dose females and one mid dose male had very slight, focal or multifocal tubular degeneration and regeneration,

involving less than 1% of the renal parenchyma. The degenerative effect was characterized by clusters of cortical and outer medullary tubules that had distended lumens and were lined by flattened epithelial cells. Regenerative tubules were lined by small cuboidal epithelial cells with increased cytoplasmic basophilia. The treatment-related degeneration and regeneration of the kidneys involved the proximal and distal tubules of the cortex, and collecting ducts of the outer zone of the medulla. Chronic interstitial inflammation and occasional necrotic tubular epithelial cells were present in the areas of tubular degeneration and regeneration.

The microscopic examination revealed an additional treatment-related effect, that of atrophy of the thymic lymphoid tissue, seen in high dose males and females. This finding was characterised by thinning of the cortical lymphoid tissue. There was no increase in the amount of cellular debris or lymphocytic necrosis in the thymus of affected dogs as compared to the control group animals. The thymic atrophy was possibly stress-related, and not an indication of primary toxicity to the thymus.

Table B.6.3.3–5 Selected microscopic pathology findings

Finding		Dietary concentration of XDE-729 Acid (ppm)							
		Males				Females			
		0	300	3000	30000	0	300	3000	15000
Number examined		2	2	2	2	2	2	2	2
Kidneys									
Tubule degeneration & regeneration, bilateral, multifocal	v slight	0	0	1	0	0	0	0	1
	slight	0	0	0	2	0	0	0	0
Tubule degeneration & regeneration, unilateral, multifocal	v slight	0	0	0	1	0	0	0	1
Thymus									
Atrophy, lymphoid tissue	v slight	0	0	0	0	0	0	0	1
	slight	0	0	0	1	0	0	0	0
	moderate	0	0	0	1	0	0	0	0

The results of the toxicokinetic investigations, conducted during the last week of the study, are summarised in Table B.6.3.3-6. For males, systemic absorption of XDE-729 Acid, based on the calculated AUC_{24h} values, was dose-proportional at the low and mid dose levels, but greater than dose proportional at the highest dose level. For females, systemic absorption of XDE-729 Acid was approximately dose-proportional at all treatment levels. Urinary elimination in the 24 hour urine samples ranged from 13 to 53% and 41 to 70% of daily intake in males and females, respectively.

Table B.6.3.3–6 Toxicokinetic investigation: mean systemic AUC_{24h} values and urinary elimination during last week of study

Parameter	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	300	3000	30000	0	300	3000	15000
Actual dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	10	79	331	0	9	78	264
AUC_{24h} for XDE-729 Acid (µg h/mL)	-	16	185	5893	-	26	167	1900
Amount of XDE-729 Acid in 24 h urine (mg/kg)	NQ	1.3	39.1	177	NQ	3.8	54.8	115
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	13	49	53	-	41	70	44

NQ = not quantifiable

CONCLUSION

Dietary administration of XDE-729 Acid for 28 days to the dog at a dose level of 3000 ppm and above (males) or 15000 ppm (females) caused adverse effects in the kidney (slight tubular degeneration and regeneration). At the highest dose level (30000 ppm in males and 15000 ppm in females) the kidney changes were accompanied by lymphoid tissue atrophy in the thymus, a slight bodyweight loss and by lower food consumption. The study NOAELs are 300 ppm for males (intake of about 11 mg/kg/day) and 3000 ppm for females (intake of about 85 mg/kg/day).

A toxicokinetic investigation showed that systemic bioavailability of XDE-729 Acid is dose-proportional at dietary levels of up to 3000 ppm in males and up to 15000 ppm in females.

[redacted] (2010)

XDE-729 Acid	
Study	IIA 5.3.3/01 90-day oral (dietary) toxicity study in Beagle dogs
Reference	[redacted] (2011)
Date performed	December 2009 – March 2010
Test facility	[redacted]
Report reference	Laboratory study no. 133-122; Dow study ID 091070
Guideline(s)	OECD 409
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot # E2978-05-0 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Beagle dogs, about 5 months old on receipt, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.3–7: Study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of animals	
		Males	Females
1	0	4	4
2	500	4	4
3	2500	4	4
4	12500	4	4

The test and control diets were prepared using Lab Diet Certified Canine #5007. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the 28-day study were within about 9% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured weekly during the study. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from all animals via the jugular vein for determination of plasma XDE-729 Acid levels at the following times: 1, 2, 6 and 16 hours after initiation of the light cycle on Day 88 and 16 hours after the day 90 food removal (on Day 91). The animals were not fasted prior to the Day 88 blood collection. Twenty-four hour urine samples were collected from all animals (non-fasted) during Week 13 for determination of XDE-729 Acid levels.

Blood samples were taken from the jugular vein before treatment commenced and during Weeks 7 and 13, after an overnight fast. A standard range of haematology and clinical chemistry parameters were measured. Urine samples were collected over 16 hour periods at the same time intervals as for blood sampling, for measurement of the standard parameters.

A necropsy was conducted on all animals the end of the 13 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The fixed organs from all animals were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.3–8 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	500	2500	12500
Males	16.75	80.6	424
Females	18.7	79.9	415

There were no unscheduled deaths or treatment-related clinical signs of toxicity.

Bodyweight gain was reduced among males, and to a lesser extent in females, at 12500 ppm. By the end of the treatment period bodyweights of males and females at this dose level were 14% and 8% lower, respectively, than controls (see Table B.6.3.3-9). The lower bodyweights of males at 12500 ppm were statistically significantly different from controls. The reduced bodyweight gain at 12500 ppm correlated with slightly lower food consumption in this group, though statistical significance was not achieved (see Table B.6.3.3-10).

Table B.6.3.3–9 Group mean bodyweights, selected values (kg)

Day	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	12500	0	500	2500	12500
-1	6.91	6.99	7.03	6.98	6.09	6.19	6.24	6.26
7	7.26	7.34	7.31	6.94	6.32	6.30	6.58	6.32
14	7.38	7.75	7.67	7.05	6.52	6.55	6.85	6.28
49	8.16	8.66	8.56	6.94	6.71	6.75	7.18	6.15
90	8.91	9.73	9.56	7.62*	7.33	7.58	7.81	6.74

*significantly different from control $p<0.05$, taking account of time and dose

Table B.6.3.3–10 Group mean food consumption, selected values (g/animal/day)

Week	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	12500	0	500	2500	12500
1	252	284	280	215	215	202	226	193
2	255	305	293	236	217	252	235	181
5	278	319	312	250	335	280	297	220
7	244	269	245	220	272	233	214	214
10	254	274	280	249	253	251	213	219
12	265	285	276	276	306	276	250	225

There were no treatment-related ophthalmoscopy findings.

There were no differences in clinical chemistry or urinalysis findings that were attributable to XDE-729 Acid treatment.

Haematology findings are summarised in Table B.6.3.3-11. At 12500 ppm, in both sexes, there were decreases in red cell mass (RBC, haemoglobin concentration and haematocrit) at weeks 7 and 13, although the differences were not statistically significant. No effects on reticulocyte count were apparent. These findings, observed only at the highest dose level, were considered likely to be a secondary effect of XDE-729 Acid treatment, related to the decreases in bodyweight gain.

Table B.6.3.3–11 Selected group mean haematology findings

Parameter	Week	Dietary concentration of XDE-729 Acid (ppm)							
		Males				Females			
		0	500	2500	12500	0	500	2500	12500
RBC (10^6/µL)	Pretest	6.26	6.38	5.90	5.99	6.41	6.56	6.51	5.76
	7	7.16	6.87	6.78	6.00	7.04	6.74	6.94	6.18
	13	7.20	6.96	6.78	5.73	7.24	6.88	6.95	5.61
Haemoglobin (g/dL)	Pretest	13.48	13.35	12.55	12.98	13.83	14.23	13.98	12.63
	7	15.60	14.85	14.75	13.20	15.58	14.90	15.20	13.78
	13	15.78	15.18	14.88	12.93	16.35	15.48	15.65	12.78
Haematocrit (%)	Pretest	42.23	42.35	39.23	40.85	43.38	44.65	43.63	39.68
	7	47.33	45.35	44.93	40.40	46.58	45.23	46.23	42.15
	13	47.30	45.73	44.53	39.03	48.23	45.70	46.05	38.83

The organ weight analysis revealed significantly increased bodyweight-related liver weight in males (by 20%, in comparison with controls) and females (by 51%) at 12500 ppm (see Table B.6.3.3-12).

Table B.6.3.3–12 Selected group mean organ weights

Parameter	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	12500	0	500	2500	12500
Final bodyweight (kg)	8.71	9.50	9.39	7.26*	7.10	7.20	7.48	6.45
Liver weight (g)	228	230	247	224	192	206	199	265**
Liver weight (% bodyweight)	2.61	2.42	2.64	3.13*	2.74	2.89	2.66	4.14**
Liver/brain weight ratio	3.12	3.03	3.42	3.13	2.62	2.99	2.67	3.64*

*significantly different from control p<0.05 **significantly different from control p<0.01

There were no treatment-related macroscopic necropsy findings. The key microscopic pathology changes are summarised in Table B.6.3.3-13. Treatment-related changes occurred only at 12500 ppm, with the kidney being identified as the main target organ in both males and females. The changes in the kidney consisted of slight degeneration with regeneration of renal proximal and distal tubules, slight degeneration of collecting ducts and the presence of a focal granuloma in the cortex or medulla. The kidney effects involved less than 1% of the renal parenchyma in most of the affected animals. The tubular degeneration was characterized by clusters of proximal and distal tubules in the cortex with distended lumens which were lined by flattened epithelial cells, and sometimes containing proteinaceous fluid. Regenerative proximal and distal tubules were lined by small cuboidal epithelial cells with increased cytoplasmic basophilia. A few necrotic epithelial cells were present in some areas of tubular degeneration in two high-dose males. Chronic interstitial inflammation and fibrosis were present in the areas of tubular degeneration and regeneration. The degeneration of collecting ducts was characterized by dilated ducts lined by flattened or hypertrophic epithelial cells, with associated interstitial fibrosis. Some affected collecting ducts contained exfoliated epithelial cells and proteinaceous fluid.

Other changes present at 12500 ppm were slight atrophy of the thymus (possibly stress related) hyperplasia of the bone marrow (characterised by an increase in myelopoiesis) and, in females only, extramedullary haematopoiesis of the liver and spleen. Microscopic examination of the

peripheral blood smears of the female with moderate bone marrow hyperplasia and a left-shifted myeloid cell line of the bone marrow revealed neutropenia, characterised by a complete lack of mature segmented neutrophils at the final sampling time, and low numbers of band stage neutrophils. The predominant white blood cell types in the peripheral blood of this animal were reactive lymphocytes and monocytes. These findings were consistent with ineffective myelopoiesis and maturation arrest.

Table B.6.3.3–13 Selected microscopic pathology findings: no. of affected animals

Finding	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	12500	0	500	2500	12500
Number examined	4	4	4	4	4	4	4	4
Kidneys								
Degeneration with regeneration, tubule, bilateral, multifocal v slight	0	0	0	3	0	0	0	2
slight	0	0	0	1	0	0	0	0
Degeneration, collecting duct, unilateral, multifocal v slight	0	0	0	0	0	0	0	1
Degeneration, collecting duct, bilateral, multifocal v slight	0	0	0	3	0	0	0	1
Granuloma, cortex, focal v slight	0	0	0	1	0	0	0	1
Granuloma, medulla, focal v slight	0	0	0	1	0	0	0	0
Thymus								
Atrophy, lymphoid tissue v slight	0	0	0	2	0	0	0	1
slight	0	0	0	0	0	0	0	1
Bone marrow								
Hyperplasia slight	0	0	0	1	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Liver								
Extramedullary haematopoiesis, multifocal slight	0	0	0	0	0	0	0	1
Extramedullary haematopoiesis, diffuse moderate	0	0	0	0	0	0	0	1
Spleen								
Extramedullary haematopoiesis, multifocal slight	0	0	0	0	0	0	0	1
Extramedullary haematopoiesis, diffuse moderate	0	0	0	0	0	0	0	1
Hematopoietic/lymphoid System								
Neutropenia moderate	0	0	0	0	0	0	0	1

The results of the toxicokinetic investigations, conducted during the last week of the study, are summarised in Table B.6.3.3-14. For both genders, systemic absorption of XDE-729 Acid, based on the calculated AUC_{24h} values, was dose-proportional at the low and mid dose levels, but much greater than dose proportional at the highest dose level. Urinary elimination as a proportion intake was lower at the mid and high exposure groups, suggesting that the elimination processes operating at 500 ppm may be saturated at these higher exposure levels.

Table B.6.3.3–14 Toxicokinetic investigation: mean systemic AUC_{24h} values and urinary elimination during last week of study

Parameter	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	12500	0	500	2500	12500
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	12.5	60.51	389.1	0	16.6	60.3	400.1
AUC_{24h} for XDE-729 Acid (µg h/mL)	-	41	183	2917	-	44	212	3794
Plasma elimination $t_{½}$ (h)	-	ND	ND	2.37	-	ND	ND	4.89
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	0	12.55	44.3	107.1	0	16.8	29.6	134.5
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	102	73	28	-	106	47	32

ND = not determined because plasma concentrations of XDE-729 at terminal sacrifice were below the limit of quantitation

CONCLUSION

Dietary administration of XDE-729 Acid for 90 days to the dog at a dose level of 12500 ppm caused adverse effects primarily in the kidney (slight tubular degeneration and regeneration, degeneration of collecting ducts, focal granuloma in the cortex or medulla), accompanied by microscopic changes in thymus (lymphoid atrophy), bone marrow (hyperplasia), spleen and liver (extramedullary haematopoiesis, females only), liver weight increases, and decreased bodyweight and food consumption. A study NOAEL of 2500 ppm (intake of about 80 mg/kg/day in both sexes) was identified.

A toxicokinetic investigation showed that systemic bioavailability of XDE-729 Acid is dose-proportional at the low (500 ppm) and mid (2500 ppm) dose levels, but much greater than dose proportional at the highest dose level (12500 ppm). Urinary elimination processes occurring at 500 ppm appeared to be saturated at the mid and high dose levels.

[redacted] (2011)

XDE-729 Acid	
Study	IIA 5.3.4/01 One-year oral (dietary) toxicity study in Beagle dogs
Reference	[redacted] (2012)
Date performed	December 2010 – December 2011
Test facility	[redacted]
Report reference	Laboratory study no. 1797-006; Dow study ID 101163
Guideline(s)	OECD 452
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot # E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Beagle dogs, 5-6 months old on receipt, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.3–15: Study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of animals	
		Males	Females
1	0	4	4
2	500	4	4
3	2500	4	4
4	10000/5000[a]	4	4

[a]for females only the dose level was reduced to 5000 ppm on day 47 following the observation of bodyweight loss

The test and control diets were prepared using Lab Diet Certified Canine #5007. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the 28-day study were within about 3% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured weekly during the study. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

Toxicokinetic investigations were conducted at part of this study. Blood samples (non-fasted) were taken from all animals via the jugular vein for determination of plasma XDE-729 Acid levels at the following times: 1, 2, 6 and 16 hours after initiation of the light cycle on one day at 3, 6 and 12 months. Twenty-four hour urine samples (non-fasted) were collected from all animals on one day during Weeks 13, 26 and 52 for determination of XDE-729 Acid levels.

Blood samples were taken from the jugular vein before treatment commenced and at 3, 6 and 12 months, after an overnight fast. A standard range of haematology and clinical chemistry parameters were measured. Urine samples were collected over 16 hour periods at the same time intervals as for blood sampling, for measurement of the standard parameters.

A necropsy was conducted on all animals the end of the 12 month treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The fixed organs from all animals were subjected to microscopic (light) examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.3.3–16 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	500	2500	10000/5000
Males	13.9	82.4	354.8
Females	16.7	89.9	219.8

There were no unscheduled deaths or treatment-related clinical signs of toxicity.

Group mean bodyweights are summarised in Table B.6.3.3-17. Bodyweights of females at the highest treatment level were reduced during the early weeks of the study, with mean weight being about 9% lower than pre-dose values. Because of this bodyweight loss the dose level for high dose females was reduced from 10000 ppm to 5000 ppm during week 6. By week 26 and for the remainder of the study, bodyweight of females at the highest dose level appeared not to be affected by XDE-729 Acid treatment. Bodyweights of males were not affected by treatment. There were no treatment related effects on food consumption.

Table B.6.3.3–17 Group mean bodyweights, selected values (kg)

Week	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	10000	0	500	2500	10000/ 5000
-1	7.84	7.84	8.11	8.09	6.55	6.75	6.69	6.55
6	7.59	8.62	8.48	8.18	6.71	7.30	7.06	5.99
13	8.10	9.20	8.64	8.66	7.28	7.56	7.22	6.34
26	8.61	9.53	8.98	9.23	7.49	7.92	7.48	7.60
39	9.16	9.85	9.46	9.78	7.94	8.41	8.29	8.15
52	9.06	9.92	9.59	9.78	8.01	8.48	8.48	8.18

There were no treatment-related ophthalmoscopy findings. There were no differences in the haematology, clinical chemistry or urinalysis findings that were attributable to XDE-729 Acid treatment.

There were no treatment-related organ weight differences or macroscopic necropsy findings.

Treatment-related microscopic pathology findings were present in the kidneys only, in males at 10000 ppm and in females at 2500 and 10000/5000 ppm, as summarised in Table B.6.3.3-18. The kidney changes were all graded as very slight, and involved less than 1% of the renal parenchyma in all of the affected animals. The tubular degeneration was characterised by clusters of proximal and distal tubules in the cortex that had distended lumens, were lined by flattened epithelial cells, and sometimes contained proteinaceous fluid. Regenerative proximal and distal tubules were lined by small cuboidal epithelial cells with increased cytoplasmic basophilia. Interstitial fibrosis and occasional interstitial lymphocytes were present in some areas of tubular degeneration and regeneration. The degeneration of collecting ducts was characterised by dilated ducts lined by flattened or hypertrophic epithelial cells, with or without associated interstitial fibrosis. Some affected collecting ducts contained exfoliated epithelial cells and proteinaceous fluid. The granulomas were present in the outer cortex of both affected animals. The high dose male with cortical granulomas also had circular areas of interstitial fibrosis of the outer cortex of both kidneys, which were consistent with resolved granulomas. The glomerulosclerosis was characterized by thickened collagen of Bowman's capsule, with or without thickening of the glomerular mesangium and atrophy of glomerular tufts.

Table B.6.3.3–18 Selected microscopic pathology findings: no. affected animals

Finding	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	10000	0	500	2500	10000/ 5000
Number examined	4	4	4	4	4	4	4	4
Kidneys								
Degeneration with regeneration, tubule, bilateral, multifocal v slight	0	0	0	2	0	0	1	1
Degeneration, collecting duct, bilateral, multifocal v slight	0	0	0	2	0	0	1	0
Fibrosis, cortex, bilateral, multifocal v slight	0	0	0	1	0	0	0	0
Glomerulosclerosis, bilateral, multifocal v slight	0	0	0	2	0	0	1	0
Granuloma, cortex, bilateral, multifocal v slight	0	0	0	1	0	0	0	0
Granuloma, cortex, unilateral, focal v slight	0	0	0	0	0	0	0	1

The results of the toxicokinetic investigations, conducted at 3, 6 and 12 months, are summarised in Table B.6.3.3-19. Based on the calculated AUC_{24h} values, systemic absorption of XDE-729 Acid was approximately dose-proportional at all dose levels in females at 3 and 6 months. However, absorption was dose-proportional only at the low and mid dose levels in males at 3 and 6 months, but greater than dose proportional at the highest dose level. At 12 months, there were too few plasma samples with quantifiable levels of XDE-729 Acid to assess kinetic linearity across doses. On average, males excreted 40% of the dose intact in the urine and females excreted 55% of the dose intact in urine. The amount of XDE-729 Acid excreted in the urine over 24 hours exhibited a linear relationship for all three dose levels in females and at the low and middle doses in males. At the high dose level for males, urinary excretion of XDE-729 Acid appeared to be less than dose proportional at all time points, though this sublinear relationship was not confirmed in the statistical analysis.

Table B.6.3.3–19 Toxicokinetic investigation: mean systemic AUC_{24h} values and urinary elimination at 3, 6 and 12 months

Parameter	Dietary concentration of XDE-729 Acid (ppm)							
	Males				Females			
	0	500	2500	10000	0	500	2500	10000/ 5000
3 months								
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	13	86	392	0	18	89	243
Plasma AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	32	177	1220	NQ	36	200	486
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	NQ	5.1	29.4	71.0	NQ	11.5	49.3	111.0
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	38.1	38.1	18.2	-	63.7	55.5	41.5
6 months								
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	12	78	331	0	16	87	177
Plasma AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	33	196	1368	NQ	43	182	735
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	NQ	5.6	42.7	106.6	NQ	10.1	54.3	82.1
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	47.9	54.8	30.9	-	66.5	61.9	46.0
12 months								
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	12	68	311	0	15	73	154
Plasma AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	NQ	NQ	541	NQ	NQ	NQ	285
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	NQ	4.8	44.9	99.9	NQ	9.5	31.8	81.5
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	41.5	68.9	31.3	-	61.8	42.3	49.7

NQ= not quantifiable

CONCLUSION

Dietary administration of XDE-729 Acid for 1 year to the dog at a dose level of 10000 ppm (males) or 2500 ppm and above (females) caused adverse effects in the kidney (tubular degeneration and regeneration, degeneration of collecting ducts, fibrosis or focal granuloma in cortex, glomerulosclerosis, all graded very slight). At 10000 ppm in females, these effects were accompanied by bodyweight loss. Study NOAELs of 2500 ppm in males (intake of about 82 mg/kg/day) and 500 ppm in females (intake of about 16.7 mg/kg/day) were identified.

A toxicokinetic investigation showed that systemic bioavailability of XDE-729 Acid is approximately dose-proportional at all dose levels in females and at the low (500 ppm) and mid (2500 ppm) dose levels in males. Respectively, males and females excreted on average 40% and 55% of the dose as intact XDE-729 Acid in the urine.

[redacted] (2012)

B.6.3.4 Short-term dermal toxicity in the rat (IIA 5.3.7)

XDE-729 Acid	
Study	IIA 5.3.7/01 28-day dermal toxicity study in F344/ DuCrl rats
Reference	[redacted] (2010)
Date performed	April – May 2010
Test facility	[redacted]
Report reference	Laboratory study ID 101031
Guideline(s)	OECD 410
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 8 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.3.4–1: Study design

Test group	Dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	10	10
2	100	10	10
4	300	10	10
5	1000	10	10

The test substance was administered as an aqueous suspension in 0.5% methylcellulose, at a dose volume of 4 ml/kg bodyweight. The stability and homogeneity of the test substance in the vehicle was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of formulations used on the study were within 7% of the nominal, demonstrating satisfactory preparation of the dosing formulations.

Exposure was for 6 hours/day, 7 days/week, for 28 days. The dosing suspensions were applied to a clipped area on the back that was not less than 10% of the total body surface. The exposure site was semi-occluded with gauze dressing and non-absorbent cotton. The animal was then wrapped in an elastic bandage to hold the test material, gauze dressing and cotton in place. The dressings were removed at the end of each 6 hour exposure period and any residual test substance was wiped or washed from the site.

General clinical observations were recorded at least once daily and a more detailed clinical examination, which included an evaluation of the dosing site for irritation, was conducted weekly. Bodyweights and food consumption were measured at least weekly. Ophthalmoscopy examinations were conducted before dosing commenced and at the end of the study.

At the end of the study, and after an overnight fast, blood samples were taken from the retroorbital venous plexus and a standard range of haematology and clinical chemistry parameters were measured. Additionally, towards the end of the study, overnight urine samples were collected and a standard range of urinalyses parameters were assessed.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. Organs from only the control and high dose animals were subjected to microscopic (light) examination.

RESULTS

There were no treatment related deaths. No signs of local effects or clinical signs indicative of systemic toxicity were observed.

Bodyweights and food consumption were not affected by treatment.

There were no treatment related ophthalmoscopy, haematology or clinical chemistry findings.

There were no organ weight differences or macroscopic and microscopic pathology observations that could be attributed to treatment.

CONCLUSION

Dermal administration of XDE-729 Acid to the rat at dose levels of up to 1000 mg/kg/day, 6 hours/day for 4 weeks, did not cause any local effects or systemic toxicity. Therefore, a study NOAEL of 1000 mg/kg/day is identified.

[redacted] (2010)

B.6.3.5 Short-term inhalation toxicity in the rat (IIA 5.3.3)

XDE-729 has a vapour pressure of 5.9×10^{-9} Pa (4.4×10^{-11} mmHg) at 25 °C (Annex IIA 2.3.1. As the vapour pressure is less than 10^{-2} Pa, assessment of short-term (28-day or 90-day) inhalation toxicity is not required.

B.6.3.6 Summary of short-term toxicity studies

Table B.6.3.6 Summary of short-term toxicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
Rat, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000/500 mg/kg/day target	M: 270 mg/kg/day	M: 734 mg/kg/day	M: ↓ bodyweight gain & food consumption; mild regenerative anemia; kidney: tubular degenerative changes, hypertrophy & vacuolation of collecting duct epithelium; spleen: extramedullary haematopoiesis; urine: ↑ volume & ↓ specific gravity	[redacted] 2009 (081115)
	F: 250 mg/kg/day	F: 982 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: tubular degenerative changes, hypertrophy & vacuolation of collecting duct epithelium; urine: ↑ volume & ↓ specific gravity	
Rat, 28 day oral dietary XDE-729 Methyl 0-10-52-261-782 mg/kg/day target	M&F: 10.7 mg/kg/day	M&F: 55.5 mg/kg/day	M: liver: ↑ weight, hepatocyte hypertrophy, hepatocytes in mitosis, slight hepatocellular vacuolisation; thyroids: diffuse hypertrophy of follicular cells	[redacted] 2011 111005
			F: liver: ↑ weight	
Rat, 90 day oral dietary XDE-729 Acid 0-10-50-250-750 mg/kg/day target	M: 262 mg/kg/day	M: 782 mg/kg/day	M: ↓ bodyweight gain; kidney: ↑ weight, hypertrophy, vacuolation, necrosis of collecting duct epithelium, tubular dilatation; urine: ↑ volume & ↓ specific gravity	[redacted] 2010 (091016)
	F: 252 mg/kg/day	F: 758 mg/kg/day	F: kidney: ↑ weight, hypertrophy, vacuolation, necrosis of collecting duct epithelium, tubular dilatation; urine: ↑ volume & ↓ specific gravity	
Rat, 90 day oral dietary XDE-729 Methyl 0-3-10-52-261-500 mg/kg/day target	M: 10.3 mg/kg/day	M: 53.5 mg/kg/day	M: liver hepatocellular vacuolisation, consistent with fatty change	[redacted] 2012 (111082)
	F: 10.1 mg/kg/day	F: 52.3 mg/kg/day	F: ↑ serum cholesterol concentration; liver: ↑ weight	
Mouse, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000 mg/kg/day target	M: 269 mg/kg/day	M: 1025 mg/kg/day	M: slight ↓ RBC	[redacted] 2009 (081116)
	F: 958 mg/kg/day	F: -	F: no adverse effects observed	
Mouse, 90 day oral dietary XDE-729 Acid 0-50-250-500-1000 mg/kg/day target	M: 495 mg/kg/day	M: 989 mg/kg/day	M: urinary bladder: slight inflammation of mucosa or wall, submucosal oedema	[redacted] 2010 (091056)
	F: 1008 mg/kg/day	F: -	F: no adverse effects observed	
Dog, 28 day oral dietary XDE-729 Acid 0-300-3000-15000(F)-30000(M) ppm	M: 300 ppm 11 mg/kg/day	M: 3000 ppm 80 mg/kg/day	M: kidney: slight tubular degeneration & regeneration	[redacted] 2010 (081127)
	F: 3000 ppm 85 mg/kg/day	F: 15000 ppm 323 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: slight tubular degeneration & regeneration; thymus: lymphoid atrophy	
Dog, 90 day oral dietary XDE-729 Acid 0-500-2500-12500 ppm	M&F: 2500 ppm 80 mg/kg/day	M: 12500 ppm 424 mg/kg/day	M: ↓ bodyweight gain & food consumption; kidney: slight tubular degeneration & regeneration, degeneration of collecting ducts, focal granuloma in cortex/medulla; liver: ↑ weight; thymus: lymphoid atrophy; bone marrow: hyperplasia	[redacted] 2011 (091070)
		F: 12500 ppm 415 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: slight tubular degeneration & regeneration, degeneration of collecting ducts, focal granuloma in cortex/medulla; liver: ↑ weight, extramedullary haematopoiesis; thymus: lymphoid atrophy; bone marrow: hyperplasia; spleen:	

			extramedullary haematopoiesis	
Dog, 1 year day oral dietary XDE-729 Acid 0-500-2500-10000/5000 ppm	M: 2500 ppm 82 mg/kg/day	M: 10000 ppm 355 mg/kg/day	M: kidney: very slight tubular degeneration & regeneration, degeneration of collecting ducts, fibrosis or focal granuloma in cortex, glomerulosclerosis	[redacted], 2012 (101163)
	F: 500 ppm 16.7mg/kg/day	F: 2500 ppm 90mg/kg/day	F: kidney: very slight tubular degeneration & regeneration, degeneration of collecting ducts, glomerulosclerosis	
Rat, 28 day dermal XDE-729 Acid 0-100-300-1000 mg/kg/day	M&F: 1000 mg/kg/day	M&F: -	M&F: no systemic of local effects observed	[redacted] 2010 (101031)
Rat, inhalation	Studies not required			

The short-term toxicity of XDE-729 Acid has been investigated in 28 and 90 day oral feeding studies in rats, mice and dogs, and in a 1-year feeding study in dogs. Additionally, the toxicity of XDE-729 Methyl has been investigated in 28 and 90 day oral feeding studies in rats. Also, a 28 day repeated dose dermal exposure study for XDE-729 Acid has been conducted. Inhalation studies have not been conducted because XDE-729 Acid and XDE-729 Methyl are non-volatile substances and inhalation is not expected to be a significant route of exposure to humans.

XDE-729 Acid

The oral dietary studies demonstrate that the main target organ for XDE-729 Acid is the kidney in rats and dogs. The primary changes in the rat kidney are renal tubular degeneration together with hypertrophy and vacuolation of the collecting duct epithelium. In the dog, degenerative and regenerative changes in the tubules and slight degeneration of collecting ducts are induced at 28 and 90 days and, additionally, slight fibrosis or focal granuloma in cortex and slight glomerulosclerosis are induced at 1 year. Other findings in the dog include decreased red blood cell (RBC) mass and subsequent extramedulary hematopoiesis in the spleen and liver. In mice the only noteworthy findings are a slight inflammation of the urinary bladder mucosa in the 90 day study and a reduction in red blood cell count in the 28 day study. The most sensitive NOAELs for short-term toxicity are 252 mg/kg/day in rats, 11 mg/kg/day in dogs (this is from the 28 day study, which identified a lower NOAEL than did the 90 day and 1 year dog studies) and 269 mg/kg/day in mice

By the dermal route, XDE-729 Acid does not cause local effects or systemic toxicity in rats.

XDE-729 Methyl

Standard short-term repeated dose toxicity studies are available in rats only. In contrast to the findings for the acid, oral dietary studies identify the liver as the main target for XDE-729 Methyl in rats. The primary changes in the liver are hepatocyte hypertrophy, mitotic figures and vacuolisation consistent with fatty change, observed at exposure levels of 52 mg/kg/day and above. Other targets, affected only at higher exposure levels, are kidneys, thyroids, thymus and blood. The kidney changes are slight multifocal hypertrophy of collecting duct epithelial cells; for the thyroid, organ weight is increased, accompanied by a diffuse hypertrophy of follicular cells; in the thymus, lymphoid tissue atrophy are induces; in blood, haemoglobin concentration, haematocrit and red cell count are reduced. For XDE-729 Methyl, a short-term NOAEL of 10 mg/kg/day is identified in the rat, based on the presence liver toxicity at 52 mg/kg/day. However, in a XDE-729 Methyl rabbit developmental toxicity study (Ellis-Hutchings et al 2012, IIA

5.6.11/04; see Section 6.6) a lower oral dietary NOAEL for maternal toxicity of 5.78 mg/kg/day is identified, based on the observation of increased liver weight and slight hypertrophy and altered tinctorial properties of periportal hepatocytes at 18.5 mg/kg/day.

Toxicokinetic investigations, conducted as part of the short-term repeat dose studies, show that following XDE-729 Methyl administration post-hepatic exposure to the parent is negligible. In blood, the administered dose is present mainly as the XDE-729 Acid metabolite and, to a lesser extent, to demethylated and conjugated XDE-729 Methyl and Acid metabolites. Most of the administered dose of XDE-729 Methyl is excreted as the Acid metabolite or as the demethylated and conjugated metabolites. Hepatic gene expression investigations in the 90-day XDE-729 Methyl study demonstrate increased *Cyp1a1* and *Ugt1a6* expression, notably at dose levels of 52 mg/kg/day and above, which correlates with the observed liver and thyroid changes, and provides evidence of activation of the AhR signalling pathway.

Classification

No hazard classification for repeated exposure toxicity is proposed because XDE-729 Acid and XDE-729 Methyl do not cause severe toxicity at the classification dose level triggers specified in Directive 67/548/EEC and Regulation (EC) 1272/2008.

B.6.4 Genotoxicity studies (IIA 5.4)

The genotoxicity of XDE-729 Acid, XDE-729 Methyl and a technical batch of XDE-729 Methyl have been investigated in standard *in vitro* bacterial and mammalian cell gene mutation assays and in chromosome aberration tests. Also, an *in vivo* micronucleus test has been conduced with XDE-729 Acid.

B.6.4.1 Genotoxicity *in vitro* (IIA 5.4.1)

XDE-729 Acid	
Study	IIA 5.4.1/01 *Salmonella – Escherichia coli* mammalian-microsome reverse mutation assay, pre-incubation method
Reference	Dakoulas EW, Vandyke MR (2010)
Date performed	December 2009 – January 2010
Test facility	BioReliance, Rockville, Maryland, USA
Report reference	Laboratory study 091134
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

XDE-729 Acid was evaluated in a bacterial mutagenicity assay using four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA 100) and *Escherichia coli* tester strain WP2 *uvr*A, in the presence and absence of a Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

The pre-incubation method was used. An initial assay was conducted, which established the dose ranges, followed by a confirmatory assay. XDE-729 Acid concentrations ranging from 1.5 µg/plate to the test limit concentration of 5000 µg/plate were used in the initial assay. Concentrations of 50 to 5000 µg/plate were used for the confirmatory assay. The study design included the testing of appropriate positive and solvent (DMSO) controls. For all test substance, solvent control and positive control treatments, duplicate plates were used in the initial assay and triplicate plates were used in the confirmatory assay.

RESULTS

There were no increases in the number of revertants in the XDE-729 Acid plates. The expected responses were observed in the positive control plates.

No evidence of toxicity to the bacteria, or precipitation of the test substance, was reported.

CONCLUSION

XDE-729 Acid is non-mutagenic in this *Salmonella typhimurium* - *Escherichia coli* reverse mutation assay.

Dakoulas EW, Vandyke MR (2010)

XDE-729 Methyl	
Study	IIA 5.4.1/02 *Salmonella – Escherichia coli* mammalian-microsome reverse mutation assay, pre-incubation method
Reference	Dakoulas EW, Vandyke MR (2011)
Date performed	February – March 2011– January 2010
Test facility	BioReliance, Rockville, Maryland, USA
Report reference	Laboratory study 111006
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl was evaluated in a bacterial mutagenicity assay using four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA 100) and *Escherichia coli* tester strain WP2 *uvr*A, in the presence and absence of a Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

The pre-incubation method was used. An initial assay was conducted, which established the dose ranges, followed by a confirmatory assay. XDE-729 Methyl concentrations ranging from 1.5 µg/plate to the test limit concentration of 5000 µg/plate were used in the initial assay. Concentrations of 50 to 5000 µg/plate were used for the confirmatory assay. The study design included the testing of appropriate positive and solvent (DMSO) controls. For all test substance, solvent control and positive control treatments, duplicate plates were used in the initial assay and triplicate plates were used in the confirmatory assay.

RESULTS

There were no increases in the number of revertants in the XDE-729 Methyl plates. The expected responses were observed in the positive control plates and tubes.

No evidence of toxicity to the bacteria was reported. Precipitation of the test substance occurred at concentrations of 150 or 500 µg/plate and above.

CONCLUSION

XDE-729 Methyl is non-mutagenic in this *Salmonella typhimurium - Escherichia coli* reverse mutation assay.

Dakoulas EW, Vandyke MR (2011)

XDE-729 Acid TGAI	
Study	IIA 5.4.1/03 Bacterial Reverse Mutation Test using *Salmonella typhimurium*
Reference	Nagane RM (2012)
Date performed	May – June 2012
Test facility	Jai Research Foundation, Gujarat, India
Report reference	Laboratory study 481-1-06-4562, Dow study ID 120589
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl TGAI, Lot 201101758-63B, TSN302167, 93.3% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl TGAI was evaluated in a bacterial mutagenicity assay using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA 100, TA 102), in the presence and absence of a Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

The plate incorporation method was used. An initial cytotoxicity assay was conducted, which established that no toxicity was present at the limit dose of 5000 µg/plate. A dose range of 156.25 to 5000 µg/plate was used in a first assay, and a range of 51.2 to 5000 µg/plate was used in a confirmatory assay. The study design included the testing of appropriate positive and solvent (DMSO) controls. For all test substance, solvent control and positive control treatments, triplicate plates were used.

RESULTS

There were no increases in the number of revertants in the XDE-729 Methyl TGAI plates. The expected responses were observed in the positive control plates.

There was no evidence of toxicity to the bacteria in the main tests. Precipitation of the test substance was reported at concentrations of 2500 and 5000 µg/plate in a preliminary solubility trial, but precipitation was not reported in the main assays.

CONCLUSION

XDE-729 Methyl TGAI is non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

Nagane RM (2012)

XDE-729 Acid	
Study	IIA 5.4.2/01 Chromosome aberration assay utilising rat lymphocytes
Reference	[redacted] (2010)
Date performed	December 2009 – January 2010
Test facility	[redacted]
Report reference	Laboratory study 091116
Guideline(s)	OECD 473
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

XDE-729 Acid was evaluated in an *in vitro* chromosomal aberration assay using rat lymphocytes, in the presence and absence of Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

Approximately 48 hours after the initiation of whole blood cultures, cells were treated for 4 hours in the absence of S9 at XDE-729 Acid concentrations ranging from 0 to 3400 µg/ml or in presence of S9 activation with concentrations ranging from 0 to 3000 µg/ml. Cultures were also treated for 24 hours in the absence of S9 at XDE-729 Acid concentrations ranging from 0 to 1500 µg/ml.

Based on the presence of marked mitotic index reductions at higher concentrations, cultures treated for 4 hours with concentrations of 0, 800, 1500, and 2000 µg/ml in the absence of S9 activation and concentrations of 0, 400, 800, and 1500 µg/ml in the presence of S9 activation were selected for determining the incidence of chromosomal aberrations. Cultures treated for 24 hours with 0, 100, 250, and 300 µg/ml in the absence of S9 were selected for determining the incidence of chromosomal aberrations.

Solvent (DMSO) and positive controls (mitomycin C and cyclophosphamide) were included in each experiment.

Coded slides were evaluated using a light microscope. Chromosomes of approximately 200 metaphases per concentration, i.e. 100 metaphases from each of two parallel cultures, were examined.

RESULTS

The chromosome assessment did not reveal any relevant or statistically significant increases in numbers of metaphases with aberrations at any time points in the XDE-729 Acid treated cultures, either in the presence or absence of S9.

The sensitivity of the system was demonstrated by the significant increases in metaphases with aberrations in the positive control cultures, both with and without S9 mix.

CONCLUSION

XDE-729 Acid is not clastogenic in rat lymphocytes treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2010)

XDE-729 Methyl	
Study	IIA 5.4.2/02 Chromosome aberration assay utilising rat lymphocytes
Reference	[redacted] (2012)
Date performed	April 2011
Test facility	[redacted]
Report reference	Laboratory study 101203
Guideline(s)	OECD 473
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl was evaluated in an *in vitro* chromosomal aberration assay using rat lymphocytes, in the presence and absence of Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

Approximately 48 hours after the initiation of whole blood cultures, cells were treated for 4 hours in the absence of S9 or in presence of S9 activation with at XDE-729 Methyl concentrations from 0 (solvent control) to 50 µg/ml. Cultures were also treated for 24 hours in the absence of S9 at XDE-729 Methyl concentrations ranging from 0 (solvent control) to 50 µg/ml; a confirmatory 24 h treatment assay was conducted because of an unusual positive control response in the original assay.

Cultures were treated for 4 hours with targeted concentrations of 0 (solvent control), 12.5, 25, and 50 µg/ml in the absence (4 and 24 hour treatment) and presence of S9 (4 hour treatment) were selected for determining the incidence of chromosomal aberrations. The choice of highest concentration was based on the observation of a slight reduction in mitotic index and on the limit of solubility of the test substance in the treatment medium.

Solvent (DMSO) and positive controls (mitomycin C and cyclophosphamide) were included in each experiment.

Coded slides were evaluated using a light microscope. Chromosomes of approximately 200 metaphases per concentration, i.e. 100 metaphases from each of two parallel cultures, were examined.

RESULTS

The chromosome assessment did not reveal any relevant or statistically significant increases in numbers of metaphases with aberrations at any time points in the XDE-729 Methyl treated cultures, either in the presence or absence of S9.

The sensitivity of the system was demonstrated by the significant increases in metaphases with aberrations in the positive control cultures with one exception, both with and without S9 mix. The exception was the lack of a significant increase in aberrations in cells treated with 0.075 µg/ml mitomycin C in the 24 h assay; however, a significant increase was seen in a concurrent positive control group exposed to a slightly higher concentration of mitomycin C (0.05 µg/ml).

Mitotic indices were slightly reduced at the highest concentration tested, which was 50 µg/ml. Relative mitotic indices of 90.4, 75.7 and 88.6 were reported at this concentration for the 4 h treatment in absence of S9, 4 h treatment in presence of S9 and 24 hour treatment, respectively. However, mitotic indices were not reduced in a confirmatory 24 hour treatment assay. Precipitation of the test substance in the treatment medium was present in all cultures at 25 µg/ml and 50 µg/ml (described as 'slight' at 25 µg/ml).

CONCLUSION

XDE-729 Methyl is not clastogenic in rat lymphocytes treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2012)

XDE-729 Methyl TGAI	
Study	IIA 5.4.2/05 *In vitro* chromosome aberration test in human peripheral blood lymphocytes
Reference	[redacted] (2012)
Date performed	May – June 2012
Test facility	[redacted]
Report reference	Laboratory study 481-1-06-4564, Dow study ID 120590
Guideline(s)	OECD 473
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl TGAI, Lot 201101758-63B, TSN302167, 93.3% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl TGAI was evaluated in an *in vitro* chromosomal aberration assay using human peripheral blood lymphocytes, in the presence and absence of Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

Approximately 48 hours after the initiation of whole blood cultures, cells were treated in the presence or absence of S9 with XDE-729 Methyl TGAI at concentrations ranging from 15.6 to 500 µg/ml. The concentration range was selected on the basis of a preliminary solubility and cytotoxicity studies which showed that XDE-729 Methyl TGAI did not cause cytotoxicity and that 500 µg/ml was the highest feasible concentration taking account of the solubility of the test substance in the DMSO vehicle. In phase I of the study, cultures were treated for 3.5 h in the presence or absence of S9. In phase II, cultures were treated for 24 h in the absence of metabolic activation. In phase III, cultures were treated for 3.5 h in the presence of metabolic activation.

Solvent (DMSO) and positive controls (mitomycin C and cyclophosphamide) were included in the study.

In all phases, concentrations of 125, 250 and 500 µg/ml were selected for evaluation, by light microscopy. Chromosomes of approximately 200 metaphases per concentration, i.e. 100 metaphases from each of two parallel cultures, were scored for chromosome aberrations.

RESULTS

The chromosome assessment did not reveal any relevant or statistically significant increases in numbers of metaphases with aberrations at any time points in the XDE-729 Methyl TGAI treated cultures, either in the presence or absence of S9.

Precipitation of the test substance in the culture medium was observed at concentrations of 250 and 500 µg/ml, and turbid appearance of the solution was observed at 62.5 and 125 µg/ml, both in the absence and presence of metabolic activation. Significant cytotoxicity was observed only in cultures treated for 24 h in absence of metabolic activation at a concentration of 500 µg/ml

The sensitivity of the system was demonstrated by the significant increases in metaphases with aberrations in the positive control cultures, both with and without S9 mix.

CONCLUSION

XDE-729 Methyl TGAI is not clastogenic in peripheral blood lymphocytes treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2012)

XDE-729 Acid	
Study	IIA 5.4.3/01 *In vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay.
Reference	[redacted] (2010)
Date performed	February 2010
Test facility	[redacted]
Report reference	Laboratory study 091118
Guideline(s)	OECD 476
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

XDE-729 Acid was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HGPRT locus in Chinese hamster ovary cells.

Two independent sets of experiments were conducted in the presence and absence of a rat liver (Aroclor 1254 induced) derived metabolic activation system (S9-mix). Concentrations of 187.5 to 3400 µg/mL were tested, using a 4 hour XDE-729 Acid exposure period. The highest concentration was based on the 10 mM limit in the assay system and exceeded the solubility of the test material in the treatment medium.

For each treatment, duplicate cultures were set up. The study design included the testing of appropriate positive controls (ethyl methanesulfonate, EMS, without S9; 20-methylcholanthrene (20-MCA, with S9) and solvent controls (DMSO). After the exposure period, treatment media were replaced by culture medium and the cells were incubated for 8 days for expression of mutant cells. This was followed by a 9 day incubation of cells in selection medium containing 6-thioguanine.

RESULTS

There were no increases in the numbers of mutant colonies in the XDE-729 Acid treated cultures. The expected responses were observed in the positive control cultures.

Toxicity, seen as a marked reduction in relative cell survival, occurred at the highest concentration tested, 3400 µg/mL. Precipitation of the test substance was observed at concentrations of ≥1500 µg/mL

CONCLUSION

XDE-729 Acid is not mutagenic in Chinese hamster ovary cells treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2010)

XDE-729 Methyl	
Study	IIA 5.4.3/02 *In vitro* Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward gene mutation assay.
Reference	[redacted] (2011)
Date performed	May 2011
Test facility	[redacted]
Report reference	Laboratory study 101204
Guideline(s)	OECD 476
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HGPRT locus in Chinese hamster ovary cells.

Two independent sets of experiments were conducted in the presence and absence of a rat liver (Aroclor 1254 induced) derived metabolic activation system (S9-mix). Concentrations of 3.13 to 50 µg/mL were tested, using a 4 hour XDE-729 Methyl exposure period. The selection of the highest concentrations was based on the limit of solubility of the test substance in the treatment medium.

For each treatment, duplicate cultures were set up. The study design included the testing of appropriate positive controls (ethyl methanesulfonate, EMS, without S9; 20-methylcholanthrene (20-MCA, with S9) and solvent controls (DMSO). After the exposure period, treatment media were replaced by culture medium and the cells were incubated for 8 days for expression of mutant cells. This was followed by a 9 day incubation of cells in selection medium containing 6-thioguanine.

RESULTS

There were no increases in the numbers of mutant colonies in the XDE-729 Methyl treated cultures. The expected responses were observed in the positive control cultures.

Precipitation of the test substance in the culture medium was observed at concentrations of 25 µg/mL and above.

CONCLUSION

XDE-729 Methyl is not mutagenic in Chinese hamster ovary cells treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2011)

XDE-729 Methyl TGAI	
Study	IIA 5.4.3/03 *In vitro* mammalian cell gene forward mutation test at the HGPRT locus of the Chinese hamster ovary cell (CHO)-K1 cell line
Reference	[redacted] (2012)
Date performed	May – July 2012
Test facility	[redacted]
Report reference	Laboratory study 482-1-06-4563, Dow study ID 120591
Guideline(s)	OECD 476
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl TGAI, Lot 201101758-63B, TSN302167, 93.3% purity
Study acceptable	Yes

METHODS

XDE-729 Methyl TGAI was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HGPRT locus in Chinese hamster ovary cells.

Two independent sets of experiments were conducted in the presence and absence of a rat liver (Aroclor 1254 induced) derived metabolic activation system (S9-mix), using a 4 h XDE-729 Methyl TGAI exposure period. In the first experiment, concentrations of 15.6 – 500 µg/mL were tested and in the second, concentrations of 18.75 – 500 µg/mL were tested. The highest concentration was selected as the highest one that could feasibly be tested based on the solubility of the test substance in the DMSO solvent, as determined in a preliminary experiment.

For each treatment, duplicate cultures were set up. The study design included the testing of appropriate positive controls (ethyl methanesulfonate, EMS, without S9; benzo(a)pyrene with S9) and solvent controls (DMSO). After the exposure period, treatment media were replaced by culture medium and the cells were incubated for 8 days for expression of mutant cells. This was followed by an 8 day incubation of cells in selection medium containing 6-thioguanine.

RESULTS

There were no increases in the numbers of mutant colonies in the XDE-729 Methyl TGAI treated cultures. The expected responses were observed in the positive control cultures.

Evidence of slight toxicity, seen as a marginal reduction in relative cloning efficiency, was present in the XDE-729 Methyl TGAI cultures in both main experiments. Whether or not precipitation of the test substance occurred in the main experiments was not reported.

CONCLUSION

XDE-729 Methyl TGAI is not mutagenic in Chinese hamster ovary cells treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2012)

B.6.4.2 Genotoxicity *in vivo* (IIA 5.4.2)

XDE-729 Acid	
Study	IIA 5.4.4/01 *In vivo* peripheral blood micronucleus assay in the mouse
Reference	[redacted] (2010)
Date performed	December 2009
Test facility	[redacted]
Report reference	Laboratory study 091117
Guideline(s)	OECD 474
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

XDE-729 Acid was evaluated for its ability to induce micronucleated reticulocytes in the peripheral blood in groups of 6 male Crl:CD1(ICR) mice, aged about 8 weeks. Two gavage doses of the test substance were administered on consecutive days at levels of 0 (0.5% methocel™ vehicle control), 500, 1000 and 2000 mg/kg/day. The dose levels were chosen on the basis of the results micronucleus test using a different batch of XDE-729 Acid. The highest dose is the limit dose for this type of assay. A positive control group received a single gavage does of 40 mg/kg cyclophosphamide.

The concentration of the test substance in the vehicle was analytically verified.

Clinical signs, bodyweight and body temperature were monitored during the study. The animals were killed 48 hours after the second dose for collection of peripheral blood and the evaluation of micronucleated reticulocytes by flow cytometry. About 5000 reticulocytes per animal were assessed.

RESULTS

There no indications of general toxicity in the 500 or 1000 mg/kg/day groups. At 2000 mg/kg/day, general toxicity was elicited in one male. This individual had slow and shallow respiration, decreased/absent activity, partially closed eye lids, fixed posture, and was cold to touch with a body temperature of 25.9°C five hours post-dosing on the second day of dosing. This animal was found dead at the end of the second day. There were no abnormal macroscopic necropsy findings.

There were no increases in the proportion of micronucleated reticulocytes in the XDE-729 Acid treated groups, as shown in Table B.6.4.2-1. The proportion of micronucleated reticulocytes was significantly increased in the positive control group, demonstrating the sensitivity of the test system.

Table B.6.4.2-1 Numbers of micronucleated reticulocytes (MN) and % reticulocytes (± SD)

Treatment group (mg/kg/day)	No. animals assessed	Number of MN/1000 reticulocytes	% reticulocytes
Vehicle control	6	1.43 ± 0.70	1.85 ± 0.24
XDE-729 Acid 500	6	1.56 ± 0.37	1.70 ± 0.15
1000	5[a]	1.16 ± 0.33	1.92 ± 0.74
2000	6	1.07 ± 0.41	1.50 ± 0.51
Positive control Cyclophosphamide 400	6	13.3 ± 5.0*	0.32 ± 0.10*

*significantly different from control, p≤0.05 [a] one animal died due to gavage dosing error

CONCLUSION

XDE-729 Acid is not clastogenic in this *in vivo* peripheral blood micronucleus assay.

[redacted] (2010)

B.6.4.3 Summary of genotoxicity studies

Table B.6.4.3-1 Summary of genotoxicity studies

Study	Test system	Result	Reference (study ID)
In vitro			
Bacterial mutation: XDE-729 Acid	*S. typhimurium*, TA 98, TA 100, TA 1535 & TA 1537; *E. coli* WP2*uvr*A	Negative	Dakoulas & VanDyke, 2010 (091134)
Bacterial mutation: XDE-729 Methyl	*S. typhimurium*, TA 98, TA 100, TA 1535 & TA 1537; *E. coli* WP2*uvr*A	Negative	Dakoulas & VanDyke, 2011 (111006)
Bacterial mutation: XDE-729 Methyl TGAI	*S. typhimurium*, TA 98, TA 100, TA 102, TA 1535 & TA 1537	Negative	[redacted], 2012 (120589)
Chromosome aberration: XDE-729 Acid	Rat blood lymphocytes	Negative	[redacted] 2010 (091116)
Chromosome aberration: XDE-729 Methyl	Rat blood lymphocytes	Negative	[redacted], 2012 (101203)
Chromosome aberration: XDE-729 Methyl TGAI	Human peripheral blood lymphocytes	Negative	[redacted] , 2012 (101590)
Mammalian cell mutation: XDE-729 Acid	Chinese hamster ovary cells (CHO/HGPRT)	Negative	[redacted], 2010 (091118)
Mammalian cell mutation: XDE-729 Methyl	Chinese hamster ovary cells (CHO/HGPRT)	Negative	[redacted], 2011 (101204)
Mammalian cell mutation: XDE-729 Methyl TGAI	Chinese hamster ovary cells (CHO/HGPRT)	Negative	[redacted] 2012 (120591)
In vivo			
Micronucleus: XDE-729 Acid	Mouse peripheral reticulocytes	Negative	[redacted], 2010 (091117)

The genotoxicity of XDE-729 Acid has been adequately investigated in the standard tests. This substance tests negative in *in vitro* assays for gene mutation and clastogenicity and in an *in vivo* micronucleus test. It is therefore concluded that XDE-729 Acid is not genotoxic.

XDE-729 Methyl also tests negative in standard *in vitro* assays for gene mutation and clastogenicity, and it can therefore be concluded that XDE-729 Methyl is not genotoxic. A

technical batch of XDE-729 Methyl (Lot 201101758-63B, TSN302167, 93.3% purity) is also negative in standard *in vitro* assays for gene mutation and clastogenicity. An *in vivo* genotoxicity assay on XDE-729 Methyl is not necessary because, firstly, the negative *in vitro* genotoxicity assays indicate that the ester form is unlikely to express genotoxic activity *in vivo* and, secondly, systemic exposure in an *in vivo* assay of the ester form will be mainly to XDE-729 Acid (see section B.6.1.3) which has been testing in an *in vivo* assay.

B.6.5 Chronic toxicity and carcinogenicity studies (IIA 5.5)

A chronic/carcinogenicity study in rats and a carcinogenicity study in mice have been conducted with XDE-729 Acid. Additionally, a series of liver mode of action (MoA) investigations have been conducted, with the aim of providing support for bridging from toxicity studies conducted with XDE-729 Acid.

B.6.5.1 Chronic toxicity and carcinogenicity in the rat

XDE-729 Acid	
Study	IIA 5.5.1/01 Two-year chronic toxicity/oncogenicity study in F344/DuCrL rats
Reference	[redacted] (2012)
Date performed	January 2010 – January 2012
Test facility	[redacted]
Report reference	Laboratory study ID 091121
Guideline(s)	OECD 453 (2009)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot # E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

F334/DuCrl rats, about 6 weeks old at the start of the study, were randomly assigned to the test groups as shown in the table below.

Table B.6.5.1–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals			
		Carcinogenicity study (terminated at 24 months)		Chronic toxicity study (terminated at 12 months)	
		Males	Females	Males	Females
1	0	50	50	10	10
2	20	50	50	10	10
3	100	50	50	10	10
4	400	50	50	10	10
5	625	50	-	10	-
	750	-	50[a]	-	10

[a]females receiving 750 mg/kg/day were terminated prematurely at 18 months because of excessive mortality (68%)

The test substance was administered by the dietary route. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved

concentrations of XDE-729 Acid in analysed samples of test diet used on the study were all within 4.4% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

A cage-side examination of all animals was conducted at least once daily. General clinical observations, including an examination for unusual swellings and palpable masses, were conducted every two weeks. More detailed clinical examination was conducted monthly on the first 10 surviving animals/sex/dose. Bodyweights and food consumption were measured weekly for the first 13 weeks and monthly thereafter. Ophthalmoscopy examinations were conducted before dosing commenced and just prior scheduled terminations at 12 and 24 months.

At 3, 6, 12, 18 and 24 months, and after an overnight fast, blood samples were taken from the retroorbital venous plexus of 10 animals/sex/dose and a standard range of haematology and clinical chemistry parameters were measured. In addition, at 24 months, an automated white blood cell (WBC) count and differential WBC count was performed on all animals remaining on study. Overnight urine samples were collected from all animals at 3, 6 and 12 months and from 10 animals/sex/dose and a standard range of urinalyses parameters were assessed.

Toxicokinetic investigations were conducted at part of this study. On one day at months 6 and 12 blood samples were taken from the jugular vein of 4 non-fasted animals/sex/dose from each group at 06.00, 09.00 and 17.00 hours, for determination of plasma XDE-729 Acid levels. Additional blood samples were taken at the 12 month termination from 4/animals/sex/dose, after fasting, for determination of plasma XDE-729 Acid levels. Overnight urine samples were collected at 6 and 12 months from 4 non-fasted animals/sex/dose for determination of XDE-729 Acid levels.

A necropsy was conducted all animals. The weights of major organs were recorded and macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. All tissues from the following groups were subject to microscopic examination: control males and females killed at 12 and 24 months; high dose males (625 mg/kg/day) and females (750 mg/kg/day) killed at 12 months; high dose males (625 mg/kg/day) and females (400 mg/kg/day) killed at 24 months; all animals dying/killed prematurely. Microscopic examination of tissues from the low and intermediate dose 12 month groups was limited to the kidneys and relevant gross lesions. Microscopic examination tissues from survivors from the low and intermediate dose 24 month groups was limited to the kidneys and relevant gross lesions for both sexes, and the urinary bladder, adrenal glands, and mesenteric tissues for males.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.5.1–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	20	100	400	625	750
Males- chronic study	20.4	102	409	640	-
Males- carcinogenicity study	20.2	101	404	633	-
Females- chronic study	20.4	102	408	-	548
Females- carcinogenicity study	20.3	102	407	-	766

Mortality for the carcinogenicity study is presented in Table B.6.5.1-3. Mortality was increased for both the males and females receiving the highest dose level, which was probably related to the high incidence of treatment-related renal disease, based on macroscopic and microscopic pathology findings (see Tables B.6.5.1–10 and B.6.5.1–12). The increased mortality was also associated with a marked reduction in bodyweight gain (see below). There were no premature deaths among animals assigned to the chronic study.

There were no treatment-related clinical signs of toxicity.

Table B.6.5.1–3 Mortality at 24 months, expressed as %

Target dose level of XDE-729 Acid (mg/kg/day)									
Males					Females				
0	**20**	**100**	**400**	**625**	**0**	**20**	**100**	**400**	**750**
32	44	44	34	62	16	10	28	30	68[a]

[a]mortality at 18 months is presented, at which time this group was terminated

Group mean bodyweights for the chronic and carcinogenicity studies combined are summarised in Table B.6.5.1-4. XDE-729 Acid treatment at 400 and 625 mg/kg/day caused a reduction in bodyweight gain in males; at termination mean bodyweight gain for these two groups was 4% and 10% lower than controls, respectively. Female bodyweights at the highest dose level (750 mg/kg/day) only were also reduced, from about 11 months; when this group was terminated at 18 months, bodyweight gain was 12% lower than controls. Food consumption was not affected by XDE-729 Acid treatment.

Table B.6.5.1–4 Group mean bodyweights, selected values (g)

Day	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	**20**	**100**	**400**	**625**	**0**	**20**	**100**	**400**	**750**
1	132	131	131	131	131	84	84	83	83	83
50	269	268	266	268	261*	159	159	161	159	159
176	378	375	377	369*	356*	190	190	193	190	190
344	438	436	433	423*	410*	217	215	220	215	211*
512	471	475	465	452*	432*	264	260	268	258	232*
729	437	439	434	424	402*	297	300	298	290	-
Gain d 1-50[a]	137	136	134	136	130	74	74	78	77	75
Gain d 1-512	339	342	334	320	300	179	176	185	175	148
Gain d 1-729	304	305	302	293	273	212	216	215	208	-

[a]bodyweight gains were not subjected to statistical analyses *significantly different from control $p<0.05$

The haematology investigations revealed some slight changes in red blood cell (RBC) parameters in females only at 750 mg/kg/day, which are shown in Table B.6.5.1-5. Haemoglobin concentrations at 3 and 6 months were significantly lower than controls. At 3 and 6 months, RBC count and haematocrit for this group were also lower than the controls, and reticulocyte count was slightly higher, though the differences did not achieve statistical significance. Historical control ranges were reported of three of these parameters (see Table B.6.5.1-5), though the relevance of a comparison with historical data is debatable because RBC count for the concurrent controls at 3 months was outside the historical control range. As the reduced RBC correlated

with an increased reticulocyte count, these slight changes are considered likely to be treatment related.

Table B.6.5.1–5 Group mean selected haematology findings; females

Parameter	Month	Target dose level of XDE-729 Acid (mg/kg/day)					Laboratory historical control range[a]
		0	20	100	400	750	
RBC count ($E^6/\mu l$)	3	9.35	9.35	9.32	9.18	9.11	7.57 - 9.05
	6	9.05	8.97	8.91	8.85	8.59	7.86 - 9.12
	12	8.41	8.39	8.59	8.10	8.12	7.89 - 8.65
	18	9.27	9.37	9.20	8.62	-	7.99 - 9.11
	24	8.23	8.63	8.29	8.51	-	7.95 - 8.61
Haemoglobin concentration (g/dl)	3	16.1	16.2	16.0	15.8	15.5*	13.5 -16.8
	6	15.6	15.4	15.4	15.2	14.7*	14.7 - 16.2
	12	15.0	15.0	15.2	14.5	14.5	14.7 - 15.4
	18	16.7	16.7	16.6	15.5	-	15.0 - 16.5
	24	15.1	15.7	15.3	15.5	-	14.2 - 15.9
Haematocrit (%)	3	49.4	49.5	49.4	48.3	48.0	38.7 - 49.4
	6	47.9	47.6	47.2	46.8	45.5	40.7 - 48.9
	12	47.7	47.4	48.4	46.3	46.1	45.5 - 47.1
	18	49.8	50.3	49.4	46.5	-	42.0 - 49.5
	24	45.6	47.4	46.1	46.9	-	43.7 - 47.1
Reticulocyte count (E^6/L)	3	173.8	174.9	176.0	171.1	186.4	Historical control range not reported
	6	147.8	149.0	151.3	158.4	164.6	
	12	202.0	207.5	182.8	278.4	217.1	
	18	225.7	217.0	216.5	234.5	-	
	24	249.9	193.4	236.3	204.9	-	

[a] historical control range from 10 studies conducted 2007 - 2011 (3 months) or 4 studies conducted 2007 – 2010
*significantly different from control $p<0.05$

The clinical chemistry investigations revealed one change considered to be treatment-related. Blood urea nitrogen (BUN) in females at 750 mg/kg/day was significantly increased at 12 months (see Table B.6.5.1–7). This corresponded with microscopic changes identified in the kidney (see below). BUN in males at 625 mg/kg/day was significantly increased at 6 months (Table B.6.5.1–7), which was considered less likely to be treatment-related because the value was within the historical control range and no increases were seen for this parameter at other time points. Females at 400 mg/kg/day had a higher mean BUN (though not statistically significant) at 18 months; however this group mean value was driven by one female that was prematurely killed because of poor clinical condition 2 weeks after this sampling interval, due to a leiomyosarcoma of the uterus. Significantly higher cholesterol levels were reported for females at 750 mg/kg/day at 3 months and for females at 400 mg/kg/day at 24 months. These higher cholesterol levels were interpreted to be unrelated to treatment because the values were within or near the historical control ranges, and were not significantly higher at any other time points; additionally in addition, males at the highest dose level cholesterol levels were actually slightly lower at 6, 12, 18, and 24 months.

Table B.6.5.1–6 Group mean selected clinical chemistry findings: males

Parameter	Month	Target dose level of XDE-729 Acid (mg/kg/day)					Laboratory historical control range[a]
		0	20	100	400	625	
Urea Nitrogen (mg/dl)	3	20	18	19	19	20	14 - 19
	6	15	16	16	16	18*	16 - 21
	12	14	14	14	14	15	14 - 16
	18	15	16	15	15	15	15 - 16
	24	15	15	14	14	15	15 - 19
Cholesterol (mg/dl)	3	62	62	58	59	63	47 - 74
	6	77	75	74	70	74	56 - 78
	12	85	86	81	80	81	76 - 100
	18	144	144	116	133	108*	111 - 151
	24	169	175	128	141	116	159 - 201

[a] historical control range from 10 studies conducted 2007 - 2011 (3 months) or 4 studies conducted 2007 – 2010
*significantly different from control p<0.05

Table B.6.5.1–7 Group mean selected clinical chemistry findings: females

Parameter	Month	Target dose level of XDE-729 Acid (mg/kg/day)					Laboratory historical control range[a]
		0	20	100	400	750	
Urea Nitrogen (mg/dl)	3	19	20	20	20	21	15 – 18
	6	19	18	17	19	19	17 – 19
	12	16	17	17	16	19*	17
	18	16	17	17	27	-	16 - 18
	24	14	15	14	15	-	13 - 17
Cholesterol (mg/dl)	3	81	76	88	77	92*	65 - 93
	6	96	97	104	102	109	92 - 116
	12	135	129	142	139	148	117 - 143
	18	135	143	158	149	-	123 - 133
	24	136	141	131	166*	-	117 - 152

[a] historical control range from 10 studies conducted 2007 - 2011 (3 months) or 4 studies conducted 2007 – 2010
*significantly different from control p<0.05

There were number of treatment-related changes in urinalysis parameters. As shown in Table B6.5.1-7, urine volume was increased and specific gravity was decreased in both males and females at 400 and 625/750 mg/kg/day. Specific gravity was also significantly lower in females at 100 mg/kg/day at 18 months; however, the difference at this dose level was considered unlikely to represent a treatment-related change as the observed mean value was within the laboratory historical control range (1.004-1.056, from 4 studies conducted 2007-2010) and significant differences for this parameter at 100 mg/kg/day were not present at any other time points. Although not shown on the table, there was a general trend for slightly lower urinary pH in both genders at 400 and 625/750 mg/kg/day. This is considered to be a non-adverse change, caused by the presence of the acidic parent (XDE-729 Acid) in urine. Additionally, there was an absence of microscopically observable triple phosphate and amorphous phosphate urinary crystals in both genders at 400 and 625/750 mg/kg/day; these crystals tend to form in the presence of more alkaline urine, so their absence is likely to be the result of the lower urinary pH.

Table B.6.5.1–7 Group mean selected urinalysis findings

Parameter	Month	Target dose level of XDE-729 Acid (mg/kg/day)									
		Males					Females				
		0	20	100	400	625	0	20	100	400	750
Urine volume (ml)	3	2.7	3.3	3.1	3.9	8.3*	2.5	2.7	2.8	3.8	6.9*
	6	3.1	4.1	3.5	6.6*	11.5*	3.9	4.8	5.5	7.1*	11.0*
	12	3.5	3.9	4.1	7.5*	9.8*	5.0	4.7	5.1	7.8*	10.5*
	18	5.6	5.1	4.8	8.3	9.9*	6.2	6.2	8.5	12.0*	-
	24	6.8	7.2	6.3	8.7	10.4	8.3	8.3	6.5	11.9*	-
Urine specific gravity	3	1.089	1.077	1.077	1.071	1.044*	1.071	1.067	1.067	1.058*	1.037*
	6	1.081	1.074	1.079	1.055*	1.036*	1.060	1.055	1.048	1.039*	1.029*
	12	1.075	1.071	1.073	1.049*	1.040*	1.053	1.055	1.050	1.037*	1.031*
	18	1.061	1.064	1.063	1.048*	1.039*	1.053	1.052	1.045*	1.031*	-
	24	1.050	1.049	1.048	1.039*	1.033*	1.039	1.039	1.047	1.030*	-

*significantly different from control $p<0.05$

At 12 months, the organ weight analysis revealed treatment-related differences for the kidney. Absolute and/or relative kidney weight was significantly increased in both genders at 400 and 625/750 mg/kg/day (see Table B.6.5.1-8). These changes corresponded to the microscopic pathology finding of hypertrophy of renal collecting duct epithelium in these groups. Relative kidney weight was also significantly higher for males at 100 mg/kg/day; however, this was considered unlikely to represent a treatment-related adverse effect as this did not correlate with any microscopic pathology findings in the kidney, the mean value was within the historical control range, and a similar difference was not present at 24 months. At 12 months, relative liver weight was significantly higher for females at 400 and 750 mg/kg/day; however, in the absence of correlating clinical chemistry or microscopic pathology findings and because the increase was less than 10% in comparison with controls, these liver weight differences were considered not to be adverse.

Consistent with the findings at 12 months, the 24 month organ weight analysis identified a treatment related increase in absolute and relative kidney weights in males at 400 and 650 mg/kg/day and females at 400 mg/kg/day (see Table B.6.5.1-9). The statistical analysis identified significant weight differences for other organs at 12 and 24 months, but these were not considered to be treatment related because dose-response relationships were not present and/or corroborating microscopic pathology finding were not observed and/or values were within the historical control ranges.

Table B.6.5.1–8 Selected group mean organ weights: chronic toxicity study

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	20	100	400	625	0	20	100	400	750
Terminal bodywt. (g)	421	410	416	390*	398	203	202	211	203	204
Kidney (g)	2.49	2.51	2.60	2.57	2.68*	1.465	1.472	1.545	1.627*	1.710*
Kidney (% of bodywt.)	0.591	0.613	0.627*	0.659*	0.675*	0.722	0.729	0.733	0.803*	0.838*
Historical control	0.602 – 0.654					0.683 – 0.741				
Liver (g)	10.317	10.065	10.119	10.005	10.017	5.203	5.194	5.397	5.516	5.613
Liver (% of bodywt.)	2.449	2.455	2.432	2.565	2.516	2.565	2.570	2.558	2.718*	2.746*
Historical control[a]	Not reported					2.501 – 3.119				

[a]historical control range from 4 studies conducted 2007 – 2010 *significantly different from control p<0.05

Table B.6.5.1–9 Selected group mean organ weights: carcinogenicity study

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	20	100	400	625	0	20	100	400	750
Terminal bodywt. (g)	413	418	414	402	381	281	284	284	274	-
Kidney (g)	2.868	2.898	2.904	3.012*	2.902	1.856	1.864	1.907	2.307*	-
Kidney (% of bodywt.)	0.703	0.696	0.702	0.753*	0.762*	0.662	0.958	0.673	0.751*	-
Historical control[a]	0.703 – 0.840					0.695 – 0.740				

[a]historical control range from 4 studies conducted 2007 – 2010 *significantly different from control p<0.05

No treatment-related gross pathology changes were found in the chronic toxicity study.

In the carcinogenicity study the main change detected in the macroscopic pathology examination was in the kidneys, observed as an increased incidence of roughened surface of the organ in males at 625 mg/kg/day and females at 750 mg/kg/day (see Table B.6.5.1–10 for a summary of gross findings). Additionally, in males at 750 mg/kg/day, a calculus was present in the kidney of animal and urinary bladder of another, which was possibly treatment-related. There were increased incidences of decreased body fat in high dose males and females and excessive gastrointestinal gas in high dose males, which was considered to be secondary to kidney disease-related morbidity. The lower incidences pituitary masses and increased spleen size, indicators of the possible presence of tumours, in high dose males and females was considered to be the result of increased mortality.

Table B.6.5.1–10 Selected gross pathology findings: carcinogenicity study, no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	20	100	400	625	0	20	100	400	750
No. surviving to terminal kill at 24 mo.	34	28	28	33	19	42	45	36	35	a
General; decreased amount of fat	0	1	1	1	6	0	0	2	1	19
General; gas, gastrointestinal tract	1	3	3	2	9	0	0	1	0	1
Kidneys; roughened surface	5	5	3	6	32	0	0	1	2	39
Kidneys, calculus, left, pelvis	0	0	0	0	1	0	0	0	0	0
Liver; increased size	7	8	8	7	0	4	2	4	3	0
Liver; roughened surface	13	11	9	8	2	5	1	5	6	0
Pituitary gland; mass/nodule 0.2 - 0.5 cm	9	7	8	11	4	8	12	9	9	1
Pituitary gland; mass/nodule 0.6 - 1.0 cm	7	4	5	5	0	7	1	6	5	1
Spleen; increased size, probable lymphoid tumour	15	14	11	10	1	8	2	9	7	0
Urinary bladder, calculus	0	0	0	0	1	0	0	0	0	0

a = group terminated prematurely at 18 months because of excessive mortality; 16 were alive at the time of the premature termination

The key microscopic findings at 12 months are summarised in Table B.6.5.1–11. Treatment-related changes were present in only the kidneys, in males and females at 400 mg/kg/day and 625/750 mg/kg/day. The effects consisted of increased incidence of chronic progressive glomerulonephropathy (females only at 400 or 750 mg/kg/day), hyperplasia of the pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in the collecting duct epithelium, chronic interstitial inflammation of the medulla, necrosis of collecting duct epithelium (females only at 750 mg/kg/day) and vacuolisation of collecting duct epithelium in the papilla.

Hyperplasia of the pelvic epithelium was noted most commonly at the base of the renal pelvis, with a focal or multifocal distribution. Hypertrophic epithelial cells in the collecting ducts were present in the renal papilla and inner and outer zones of the medulla. Hypertrophic epithelial cells had increased cytoplasmic basophilia, and sometimes had nuclei which were two or three times larger than normal. The hypertrophy occurred in principal and intercalated cells of the collecting ducts. The chronic inflammation consisted of small clusters of interstitial lymphocytes around the hypertrophic collecting ducts in the inner medulla and papilla. Necrosis of collecting duct epithelial cells was characterised by individual epithelial cells in the inner medulla and papilla that had pyknotic or karyorrhectic nuclei. The vacuolisation of collecting duct epithelial cells was characterised by cytoplasmic clearing in individual cells near the tip of the renal papilla.

Table B.6.5.1–11 Selected microscopic pathology findings: chronic toxicity study, no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	20	100	400	625	0	20	100	400	750
No. of animals examined	10	10	10	10	10	10	10	10	10	10
Kidneys										
Chronic progressive glomerulonephropathy v slight	10	10	10	9	10	2	2	4	6	10
Hyperplasia, pelvic epithelium, uni or bilateral, focal, multifocal or diffuse, slight or moderate	2	0	0	4	6	0	0	1	6	9
Hypertrophy, epithelium, collecting duct, bilateral, multifocal, slight or v slight	0	0	0	10	10	0	0	0	10	10
Increased no. of mitotic figures, epithelium, collecting duct, uni or bilateral, v slight	0	0	0	3	1	0	0	0	3	5
Inflammation, chronic, interstitium, medulla, uni or bilateral, focal or multifocal, v slight	0	0	0	1	6	1	0	1	4	7
Necrosis, epithelium, collecting duct, uni or bilateral, multifocal, v slight	0	0	0	0	0	0	0	0	0	8
Vacuolisation, papilla, collecting duct, uni or bilateral, multifocal, v slight	0	0	0	4	4	0	0	0	9	10

The key microscopic non-neoplastic findings in the carcinogenicity study are summarised in Table B.6.5.1–12. The primary treatment-related changes were present in the kidneys and urinary bladder in males at 400 and 650 mg/kg/day and females at 400 mg/kg/day (microscopic examination was not conducted on females at 750 mg/kg/day). Tubular degeneration with regeneration occurred in males at 400 and 625 mg/kg/day and females at 400 mg/kg/day. Most of the animals with degenerative tubular changes also had necrosis of individual tubular epithelial cells. The tubular degeneration/regeneration and necrosis was not seen at 12 months. The degeneration with regeneration was present in the proximal and distal tubules of the cortex and medulla, resulting in an undulant cortical surface. Degenerative tubules had reduced diameters and smaller lumens, and were lined by flattened or cuboidal epithelial cells that had increased cytoplasmic basophilia as compared to unaffected tubules. Some of the degenerative tubular epithelial cells had variably sized clear circular cytoplasmic vacuoles. Regenerative tubules were lined by enlarged cuboidal or columnar epithelial cells with increased size nuclei, prominent nucleoli, and increased cytoplasmic basophilia. Some regenerative tubules had two or more layers of epithelial cells that projected into the lumens. Some animal had a few mitotic figures in epithelial cells of the regenerative tubules. Small clusters of interstitial lymphocytes accompanied the tubular effects. In addition, most of the animals with degenerative tubular effects had necrosis of individual tubular epithelial cells. Other treatment-related changes in the kidneys at 400 and 625 mg/kg/day were similar to those seen in at 12 months in the chronic study.

One female at 100 mg/kg/day had severe bilateral degeneration with regeneration of renal tubules, and associated very slight necrosis of tubular epithelial cells. The severe tubular degeneration with regeneration was interpreted to be a spontaneous alteration because of its isolated (singular) occurrence in this dose group, and the lack of a dose-response in terms of severity (the degeneration/regeneration in females at 400 mg/kg/day was graded slight or moderate). It should be noted that the microscopic characteristics and severity of the renal lesions of this female were

similar to one male given 20 mg/kg/day that had unilateral severe degeneration with regeneration of renal tubules and associated unilateral very slight necrosis of individual tubular epithelial cells. The renal alterations of this low-dose male were interpreted to be unrelated to treatment because the contralateral kidney from this animal did not have these lesions, and there were no males from the next higher dose of 100 mg/kg/day that had similar renal alterations.

The incidence of pelvic epithelial hyperplasia in females at 100 mg/kg/day was significantly greater than controls. This was interpreted to be an equivocal finding that was unlikely to be an adverse effect of dietary administration of XDE-729. This conclusion is supported by the following points: 1) many of the cases of pelvic epithelial hyperplasia at this dose level were unilateral and focal, which is an unlikely distribution for a potential treatment-related systemic effect; 2) there were no accompanying treatment-related effects in the collecting ducts or tubules of the kidneys in any rat from this dose level; 3) there were no accompanying treatment-related effects in the urinary bladder or in urinalysis parameters at this dose level; and 4) there were no treatment related kidney effects (including pelvic epithelial hyperplasia) in females at 100 mg/kg/day at 12 months study. It should be noted that the pelvic epithelial hyperplasia in all females given 100 mg/kg/day was graded as slight, with no accompanying cellular atypia or prominent outward or inward growth of the epithelial cells. Hyperplasia of the renal pelvic epithelium has been reported to be a common spontaneous finding in Fischer 344 rats, frequently seen in association with spontaneous nephropathy. There are no laboratory historical control data for pelvic epithelial hyperplasia as this specific lesion is usually incorporated into the more generalised diagnosis of chronic progressive glomerulonephropathy

Additionally, one male at 400 mg/kg/day and two males at 625 mg/kg/day had calculi present in the pelvis of one or both kidneys, considered to be treatment-related. The calculi were pale eosinophilic and particulate, with irregular (roughened) outer surfaces.

The treatment-related microscopic changes in the urinary bladder were present in three males at 400 mg/kg/day and 7 males at 625 mg/kg/day. The effects consisted of slight to moderate, diffuse or focally extensive hyperplasia of the transitional epithelium, and very slight to slight subacute to chronic inflammation in the submucosa beneath the hyperplastic epithelium. The submucosal inflammation consisted of accumulations of mononuclear inflammatory cells. One male at 400 mg/kg/day and two at 625 mg/kg/day with the hyperplasia/inflammation also had microscopic calculi in the lumen of the urinary bladder, considered to be treatment-related. The calculi were pale eosinophilic and particulate, with irregular (roughened) outer surfaces, and were similar in appearance to the calculi previously described in the pelvis of the kidneys.

Treatment-related changes in the adrenals were present in males at 400 and 625 mg/kg/day, though likely to be secondary (an adaptive response) to the kidney toxicity. The incidence of slight hypertrophy of the zona glomerulosa of the adrenal cortex was increased; the hypertrophy was characterised by an increase in cell size and eosinophilic staining of the cytoplasm as compared to the controls. Most of the males at 625 mg/kg/day with this hypertrophy also had vacuolisation (consistent with fatty change) of the zona glomerulosa. The vacuolisation was characterised by single or multiple clear circular vacuoles in the cytoplasm of hypertrophic cells in the zona glomerulosa. All of the males with hypertrophy and/or vacuolisation of the zona glomerulosa had treatment-related tubular degeneration with regeneration of the kidneys. Males at 625 mg/kg/day had a treatment-related increase in the incidence of atrophy of mesenteric adipose tissue, most probably related to the reduced bodyweight gain in this group.

Table B.6.5.1–12 Selected microscopic non-neoplastic pathology findings: carcinogenicity study – no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)								
	Males					Females			
	0	20	100	400	625	0	20	100	400
No. of animals in group	50	50	50	50	50	50	50	50	50
Kidneys									
Degeneration with regeneration, tubule, uni or bilateral slight, moderate or severe	0	1	0	4	26*	0	0	1	4
Necrosis, individual cell, epithelium, tubule, uni or bilateral, multifocal v. slight	0	1	0	1	21*	0	0	1	2
Hyperplasia, pelvic epithelium, unilateral, focal, slight	5	7	5	5	1	4	1	11	3
Hyperplasia, pelvic epithelium, unilateral, multifocal, slight	12	14	11	17	11	2	3	5	17*
Hyperplasia, pelvic epithelium, unilateral, multifocal, moderate	0	0	0	1	2	0	0	0	0
Hyperplasia, pelvic epithelium, unilateral, diffuse, moderate	0	0	0	2	0	0	0	0	0
Hyperplasia, pelvic epithelium, bilateral, focal, slight	0	0	1	0	0	2	0	4	2
Hyperplasia, pelvic epithelium, bilateral, multifocal, slight	3	2	6	26*	35*	0	1	2	21*
Total with hyperplasia, pelvic epithelium, uni or bilateral, focal, multifocal or diffuse, slight or mod.	20	23	23	49*	48*	8	5	22*	43*
Calculi, pelvis, uni or bilateral, focal or multifocal: slight	0	0	0	1	2	0	0	0	0
Hypertrophy, epithelium, collecting duct, uni or bilateral, focal or multifocal v. slight, slight or mod.	27	29	30	48*	47*	8	5	11	49*
Necrosis, epithelium, collecting duct, uni or bilateral, multifocal v. slight	3	0	0	3	5	0	0	1	6*
Inflammation, chronic, interstitium, medulla, uni or bilateral, multifocal v. slight	14	18	17	37*	38*	9	3	14	40*
Urinary bladder									
Hyperplasia, transitional epithelium diffuse or focally extensive slight or moderate	0	1	0	3	7*	0	0	0	0
Inflammation, subacute to chronic, submucosa, multifocal or diffuse v. slight or slight	0	0	0	3	7*	0	0	0	0
Adrenal gland									
Hypertrophy, zona glomerulosa, bilateral, diffuse v. slight or slight	0	0	0	2	26*	0	0	1	0
Vacuolisation, consistent with fatty change, zona glomerulosa v. slight or slight	0	0	0	0	19*	0	0	0	0
Mesenteric tissue									
Atrophy, adipose tissue slight or moderate	4	6	3	8	15*	5	0	1	2

*significantly different from control $p<0.05$

The microscopic neoplastic pathology findings are presented in Table B.6.5.1–13. This table shows clearly that there were no treatment-related increases in the incidence of neoplasms in the XDE-729 Acid treated groups.

Table B.6.5.1–13 Microscopic neoplastic pathology findings: carcinogenicity study, no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)								
	Males					Females			
	0	20	100	400	625	0	20	100	400
No. of animals in group	50	50	50	50	50	50	50	50	50
Adrenal glands									
Adenoma; unilateral	0	0	1	0	0	0	0	0	0
Complex pheochromocytoma	0	1	0	0	0	0	0	0	0
Ganglioneuroma; benign	1	0	0	0	0	0	0	0	0
Pheochromocytoma; medulla; benign	3	4	5	7	4	1	0	0	0
Auditory sebaceous gland									
Carcinoma	0	1	0	1	0	0	0	0	0
Bone									
Chordoma; sacrum or vertebra; malignant	0	1	0	1	0	0	0	0	0
Osteosarcoma; humorous or hindlimb; malignant	1	0	0	1	0	0	0	0	0
Bone marrow									
Leukemia; lymphoid cell; malignant	0	0	1	0	0	0	0	0	0
Brain									
Astrocytoma; medulla oblongata; malignant	0	0	0	0	0	1	0	0	0
Carcinoma	2	0	1	0	0	1	0	3	1
Granular cell tumor; meninges; benign	1	0	0	0	0	0	0	0	0
Mixed glioma; cortex; malignant	0	0	1	0	0	0	0	0	0
Heart									
Schwannoma; endocardium; benign	1	0	1	0	0	1	0	0	0
Jejunum									
Adenoma; polypoid	0	1	0	0	0	0	0	0	0
Leiomyosarcoma; malignant	0	0	0	1	0	0	0	0	0
Kidneys									
Adenoma; one; cortex; tubule	0	0	0	0	0	1	0	0	0
Liposarcoma; malignant	0	0	0	0	0	1	0	0	0
Transitional cell carcinoma; pelvis	0	0	1	0	1	0	0	0	0
Liver									
Adenoma; hepatocyte	2	2	3	3	2	0	0	0	0
Carcinoma; hepatocyte	1	0	0	0	0	0	0	0	0
Haemangiosarcoma; malignant	0	0	0	0	2	0	0	0	0
Lipoma; benign	0	0	0	1	0	0	0	0	0
Lung									
Adenoma; bronchiolo - alveolar	1	1	0	0	1	0	0	0	1
Carcinoma; malignant	1	0	0	0	0	0	0	0	0
Haemangiosarcoma; malignant	0	0	0	0	1	0	0	0	0
Osteosarcoma; malignant;	1	0	0	0	0	0	0	0	0
Squamous cell carcinoma; malignant	0	0	1	0	0	0	0	0	0
Leukemia; large granular lymphocyte	0	0	0	0	0	0	0	1	0
Mammary gland									
Adenocarcinoma; malignant	0	0	0	0	0	4	3	2	0
Adenoma or fibroadenoma; benign	1	4	1	2	0	8	8	7	8
Mediastinal tissue									
Hibernoma or squamous cell carcinoma	0	0	2	0	0	0	0	0	0
Mesenteric tissue									
Carcinoma; poorly differentiated	0	0	0	0	0	0	1	0	0
Multiple organs									
Adenocarcinoma; malignant	0	1	0	0	0	0	0	0	0
Mesothelioma; malignant	1	1	2	2	0	0	0	0	0

Nasal tissue - pharynx									
Squamous cell carcinoma; malignant	0	0	2	0	0	0	0	0	1
Oral tissue									
Odontoma; malignant	0	1	0	0	0	0	0	0	0
Squamous cell carcinoma; gingival or palate	2	1	0	0	1	0	0		0
Ovaries									
Adenoma; tubulostromal; benign						0	1	0	0
Pancreas									
Adenoma; islet cell or mixed acinar	7	5	0	0	1	0	0	0	1
Carcinoma; islet cell	0	0	1	0	0	0	0	0	0
Pituitary gland									
Adenoma or carcinoma, pars distalis	27	19	18	25	18	19	14	22	17
Ganglioneuroma; pars nervosa; benign	1	0	0	0	0	0	0	1	0
Preputial/clitoral gland									
Adenoma; benign	0	0	0	0	0	0	0	0	1
Carcinoma; malignant	3	0	3	3	4	1	2	1	2
Prostate									
Adenocarcinoma; malignant	0	1	0	1	0				
Skin and subcutis									
Basal cell adenoma; benign	0	0	0	0	0	1	0	0	0
Basal cell carcinoma; malignant	0	0	0	0	0	0	1	0	0
Fibroma; benign	3	3	0	4	2	0	0	0	0
Fibrosarcoma; malignant	0	1	0	1	0	0	0	0	1
Keratoacanthoma; benign	2	1	0	3	0	1	0	1	0
Keratoacanthoma; malignant	0	0	1	0	0	0	0	0	0
Lipoma; benign	2	0	0	0	1	0	0	0	1
Neurofibrosarcoma; malignant	0	0	1	0	0	0	0	0	0
Papilloma; benign	1	1	0	1	1	0	0	0	0
Sarcoma: malignant	2	0	1	0	0	0	1	0	0
Squamous cell carcinoma	1	0	0	0	1	0	1	0	0
Trichoepithelioma; benign	0	1	0	1	0	0	0	0	0
Spleen									
Haemangiosarcoma or sarcoma; malignant	0	1	0	0	2	0	0	0	0
Leukemia; large granular lymphocyte	17	16	13	13	3	9	3	10	10
Histiocytic sarcoma; malignant	0	0	1	0	0	0	0	0	0
Testes									
Interstitial cell adenoma	42	42	44	43	36				
Thymus									
Lymphosarcoma; malignant	0	0	0	0	1	0	0	0	
Thyroid gland									
Adenoma or carcinoma; follicular or parafollicular cell; benign or malignant	14	2	1	1	3	8	0	3	2
Urinary bladder									
Transitional cell carcinoma	0	0	1	0	0	0	0	0	0
Uterus									
Adenocarcinoma						0	3	1	1
Adenoma						0	1	0	0
Carcinoma						0	1	0	0
Endometrial stromal polyp						13	4	11	11
Leiomyoma; myometrium; benign						0	0	1	0
Leiomyosarcoma; malignant						0	0	0	1
Stromal cell sarcoma; malignant						1	0	1	2
Leukemia; malignant;						0	0	0	1

The results of the toxicokinetic investigations, conducted at 6 and 12 months, are summarised in Table B.6.5.1-14. Based on the calculated AUC_{24h} values, systemic absorption of XDE-729 Acid was approximately dose-proportional at all dose levels, except for males at 6 months at the highest dose level which showed less than dose-proportionality. Urinary excretion of intact XDE-729 Acid appeared to be approximately dose proportional at all dose levels and at both time points. less than dose proportional at all time points. At 6 months a mean of 72% administered dose of XDE-729 Acid was excreted intact in the urine; at 12 months the proportion of doses excreted in the urine had declined to a mean of 41%, which may be due to an age-related decline in renal clearance. Trace amounts of XDE-729 Acid were detected in some of the urine samples from controls; the reason for this contamination is not known.

B.6.5.1–14 Toxicokinetic investigation: mean systemic AUC_{24h} values and urinary elimination at 3, 6 and 12 months

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)									
	Males					Females				
	0	20	100	400	625	0	20	100	400	750
6 months										
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	21	111	444	749	0	20	97	428	823
Blood AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	82	176	1002	1356	NQ	59	255	1151	2376
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	NQ	12.3	89	311	553	0.08	16.9	92	247	454
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	59	80	70	73	-	85	96	57	55
12 months										
Dose of XDE-729 Acid at time of blood sampling (mg/kg/day)	0	21	102	414	623	0	20	105	425	832
Bloood AUC_{24h} for XDE-729 Acid (µg h/mL)	NQ	86	197	1278	2235	NQ	56	286	1328	2603
Amount of XDE-729 Acid in 24 h urine (mg/kg/day)	0.17	7.7	36	167	274	NQ	9.2	38	212	347
Amount of XDE-729 Acid in 24 h urine as % of daily intake	-	37	35	40	44	-	45	36	50	77

NQ = not quantifiable

CONCLUSION

Dietary administration of XDE-729 Acid to rats for up to 2 years causes non-neoplastic changes, but is not carcinogenic in this species.

Regarding the non-neoplastic effects, the kidneys are identified as the principal target, with effects being observed at 400 mg/kg/day and above. The adverse effects seen at 12 months were chronic progressive glomerulonephropathy, hyperplasia of the pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in the collecting duct epithelium, chronic interstitial inflammation of the medulla, necrosis of collecting duct epithelium and vacuolization of collecting duct epithelium in the papilla, accompanied by increase kidney weight. Additional pathology changes in the kidney were observed at 24 months, namely tubular degeneration with regeneration and necrosis of individual tubular epithelial cells and, in occasional males, the presence of calculi in the pelvis. At 24 months there were also changes in the urinary bladder, characterised by hyperplasia of the transitional epithelium and inflammation

in the submucosa beneath the hyperplastic epithelium and, in one animal, the presence of microscopic calculi in the lumen. Secondary to the kidney toxicity, slight hypertrophy and vacuolisation of the adrenal zona glomerulosa was seen at 24 months. The kidney toxicity was accompanied by reduced bodyweight gain from 400 mg/kg/day and increased urinary volume and, at the highest dose level, increased blood urea nitrogen and reduced survival. Additionally, the haematology investigations revealed slightly reduced haemoglobin concentration, RBC count and haematocrit and a slightly increased reticulocyte count in females at 750 mg/kg/day. A study NOAEL of 102 mg/kg/day (target 100 mg/kg/day) is identified.

A toxicokinetic investigation showed that systemic bioavailability of XDE-729 Acid is approximately dose-proportional (except for males at the highest dose level at 6 months). A mean of 72% of the administered dose of XDE-729 Acid was excreted intact in the urine at 6 months, but at 12 months the proportion of dose excreted in the urine had declined to a mean of 41%, which may be due to an age-related decline in renal clearance.

[redacted] (2012)

B.6.5.2 Chronic toxicity and carcinogenicity in the mouse

XDE-729 Acid	
Study	IIA 5.5.3/01 18-Month dietary oncogenicity study in Crl:CD1(ICR) mice
Reference	[redacted] (2012)
Date performed	March 2010 – September 2011
Test facility	[redacted]
Report reference	Laboratory study ID 101021
Guideline(s)	OECD 451 (2009)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot # E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Crl:CD1(ICR) mice, about 6 weeks old at the start of the study, were randomly assigned to the test groups as shown in the table below.

Table B.6.5.2–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	50	50
2	50	50	50
3	250	50	50
4	750	50	-
	1000	-	50

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet

used on the study were within 4% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

A cage-side examination of all animals was conducted at least once daily. General clinical observations, including an examination for unusual swellings and palpable masses, were conducted every two weeks. More detailed clinical examination was conducted monthly on the first 10 surviving animals/sex/dose. Bodyweights and food consumption were measured weekly for the first 13 weeks and monthly thereafter. Ophthalmoscopy examinations were conducted before dosing commenced and just prior scheduled terminations at 18 months.

Toxicokinetic investigations were conducted at part of this study. On one day at months 6 and 12 blood samples were taken from the pedal vein of 4 non-fasted animals/sex/dose from each group; Spot urine samples were collected on one day at 6 and 12 months from 4 non-fasted animals/sex/dose. The animals selected for blood or urine sampling were individuals that had not scratched their food at the time of sampling. Plasma and urine samples were analysed for XDE-729 acid by LC/MS/MS.

Blood samples (non-fasted) were taken from the pedal vein at 12 months and orbital sinus at 18 months the time of scheduled necropsy. A total white blood cell (WBC) count and differential WBC count were determined from the 18 month samples.

A necropsy was conducted on all animals. Surviving animals were terminated after 18 months. Macroscopic changes were recorded. Brain, liver, kidneys, heart, adrenals, testes, epididymides, uterus, ovaries and spleen weights were recorded. A standard range of organs and tissues were removed and fixed. Microscopic examination was conducted on all tissues from the control and high dose animals and from decedents. Relevant gross lesions, liver, lung, kidneys and urinary bladder from all low and mid dose animals were subject to microscopic examination. Additionally, from males of the low and mid dose groups, adrenals, coagulating glands, seminal vesicles, prostate and spleen were examined microscopically.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values were very close to the target doses, as shown below:

Table B.6.5.2–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	50	250	750/1000
Males	50	251	751
Females	50	251	1004

There were no treatment-related clinical signs of toxicity.

Mortality rates are presented in Table B.6.5.2-3. Mortality was significantly increased for males at 750 mg/kg/day, a difference that was considered to be treatment-related. Many of the males dying or killed prematurely had treatment-related pathological changes in the kidneys and urinary bladder, as discussed below. For females mortality was slightly higher at 1000 mg/kg/day but the difference was insufficient to be ascribed to XDE-729 Acid treatment.

Table B.6.5.2–3 Mortality at 18 months, expressed as %

Target dose level of XDE-729 Acid (mg/kg/day)							
Males				Females			
0	50	250	750	0	50	250	1000
26	26	20	46*	22	28	24	34

*significantly different from control p<0.05

There were no treatment-related effects on bodyweights or food consumption.

There were no treatment-related ophthalmoscopy findings or effects on total and differential WBC counts.

There were no treatment-related organ weight differences.

The key macroscopic pathology findings are presented in Table B.6.5.2-4. Treatment-related macroscopic changes were present in the urinary bladder in males only. At 250 and 750 mg/kg/day, calculi were present in the bladder of some males, and at 750 mg/kg/day there was an increased incidence of thickened wall of bladder. The latter finding was also present in two males at 50 mg/kg/day, but in the absence of a dose-response relationship and corroborating microscopic changes this observation was not regarded as treatment-related.

Table B.6.5.2-4 also presents the incidence of liver mass nodules, which are indicative of possible tumours. The incidence of liver mass nodules in males was higher than concurrent controls in all treated groups but in the absence of a dose-response relationship this was not considered to be treatment-related. This interpretation receives additional support from a reference to historical control data, which show that the incidence of liver mass nodules in the treated males is similar to that observed in control males in two previous mouse carcinogenicity studies conducted by testing laboratory.

Table B.6.5.2–4 Selected macroscopic pathology findings: no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750	0	50	250	1000
Number examined	50	50	50	50	50	50	50	50
Urinary bladder								
Calculus	0	0	1	10	0	0	0	0
Thickened wall	0	2	0	7	0	0	0	0
Liver								
Mass nodule	5	12	9	11	3	1	0	1
Historical controls	8/50, 11/50 in two studies, completed in 2007 & 2010, respectively				Not reported			

The key microscopic non-neoplastic pathology findings are presented in Table B.6.5.2-5. Treatment-related effects occurred in the urinary bladders of males at 250 and 750 mg/kg/day, the kidneys of males and females at 750 mg/kg/day, and the adrenal glands and spleen of males at 750 mg/kg/day.

The treatment-related changes in the urinary bladder of males consisted of subacute to chronic inflammation and presence of microscopic calculi within the bladder lumen at both 250 and 750

mg/kg/day and hyperplasia of the transitional epithelium lining the mucosa, increased numbers of mitotic figures within the transitional epithelium, necrosis of individual transitional epithelial cells and ulceration of the transitional epithelium at 750 mg/kg/day only. The inflammation was characterised by the presence of mononuclear and polymorphonuclear inflammatory cells and submucosal oedema. Inflammatory reactions graded as slight were generally restricted to the mucosa and submucosa, while the moderate and severe inflammatory reactions extended into the deeper muscular layers. Hyperplastic transitional epithelial cells had variably increased amounts of cytoplasm, enlarged and vesicular nuclei, which contained prominent and multiple nucleoli and variable amounts of chromatin. The calculi were of two different types of appearance. One of the types was crystalline in appearance, light grey to colorless, and had a star-burst internal pattern. These crystals were estimated to be approximately 300-500 microns in diameter. The second type of calculi was eosinophilic but lacked the colorless/grey crystalline appearance. It was not clear whether there was a progression from the eosinophilic calculi to the crystalline calculi. The exact composition of these crystals/calculi was not determined and therefore, it is also not clear as to whether they were composed of the test material and/or its metabolites or other substances. Although the exact pathogenesis of the bladder lesions is not clear, it is likely that the treatment-related presence of urinary crystals/calculi in the bladders of male mice given 250 or 750 mg/kg/day contributed to physical injury and /or chronic irritation of the bladder mucosa resulting in varying degrees of inflammation, necrosis of individual cells of the epithelium, ulceration of the mucosa and epithelial hyperplasia. This interpretation is consistent with the observation that females given up to and including 1000 mg/kg/day had no urinary crystals/calculi in the bladders and none of the treatment-related bladder lesions observed in the affected males. A lower incidence of aggregates of mononuclear cells occurred in the urinary bladder of males given 750 mg/kg/day than that of the controls and was statistically identified. This was interpreted to be a secondary effect as a result of the significant degree of inflammation of the urinary bladder in males given 750 mg/kg/day that masked the small and often inconspicuous aggregates of mononuclear cells in the urinary bladder.

In the kidneys, treatment-related degeneration with regeneration of cortical tubules was observed in males at 750 mg/kg/day. This was characterised by the presence of small tubular epithelial cells that did not have the volume and eosinophilic staining of normal tubular epithelium while regeneration was characterised by increased numbers of similar appearing small tubular epithelial cells. The pathogenesis of this treatment-related change is not clear. Glomeruli in affected regions were atrophic but were not sclerotic or hyalinized. Tubules in kidneys of some males at 750 mg/kg/day without degeneration/regeneration demonstrated that was also interpreted to be treatment related. This lesion was characterised by an increase in the size and appearance of the tubular lumen and a flattening of the lining tubular epithelium. Enlarged cells (hypertrophy), interpreted to be intercalated cells, were microscopically observed in the collecting ducts in males and fermales at 750/1000 mg/kg/day and in some females at 250 mg/kg/day. This effect was characterised by an enlargement of the epithelial cells, estimated to be 3 to 5 times the normal size that had a granular, pale eosinophilic cytoplasm which protruded into the lumen of the collecting ducts. These cells occurred singly or up to 3 or 4 cells in a row, primarily in the outer zone of the medulla, in the region of the intersection of the inner and outer stripes and were consistent with intercalated cells. The nuclei of affected cells were also enlarged and on occasion the cells contained multiple nuclei. Hypertrophy of intercalated cells was interpreted to be a treatment-related effect that was likely compensatory in pathogenesis and not an adverse effect. Hypertrophy of intercalated cells has been reported to be a physiologic response to several factors affecting acid-base homeostasis, including acute respiratory acidosis, chronic metabolic acidosis, and hypokalaemia (Hansen *et al*., 1980; Madsen *et al*., 1991; Madsen and Tischer, 1983; 1984; 1986; Verlander *et al*., 1987).

Hansen, G. P., Tisher, C. C., and Robinson, R. R. (1980). Response of the Collecting Duct to Disturbances of Acid-Base and Potassium Balance. *Kidney Internat.* **17**, 326-337.

Madsen, K. M., Verlander, J. W., Kim, J., and Tisher, C. C. (1991). Morphological Adaptation of the Collecting Duct to Acid-Base Disturbances. *Kidney Internat.* **40 (**Suppl. 33), S57-S63.

Madsen, K. M. and Tisher, C. C. (1983). Cellular Response to Acute Respiratory Acidosis in Rat Medullary Collecting Duct. *Am. J. Physiol.* **245**(6), F670-F679.

Madsen, K. M. and Tisher, C. C. (1984). Response of Intercalated Cells of Rat Outer Medullary Collecting Duct to Chronic Metabolic Acidosis. *Lab. Invest.* **51**, 268-276.

Madsen, K. M. and Tisher, C. C. (1986). Structural-Functional Relationships Along the Distal Nephron. *Am. J. Physiol.* **250,** F1-F15.

Verlander, J. W., Kirsten M. M., and Tisher, C. C. (1987). Effect of acute respiratory acidosis on two populations of intercalated cells in rat cortical collecting duct. *Am. J. Physiol.* **253**(6), F1142-F1156

In the adrenals, seven males at 750 mg/kg/day had a slight hypertrophy of the zona glomerulosa of the cortex, which was characterised by a decrease in the normal vacuolation of the cells in the zona glomerulosa and an increase in cell size and eosinophilic staining of the cytoplasm as compared to the controls. Five of these males were found moribund and were killed prematurely and two died spontaneously. All of these males had significant kidney disease, either severe nephropathy (1/7) or the treatment-related moderate or severe tubular degeneration with regeneration (6/7) that was previously discussed. This adrenal change was interpreted being secondary to the significant degree of renal disease rather than a primary treatment-related effect.

There was an increased incidence of splenic atrophy in males at 750 mg/kg/day, characterised by a decrease in the overall size of the spleen due to decrease in the amount of splenic red and white pulp. Splenic atrophy only occurred in males that were killed prematurely due to their moribund condition or died spontaneously. There were no statistically identified differences in the splenic weights of males given 750 mg/kg/day that survived until the scheduled necropsy and in fact, they were slightly higher than that of the controls. Therefore, the splenic atrophy was interpreted to be secondary to non-specific stress possibly associated with exceeding the MTD and was not considered to be a primary adverse effect of treatment.

Table B.6.5.2–5 Selected microscopic non-neoplastic pathology findings: no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750	0	50	250	1000
No. of animals in group	50	50	50	50	50	50	50	50
Urinary bladder								
Aggregates of mononuclear cells slight or v.slight	31	31	32	11*	30	33	33	31
Inflammation, sub acute to chronic, focal or multifocal v. slight to moderate	2	2	0	4	0	0	0	0
Inflammation, sub acute to chronic, diffuse v. slight	0	3	1	1	0	0	0	0
slight	0	1	0	11	0	0	0	0
moderate	0	0	2	12*	0	0	0	0
severe	0	0	0	3				
Inflammation, acute, focal v. slight	0	0	0	1	0	0	0	0
Transitional epithelium:								
Mitotic figures, increased, slight or v. slight	0	0	0	10*	0	0	0	0
Hyperplasia, focal/multifocal/diffuse slight	0	1	0	7*	0	0	0	0
Necrosis slight or v. slight	0	1	0	11*	0	0	0	0
Ulcer	0	0	0	2	0	0	0	0
Calculi	0	0	2	10*	0	0	0	0
Kidneys								
Degeneration with regeneration, cortex, tubules, uni- or bilateral, diffuse or multifocal slight to severe	0	0	0	12*	0	0	0	0
Dilatation, tubule, bilateral v. slight to moderate	1	1	3	14*	1	0	0	0
Hypertrophy, intercalated cells, bilateral, multifocal v. slight to moderate	2	0	0	39*	1	1	8*	36*
Adrenal gland								
Hypertrophy, zona glomerulosa, cortex, bilateral slight	0	0	0	7*	0	0	0	0
Spleen								
Atrophy	1	2	2	15*	2	2	1	4

*significantly different from control $p<0.05$

The microscopic neoplastic pathology findings are presented in Table B.6.5.2–6. There were no tumours that were considered to be related to XDE-729 Acid treatment. However, the distribution of liver tumours across the dose groups and presence of haemangiosarcoma in skeletal muscle of two females at 400 mg/kg/day requires a more detailed comment.

The incidence of liver adenomas in males of the XDE-729 Acid treated groups of males was increased, with the incidence of males with one or more adenomas and/or carcinomas being statistically significantly higher than controls at the highest dose level. Despite the significant difference, the observations were considered to be chance findings, unrelated XDE-729 Acid treatment, because clear dose response relationships were not present. This interpretation receives additional support from a reference to historical control data, which show that the incidence of liver tumours in the treated males is similar to that observed in control males in two carcinogenicity studies in CD1 mice recently conducted by the test facility

Regarding haemangiosarcoma, two females at 1000 mg/kg/day had primary haemangiosarcoma in skeletal muscle, a rare tumour type not seen in control males or females in two carcinogenicity studies in CD1 mice recently conducted by the test facility (although one control male with a secondary haemangiosarcoma in skeletal muscle in one study was reported). For the following reasons, these tumours in muscle were considered not to be treatment-related. Firstly, haemangiosarcomas are malignant neoplasms of endothelial cells which can arise in any organ, and can be observed in multiple sites including spleen, uterus, liver and bone marrow in CD-1 mice (Chandra and Frith, 1992). Indeed, in the present study, haemangiosarcomas in control mice were observed in different organs including the liver in one male, multiple organs in two males (liver/heart in one and liver/spleen in another), ovary in one female, uterus in one female, skin and subcutis in one male, and the spleen in one male. The specific location of the haemangiosarcoma (i.e. skeletal muscle) should not be judged as a cause for concern, particularly when the incidence is so low (2/50) because if XDE-729 Acid were to induce malignant transformation of endothelial cells, one would reasonably expect haemangiosarcomas in significantly higher numbers in other organs (such as liver, spleen etc.) of the high dose females as compared to the controls. Actually, the combined incidence of haemangiosarcomas in different organs of control (3/50) and 400 mg/kg/day (4/50) females was very similar. Finally, the overall toxicity profile of XDE-729 Acid indicates there are no treatment-related effects on skeletal muscle, so there is no reason to suspect indirect or direct involvement of skeletal muscle in the formation of a low incidence of haemangiosarcoma in the skeletal muscle of the high dose females.

Reference

Chandra M, Frith CH (1992) Spontaneous neoplasms in aged CD-1 mice. *Toxicology Letters*, **61**, 67-74.

Table B.6.5.2–6 Microscopic neoplastic pathology findings: no. of affected animals

Finding	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750	0	50	250	1000
No. of animals in group	50	50	50	50	50	50	50	50
Adrenal glands								
Adenoma	2	0	1	1	0	0	0	1
Carcinoma	0	0	0	0	0	1	0	0
Bone marrow								
Haemangioma; benign	2	0	0	0	0	0	0	0
Cervix								
Leiomyoma; benign					0	0	1	0
Leiomyosarcoma; malignant					1	0	0	0
Stromal cell sarcoma; malignant					0	0	0	1
Duodenum								
Adenoma	0	0	0	1	0	0	0	0
Haematopoietic/lymphoid system								
Lymphosarcoma; malignant	1	1	2	1	6	2	4	1
Lacrimal/extraorbital gland								
Schwannoma; malignant	1	0	0	1	0	0	0	0
Lacrimal/Harderian gland								
Ademona	5	0	2	3	1	1	1	1
Liver								
Adenoma, hepatocyte	4	9	8	8	1	0	0	0
Historical controls	9/50, 12/50 in 2 studies[a]				Not reported			
Carcinoma, hepatocyte	0	2	2	1	0	0	0	0
Historical controls	1/50, 2/50 in 2 studies[a]				Not reported			
Total no. with adenoma and/or carcinoma	4	10	10	9*	1	0	0	0
Historical controls	10/50, 13/50 in 2 studies[a]				Not reported			
Haemangiosarcoma; malignant	1	2	1	0	1	2	0	0
Lymphosarcoma; malignant	0	1	0	0	0	0	0	0
Lung								
Adenocarcinoma; bronchiolo-alveolar	2	5	2	4	1	0	2	0
Adenoma; bronchiolo-alveolar	10	2	6	11	6	2	4	4
Carcinoma; cortex	0	0	0	0	0	1	0	0
Lymph node, mediastinal								
Adenocarcinoma; bronchiolo-alveolar	0	0	0	1	0	0	0	0
Histiocytic sarcoma; malignant	0	1	0	0	0	0	0	0
Lymph node, mesenteric								
Haemangioma; benign	0	0	0	0	1	0	0	0
Mammary gland								
Adenocarcinoma					0	0	1	0
Adenoma					0	0	1	0
Mediastinal tissue								
Adenocarcinoma; bronchiolo-alveolar	0	0	0	0	1	0	0	0
Multiple organs								

Adenocarcinoma; malignant	0	1	1	0	0	0	0	0
Carcinoma; follicular cell	0	0	1	0	0	0	0	0
Haemangiosarcoma; malignant	2	0	0	1	0	0	0	1
Histiocytic sarcoma; malignant	0	1	0	0	0	0	0	0
Stromal cell sarcoma; malignant	0	0	0	0	2	0	0	0
Lymphosarcoma; malignant	1	0	2	1	6	1	3	1
Oral tissue								
Papilloma; lip; benign	0	0	0	0	1	0	0	0
Ovaries								
Adenoma, cystic					1	0	0	0
Haemangiosarcoma; malignant					1	0	0	0
Pituitary gland								
Adenoma	0	0	0	0	1	1	0	0
Skeletal muscle								
Haemangiosarcoma; malignant	0	0	0	0	0	0	0	2
Historical controls	0/50, 0/50 in 2 studies[a]				0/50, 0/50 in 2 studies[a]			
Skin and subcutis								
Haemangiosarcoma; malignant	1	0	0	0	0	0	0	0
Spleen								
Haemangiosarcoma; malignant	1	0	0	1	0	1	0	0
Stomach								
Carcinoma	1	0	0	0	0	0	0	0
Testes								
Interstitial cell adenoma	1	0	0	0				
Thyroid glands								
Adenocarcinoma; follicular cell	0	0	1	1	0	0	0	0
Adenoma; follicular cell	1	0	0	0	0	0	0	0
Uterus								
Endometrial stromal polyp					1	2	0	1
Hemangioma; benign					1	1	0	0
Hemangiosarcoma; malignant					1	1	1	2
Leiomyoma; benign					0	0	0	1
Leiomyosarcoma; malignant					0	0	0	1
Stromal cell carcinoma; malignant					3	0	0	2

*significantly different from control p<0.05 (Peto mortality adjusted test)
[a]the two earlier studies were conducted in 2007 and 2010

The toxicokinetic findings are summarised in Table B.6.5.2-7. The plasma and urine concentration of XDE-729 Acid, measured at 6 and 12 months, were approximately dose-proportional in both genders.

Table B.6.5.2–7 Toxicokinetic investigation: mean plasma and urinary concentrations of XDE-729 Acid

Finding	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750	0	50	250	1000
6 months								
Actual dose of XDE-729 Acid at time of blood/urine sampling (mg/kg/day)	0	52.3	258	805	0	56.2	246	888
XDE-729 Acid plasma conc. (µg/g)	NQ	4.59	19.57	44.95	NQ	2.19	11.85	32.18
XDE-729 Acid urine conc. (µg/g)	<1	506	3680	8796	NQ	267	2148	4993
12 months								
Actual dose of XDE-729 Acid at time of blood/urine sampling (mg/kg/day)	0	46.2	240	792	0	56.3	228	838
XDE-729 Acid plasma conc. (µg/g)	NQ	4.20	30.78	62.30	NQ	2.57	19.37	67.18
XDE-729 Acid urine conc. (µg/g)	<1	563	2433	6358	NQ	115	1574	3723

NQ = not quantifiable

CONCLUSION

Dietary administration of XDE-729 Acid to mice for 18 months causes non-neoplastic changes, but is not carcinogenic in this species.

Regarding the non-neoplastic effects, the urinary bladder and kidneys are identified as the principal toxicity targets, affecting males. At 250 mg/kg/day and above inflammation of the urinary bladder and microscopic calculi were present in the urinary bladder lumen of males. At 750 mg/kg/day the effects on the urinary bladder were additionally characterised by hyperplasia, increased numbers of mitotic figures, necrosis of individual cells and ulceration of the transitional epithelium; also, kidney tubular degeneration with regeneration and dilatation were present. The urinary bladder and kidney toxicity was associated with increased mortality among males at 750 mg/kg/day. Treatment-related hypertrophy of the intercalated cells of the kidney was present in males at 750 mg/kg/day and females at 250 and 1000 mg/kg/day; this was interpreted as being an adaptive response, possibly related to maintaining acid-base homeostasis. Study NOAELs of 50 mg/kg/day in males and 1004 mg/kg/day (target 1000 mg/kg/day) in females are identified for non-neoplastic toxicity.

A toxicokinetic investigation indicated that systemic bioavailability and urinary excretion of XDE-729 Acid are generally dose-proportional.

[redacted] (2012)

B.6.5.3 Mode of action (MoA) and supporting data in the rat and mouse

XDE-729 Acid & XDE-729 Methyl	
Study	IIA 5.5.4/01 Hepatic gene expression and biomarker analyses in male F344/DuCrl rats administered XDE-729 Acid or XDE-729 Methyl in diet for 7 days
Reference	[redacted] (2012)
Date performed	May 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111088
Guideline(s)	N/A
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51, TSN031117-0004, 97.2% purity XDE-729 Acid, Lot #E2837-52, TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Male F334/DuCrl rats, 9-10 weeks old at the start of the study, were randomly assigned to the test groups as shown in the table below.

Table B.6.5.3–1 Study design

Test group	Treatment (dietary administration)	Number of males
1	Control	4
2	XDE-729 Methyl 782 mg/kg/day	4
3	XDE-729 Acid 750 mg/kg/day	4

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substances in the diet for the period of use was established prior to study commencement. The dose level for the XDE-729 Methyl group was selected as a dose expected to elicit liver and thyroid responses, the equivalent dose in terms of XDE-729 was selected for the XDE-729 Acid group.

General clinical observations were recorded at least once daily. Bodyweights and food consumption were monitored.

Prior to necropsy, non-fasted blood samples were taken from the jugular vein and 24-hour urine samples were taken from all animals. Liver samples were taken at necropsy, and flash frozen in liquid nitrogen. Blood, urine and liver samples were analysed for levels of parent compound, ester, and known major metabolites (O-demethylated metabolite and its conjugates, as well as the acyl glucuronide of XDE-729 Acid) using HPLC/ESI-MS/MS.

A limited necropsy was conducted on all animals the end of the 7 day treatment period, after killing by decapitation under CO_2/O_2 anaesthesia. Liver and thyroids were removed and samples processed for microscopic (light) examination.

Liver tissue from all animals was preserved in *RNA*later and gene expression analysis was conducted. RNA isolated from each individual animal was used in a reverse transcription reaction to create complementary DNA (cDNA) that then served as the template for assessment

of gene expression using a quantitative real-time polymerase chain reaction (QRTPCR) approach. The targeted gene expression studies were conducted using an Applied Biosystems (ABI) 7500 real-time Polymerase Chain Reaction system using Applied Biosystems TaqMan Gene Expression Assays. The following genes were selected as biomarkers for nuclear receptor activation and to aid in understanding possible involvement of other metabolic pathways in response to XDE-729 Methyl in F344/DuCrl rats:

1. *Cyp1a1* "AhR response gene" ABI TaqMan ID: Rn00487218_m1
2. *Cyp2b1* "CAR response gene" [*Cyp2b6* in humans, *Cyp2b10* in mice]. ABI TaqMan ID: Rn01457875_m1
3. *Cyp2b2* "CAR response gene" [*Cyp2b6* in humans, *Cyp2b10* in mice]. ABI TaqMan ID: Rn02786833_m1
4. *Cyp3a23/3a1* "PXR response gene" [aka, *Cyp3a3*; *Cyp3a4* in humans, *Cyp3a11* in mice]. ABI TaqMan ID: Rn03062228_m1
5. *Cyp4a22* "PPAR-α response gene" [aka *Cyp4a1*] ABI TaqMan ID: Rn00598510_ml
6. *Ugt1a6* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00756113_mH
7. *Ugt2b1* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00756519_m1
8. *Ugt1a1* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00754947_m1

Gene expression was quantified using the comparative Ct method (ΔΔCt). For this method, the amount of target mRNA is expressed relative to an endogenous reference mRNA (i.e. housekeeping gene such as beta-actin) and relative to a calibrator sample (i.e. vehicle control). For each individual sample well, the Ct of the housekeeping gene was subtracted from the Ct of the target gene, and a mean of these values is generated for each treatment group (ΔCt). The ΔCt results from each sample were subtracted from the vehicle control ΔCt values to generate a ΔΔCt value ($\Delta Ct_{treated} - \Delta Ct_{control} = \Delta\Delta Ct$). The expression of the amount of target mRNA, normalized to an endogenous reference, and relative to a calibrator (i.e., vehicle control), was reported as fold change compared to control by the following formula: fold = $2^{-\Delta\Delta Ct}$.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl or XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.5.3–2 Mean dose received (mg/kg/day)

Test substance	Target dose level (mg/kg/day)	Received dose (mg/kg/day)
XDE-729 Methyl	782	766
XDE-729 Acid	750	672

There were no treatment related deaths or clinical signs.

Reductions in bodyweight weight gain (by 31% vs. controls) and food consumption (15%) were observed in the XDE-729 Methyl group, which were considered to be treatment-related adverse effects (see Table B.6.5.3-3). Bodyweights and food consumption were unaffected in the XDE-729 Acid group.

Table B.6.5.3–3 Group mean bodyweights and food consumption, days 1-8 (g/animal/day)

Parameter	Treatment (mg/kg/day)		
	Control	XDE-729 Methyl (782)	XDE-729 Acid (750)
Bodgweight, day 1	191.3	191.5	191.5
Bodgweight, day 8	213.8	214.0	207.0
Bodyweight gain days 1-8	22.6	22.5	15.5
Food consumption days 1-8	15.6	14.7	13.2

Absolute and relative liver weights were increased, by about 18% compared with controls, in the XDE-729 Methyl group (see Table 6.5.3-4). These liver weight changes were associated with microscopically observed very slight panlobular hypertrophy of hepatocytes and very slight increases in the numbers of mitotic figures in the hepatocytes (see Table 6.5.3-5). Also, in the thyroid very slight diffuse hypertrophy of the collecting duct epithelium and very slight increased numbers of mitotic figures in follicular cells was observed in the XDE-729 Methyl group. There were no microscopic changes in the liver or thyroids of the XDE-729 Acid group.

Table B.6.5.3–4 Group mean liver and thyroid weights

Parameter	Treatment (mg/kg/day)		
	Control	XDE-729 Methyl (782)	XDE-729 Acid (750)
Final bodyweight (g)	213.8	214.0	207.0
Liver weight (g) [% increase vs. control]	9.05	10.65 [18%]	7.33
Liver weight (% bwt) [% increase vs. control]	4.22	4.97 [18%]	3.54
Thyroid weight (g)	0.0085	0.0110	0.0077
Thyroid weight (% bwt)	0.0040	0.0051	0.0037

Table B.6.5.3–5 Microscopic pathology findings: no. of affected animals

Parameter	Treatment (mg/kg/day)		
	Control	XDE-729 Methyl (782)	XDE-729 Acid (750)
Number examined	4	4	4
Liver			
Hypertrophy, hepatocellular panlobular very slight	0	4	0
Increased mitotic figures very slight	0	4	0
Thyroid			
Hypertrophy, epithelium, collecting duct, multifocal very slight	0	4	0
Increased number of mitotic figures, follicular cell, multifocal very slight	0	4	0

The results of the toxicokinetic investigations are summarised in Table B.6.5.3-6.

XDE-729 Methyl group: The parent was present in the liver and, at very low concentrations, in the blood. XDE-729 Acid was present in relatively higher (~3-fold) concentrations in the liver, and in significant concentrations in the blood, suggesting extensive conversion of the administered dose to XDE-729 Acid. Five metabolites were detected in liver; these were the glucuronide conjugate of O-demethyl XDE-729 Methyl, O-demethyl XDE-729 Acid and the sulphate, glucuronide and acyl glucuronide conjugates of the O-demethyl XDE-729 Acid

metabolite. In urine, only about 0.03% of the administered dose was excreted as parent, and 3.3% was excreted as a methyl metabolite, namely the glucuronide conjugate of O-demethyl XDE-729 Methyl. However, about 24.7% of the administered dose was excreted in urine as XDE-729 Acid, and a total of about 16.6% of the dose was excreted as O-demethyl XDE-729 Acid or its sulphate, glucuronide or acyl glucuronide conjugates.

XDE-729 Acid group: The parent was detected in the blood and liver, at levels slightly higher (~1.5-fold) that found in the XDE-729 Methyl treated group. XDE-729 Methyl was also found in the blood and liver, but only in trace amounts. The Applicant suspects that the presence of XDE-729 Methyl represents an artefact of the extraction method which used methanol as the solvent. The extraction method was changed for subsequent XDE-729 toxicokinetics studies; samples were collected in deuterated methanol to distinguish artefact methyl ester from XDE-729 methyl ester. None of the five identified metabolites identified in the XDE-729 Methyl group were present in blood or liver. In urine, 47.4% of the administered dose was excreted as parent, and a total of about 4.5% of the dose was excreted as O-demethyl XDE-729 Acid or its sulphate, glucuronide or acyl glucuronide conjugates.

The toxicokinetic investigations show that administered XDE-729 Methyl is extensively converted to XDE-729 Acid and excreted mainly as either XDE-729 Acid or as O-demethyl XDE-729 Acid and its sulphate, glucuronide or acyl glucuronide conjugates. O-demethylation of the parent followed by glucuronide conjugation constitutes a minor metabolic pathway for XDE-729 Methyl. In comparison, for administered XDE-729 Acid the extent of O-demethylation and subsequent sulphate, glucuronide or acyl glucuronide conjugation appears to be lower than is the case for XDE-729 Methyl.

Table B.6.5.3–6 Blood, liver and urine kinetics of XDE-729 Methyl, XDE-729 Acid and the major metabolites

Analyte	Treatment (mg/kg/day)		
	Control	XDE-729 Methyl (782)	XDE-729 Acid (750)
Blood, mean concentration of analyte as µg/g			
XDE-729 Methyl	NQ	0.04	0.03
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	NQ	NQ
XDE-729 Acid	NQ	10.29	17.97
O-demethyl XDE-729 Acid	NQ	NQ	NQ
Sulphate conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ
Glucuronide conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	NQ	NQ	NQ
Liver, mean concentration of analyte as µg/g			
XDE-729 Methyl	NQ	3.99	0.29
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	3.34	NQ
XDE-729 Acid	NQ	11.92	16.36
O-demethyl XDE-729 Acid	NQ	0.34	NQ
Sulphate conj. of O-demethyl XDE-729 Acid	NQ	2.29	NQ
Glucuronide conj. of O-demethyl XDE-729 Acid	NQ	4.91	NQ
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	NQ	2.82	NQ
Urine, total amount excreted in 24 h as mg/kg bw [as % of dose[1]]			
XDE-729 Methyl	NQ	0.2 [0.03%]	NQ
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	36.9 [3.3%]	NQ
XDE-729 Acid	NQ	181.4 [24.7%]	318.8 [47.4%]
O-demethyl XDE-729 Acid	NQ	3.9 [0.5%]	2.2 [0.3%]
Sulphate conj. of O-demethyl XDE-729 Acid	NQ	78.4 [8.9%]	18.1 [2.2%]
Glucuronide conj. of O-demethyl XDE-729 Acid	NQ	58.5 [5.3%]	4.1 [0.4%]
Acyl glucuronide conj. of O-demethyl XDE-729 Acid	NQ	21.5 [1.9%]	16.2 [1.6%]

NQ = not quantifiable
[1] based on mg equivalents of XDE-729 Methyl (Group 2) or mg equivalents of XDE-729 Acid (Group 3) excreted in urine in 24 h

The hepatic gene expression analysis results are summarised in Table B.6.5.3-6. For the XDE-729 Methyl group *Cyp1a1* and *Ugt1a6* expression were markedly increased, by over 20000- and 33-fold respectively, in comparison with controls. This profile is consistent with ligand-induced activation of the AhR pathway. Expression of *Cyp2b1* and *Cyp2b2* showed a modest increase (17-fold and 4-fold respectively) in the XDE-729 Methyl group; however, the magnitude of response is not consistent with a ligand-specific activation of the CAR pathway. *Cyp3a23* and *Cyp4a22* expression was not increased, indicating that the PXR and PPAR-α signalling pathways were not activated by XDE-729 Methyl. Similarly, no gene expression changes were noted for XDE-729 Methyl for the phase II glucuronidation-associated *Ugt1a1* or *Ugt2b1*. In contrast, in

the XDE-729 Acid group there were only minimal alterations in gene expression, considered to be of no toxicological significance.

Table B.6.5.3–7 Gene expression analysis in liver, shown as fold-change compared with controls[1]

Gene	Treatment (mg/kg/day)		
	Control	XDE-729 Methyl (782)	XDE-729 Acid (750)
Cyp1a1 AhR responsive	1	23970	4.2
Cyp2b1 CAR responsive	1	16.9	1.5
Cyp2b2 CAR responsive	1	4.0	1.3
Cyp3a23 PXR responsive	1	1.9	1.2
Cyp4a22 PPAR-α responsive	1	1.5	1.3
Ugt1a1 Thyroid hormone metab	1	1.4	1.2
Ugt1a6 Thyroid hormone metab.	1	33.6	1.9
Ugt2b1 Thyroid hormone metab.	1	3.7	1.4

[1] fold-change calculated after normalisation to an endogenous control gene (beta-actin).

CONCLUSION

Dietary administration of XDE-729 Methyl to the male rat for 7 days at a target dose level of 782 mg/kg/day elicited toxicity, observed as reduced bodyweight gain and food consumption, increased liver weight and minor microscopic changes in the liver (panlobular hypertrophy and increased number of mitotic figures in hepatocytes, graded very slight) and thyroids (diffuse hypertrophy of collecting duct epithelium and increased numbers of mitotic figures in follicular cells, graded very slight). In contrast, no evidence of toxicity was observed in male rats receiving the equivalent dose (target 750 mg/kg/day) of XDE-729 Acid.

The toxicokinetic investigations show that administered XDE-729 Methyl is extensively converted to XDE-729 Acid and excreted mainly as either XDE-729 Acid or as O-demethyl XDE-729 Acid and its sulphate, glucuronide or acyl glucuronide conjugates. O-demethylation of the parent followed by glucuronide conjugation constitutes a minor metabolic pathway for XDE-729 Methyl. By comparison, the extent of O-demethylation and subsequent sulphate, glucuronide or acyl glucuronide conjugation for administered XDE-729 Acid appears to be lower than is the case for XDE-729 Methyl.

Hepatic gene expression investigations provided evidence of activation of the AhR signalling pathway by XDE-729 Methyl, based on increased expression of *Cyp1a1* and *Ugt1a6* genes. In contrast, there was no evidence of activation of the AhR signalling pathway by XDE-729 Acid. For both XDE-729 Methyl and XDE-729 Acid, the hepatic gene expression profile indicates that the CAR, PXR or PPAR-α signalling pathways are not activated.

[redacted] (2012)

XDE-729 Methyl	
Study	IIA 5.5.4/02 7-day dietary toxicity probe study in Crl:CD1(ICR) mice
Reference	[redacted] (2012)
Date performed	November 2011
Test facility	[redacted]
Report reference	Laboratory study ID 110177
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Crl:CD1(ICR) mice, about 6-7 weeks old at the start of the study, were randomly assigned to the test groups as shown in the table below.

Table B.6.5.3–8: Study design

Test group	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of animals	
		Males	Females
1	0	5	5
2	261	5	5
3	782	5	5

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement.

General clinical observations were recorded at least once daily. Bodyweights and food consumption were monitored. Blood samples (non-fasted) were taken from the orbital sinus at the time of scheduled necropsy and a standard range of clinical chemistry parameters were measured.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from all animals (non-fasted) from the pedal vein at 06.00 h on day 7 and from obital sinus at necropsy (08.00-11.00 on day 8). Blood was analysed for XDE-729 Methyl, XDE-729 Acid, and XDE-729 Methyl minor metabolites: glucuronide conjugate of O-demethyl XDE-729, sulphate conjugate of O-demethyl DE-729, O-demethyl XDE-729, glucuronide conjugate of O-demethyl XDE-729 methyl, and acyl glucuronide conjugate of XDE-729 using HPLC. Spot urine samples were collected from all animals on day 29 for determination of XDE-729 Methyl and XDE-Acid using HPLC/ESI-MS/MS. Liver samples were taken at necropsy (approx 100-200 mg) and flash frozen by immersion in liquid nitrogen. Liver extracts were analyzed for XDE-729 Methyl, XDE-729 Acid and for XDE-729 Methyl minor metabolites (as for blood, above) using HPLC/ESI-MS/MS.

A limited necropsy was conducted on all animals the end of the 7 day treatment period. The weights of major organs were recorded. Macroscopic changes were recorded. A standard range of organs and tissues were removed and fixed. The liver and kidneys were weighed and processed for microscopic examination.

Liver tissue from all animals was preserved in *RNA*later and gene expression analysis was conducted. RNA isolated from each individual animal was used in a reverse transcription reaction to create complementary DNA (cDNA) that then served as the template for assessment of gene expression using a quantitative real-time polymerase chain reaction (QRTPCR) approach. The targeted gene expression studies were conducted using an Applied Biosystems (ABI) 7500 real-time Polymerase Chain Reaction system using Applied Biosystems TaqMan Gene Expression Assays. The following genes were selected to aid an understanding the potential mode of action and other possible metabolic pathways of XDE-729 Methyl in CH:CD(ICR) mice.

1. *Cyp1a1:* "AhR response gene" Mouse ABI TaqMan ID: Mm00487218_m1
2. *Cyp2b10:* "CAR response gene" [*Cyp2b6* in humans, *Cyp* 2b1 in rats]. Mouse ABI TagMan ID: Mm00456591_m1, a biomarker for the CAR signalling pathway
3. *Cyp3a11:* "PXR response gene" [(aka, *Cyp3a3*; *Cyp3a4* in humans, *Cyp3a23/3a1* in rats)]. Mouse ABI TaqMan ID: Mm00731567_m1
4. *Cyp4a10:* "PPAR-α response gene" Mouse ABI TaqMan ID: Mm01188913_gl

Gene expression was quantified using the comparative Ct method (ΔΔCt). For this method, the amount of target mRNA is expressed relative to an endogenous reference mRNA (i.e. housekeeping gene such as beta-actin) and relative to a calibrator sample (i.e. vehicle control). For each individual sample well, the Ct of the housekeeping gene was subtracted from the Ct of the target gene, and a mean of these values is generated for each treatment group (ΔCt). The ΔCt results from each sample were subtracted from the vehicle control ΔCt values to generate a ΔΔCt value ($\Delta Ct_{treated} - \Delta Ct_{control} = \Delta\Delta Ct$). The expression of the amount of target mRNA, normalized to an endogenous reference, and relative to a calibrator (i.e., vehicle control), was reported as fold change compared to control by the following formula: fold = $2^{-\Delta\Delta Ct}$.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.5.3-9 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Methyl (mg/kg/day)	261	782
Males	250	778
Females	276	863

There were no treatment related deaths. No treatment-related clinical signs were reported.

There were no treatment-related effects on bodyweights, food consumption or clinical chemistry parameters.

There were no treatment-related macroscopic or microscopic pathology findings. Absolute and relative liver weights were higher at 782 mg/kg/day in both males and females (see Table B.6.3.2-10). As the relative liver weight increase was less than 10% in comparison with controls, and there were no correlating microscopic findings, this organ weight difference was considered not to represent an adverse effect of treatment.

Table B.6.5.3-10 Group mean liver weights

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)					
	Males			Females		
	0	261	782	0	261	782
Final bodyweight (g)	30.0	29.0	30.5	23.8	23.7	22.9
Liver weight (g) [% increase vs. control]	1.664	1.656	1.849 [+11.1]	1.250	1.251	1.312 [+5.0]
Liver weight (% bwt) [% increase vs. control]	5.537	5.712	6.067 [9.6]	5.247	5.275	5.738 [9.4]

The toxicokinetic investigation showed that XDE-729 Methyl was present in the blood and urine at levels ~100- and ~9000-fold lower, respectively, than the levels of the metabolite XDE-72 Acid. Concentrations of XDE-729 methyl were considerably higher in liver than in blood (approximately 50-100-fold higher in liver). Concentrations of XDE-729 Acid in liver were similar to those in blood. XDE-729 Methyl and XDE-729 Acid exhibited generally linear kinetics in both sexes in blood, liver, and urine. Levels of both XDE-729 Methyl and XDE-729 Acid were generally lower in females compared to males, suggesting that female mice may eliminate XDE-729 Methyl more efficiently than male mice. These data show that in mice most of the parent XDE-729 Methyl is rapidly converted to XDE-729 Acid, as has been shown to occur in rats.

Table B.6.5.3-11 Toxicokinetic investigation: mean XDE-Methyl and XDE-Acid concentrations in blood, urine and liver

Sample	Analyte	Sampling time	Target dose level of XDE-729 Methyl (mg/kg/day)					
			Males			Females		
			0	261	782	0	261	782
Blood (µg/mL)	XDE-Methyl	Day 7	NQ	NQ	0.06	NQ	NQ	NQ
		Terminal	NQ	0.03	0.05	NQ	0.04	0.07
	XDE-Acid	Day 7	NQ	4.11	5.75	NQ	1.23	5.64
		Terminal	NQ	5.72	15.18	NQ	6.63	17.89
Urine (µg/mL)	XDE-Methyl	Day 7	NQ	NQ	0.20	NQ	NQ	0.24
	XDE-Acid	Day 7	NQ	1033	2377	NQ	636	1371
Liver (µg/g)	XDE-Methyl	Terminal	NQ	2.43	4.95	NQ	1.39	3.69
	XDE-Acid	Terminal	NQ	4.75	11.94	NQ	2.83	7.32

The glucuronide conjugate of O-demethyl XDE-729, sulphate conjugate of O-demethyl DE-729, O-demethyl XDE-729, glucuronide conjugate of O-demethyl XDE-729 methyl, and acyl glucuronide conjugate of XDE-729 were all detected in blood and liver samples for XDE-Methyl treated mice.

There were no treatment-related macroscopic microscopic pathology findings.

Examination of the hepatic gene expression responses for *Cyp1a1* in both males and females revealed a dose-responsive induction profile consistent with ligand-induced activation of the AhR pathway (see Table B.6.3.2-12). Relative to controls, *Cyp1a1* expression was markedly increased in both males and females. However, gene expression responses for *Cyp2b10, Cyp3a11*

and *Cyp4a10* suggests that XDE-729 Methyl does not activate the CAR, PXR and PPAR-α signalling pathways.

Table B.6.5.3.–12 Gene expression analysis in liver, shown as fold-change compared with controls

Gene	Target dose level of XDE-729 Methyl (mg/kg/day)					
	Males			Females		
	0	261	782	0	261	782
CYP1a1, AhR responsive	1	44.5	308.5	1	15.1	662.9
CYP2b10, CAR responsive	1	3.3	5.1	1	2.2	5.8
CYP3a11, PXR responsive	1	0.7	0.6	1	0.9	0.8
CYP4a10, PPAR-α responsive	1	0.7	0.7	1	0.9	0.5

CONCLUSION

Dietary administration of XDE-729 Methyl to mice for 7 days at target dose levels of 261 and 782 mg/kg/day for 7 days did not cause changes that are considered to be adverse, though liver weight increases were induced at the highest dose level. Nevertheless, hepatic gene expression investigations provided evidence of activation of the AhR signalling pathway in mice, based on the increased expression of *Cyp1a1*, as is the case in rats. The hepatic gene expression profile indicates that XDE-729 Methyl does not activate the CAR, PXR or PPAR-α signalling pathways. Study NOAELs of 778 mg/kg/day in males and 863 mg/kg/day in females (target 782 mg/kg/day) are identified. Comparison with the results of the 7 day in rats (LeBaron et al 2012) indicate that mice are less sensitive to XDE-729 Methyl induced liver toxicity and hepatic *Cyp1a1* gene expression than rats for short exposure period. Toxicokinetic investigations demonstrated that most of the parent XDE-729 Methyl is rapidly converted to XDE-729 Acid in mice, as has been shown to occur in rats.

[redacted] (2012)

XDE-729 Methyl	
Study	IIA 5.5.4/03 Evaluation of molecular and cellular changes in the livers of male F344/DuCrl rats after a 4 week dietary exposure and a 4 day or 28 day recovery period
Reference	[redacted] (2012)
Date performed	February – April 2012
Test facility	[redacted]
Report reference	Laboratory study ID 120037
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Male F334/DuCrl rats, 11-12 weeks old at the start of the study, were randomly assigned to the test groups as shown in the table below. Slightly older rats, in comparison to those used on previous XDE-729 Methyl studies, were used to minimise the background hepatocellular

proliferation that is usually present in younger rats which could reduce the power of the study to detect treatment-related changes.

Table B.6.5.3–13 Study design

Test group	Target dose level of XDE-729 Methyl, for 28 days (mg/kg/day)	Number of males		
		Main group, day 29 termination	4 day recovery	28 day recovery
1	0	5	3	5
2	3	5	-	-
3	10	5	3	-
4	52	5	-	5
5	261	5	-	5

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substances in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 6% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were recorded at least once daily. Bodyweights and food consumption were monitored.

All study animals (excluding the 4-day recovery group animals) were implanted with mini-osmotic pumps model 2ML1, (Alzet Corporation, Palo Alto, California) seven days prior to scheduled necropsy and infused with 5-bromo-2'-deoxyuridine (BrdU; a structural analog of thymidine). BrdU is used as a marker of hepatocellular proliferation.

Animals were submitted for necropsy on day 29 (main groups), day 33 (4 day recovery) or day 57 (28 day recovery). The liver and kidneys were weighed at necropsy. Liver tissue was preserved for microscopic examination, BrdU proliferation analysis (excluding 4-day recovery group animals), gene expression analysis and for toxicokinetic analysis.

In the hepatocellular proliferation analysis, a labeling index was calculated based on BrdU positive nuclei which were scored as percentages based on 1000 hepatocytes in each of four three hepatolobular zones: centrilobular, midzonal, periportal and panlobular regions.

Liver tissue from all animals was preserved in *RNA*later and gene expression analysis was conducted. RNA isolated from each individual animal was used in a reverse transcription reaction to create complementary DNA (cDNA) that then served as the template for assessment of gene expression using a quantitative real-time polymerase chain reaction (QRTPCR) approach. The targeted gene expression studies were conducted using an Applied Biosystems (ABI) 7500 real-time Polymerase Chain Reaction system using Applied Biosystems TaqMan Gene Expression Assays. The following genes were selected as biomarkers for nuclear receptor activation:

1. *Cyp1a1* "AhR response gene" ABI TaqMan ID: Rn00487218_m1
2. *Cyp1a2* "AhR response gene" ABI TaqMan ID: Rn00561082_m1
3. *Cyp2b1* "CAR response gene" [*Cyp2b6* in humans, *Cyp2b10* in mice]. ABI TaqMan ID: Rn01457875_m1

4. *Cyp3a23/3a1* "PXR response gene" [aka, *Cyp3a3*; *CYP3A4* in humans, *Cyp3a11* in mice]. ABI TaqMan ID: Rn03062228_m1
5. *Cyp4a22* "PPAR-α response gene" [aka, *Cyp4a1*] ABI TaqMan ID: Rn00598510_m1
6. *Ugt1a6* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00756113_mH
7. *Ugt1a1* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00754947_m1
8. *Ugt2b1* "Thyroid hormone metabolism-associated" ABI TaqMan ID: Rn00756519_m1

Gene expression was quantified using the comparative Ct method (ΔΔCt). For this method, the amount of target mRNA is expressed relative to an endogenous reference mRNA (i.e. housekeeping gene such as beta-actin) and relative to a calibrator sample (i.e. vehicle control). For each individual sample well, the Ct of the housekeeping gene was subtracted from the Ct of the target gene, and a mean of these values is generated for each treatment group (ΔCt). The ΔCt results from each sample were subtracted from the vehicle control ΔCt values to generate a ΔΔCt value ($\Delta Ct_{treated} - \Delta Ct_{control} = \Delta\Delta Ct$). The expression of the amount of target mRNA, normalized to an endogenous reference, and relative to a calibrator (i.e., vehicle control), was reported as fold change compared to control by the following formula: fold $= 2^{-\Delta\Delta Ct}$.

Toxicokinetic investigations were conducted at part of this study. Blood (non-fasted) and liver samples taken at necropsy and overnight urine samples obtained in the week prior to necropsy were analysed for XDE-729 Methyl and XDE-729 Acid using HPLC/ESI-MS/MS.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl or XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.5.3–14 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Methyl (mg/kg/day)	3	10	52	261
Main group, day 29 termination	3.18	10.6	55.5	280
4 day recovery group	-	10.3	-	-
28 day recovery group	-	-	53.7	277

There were no treatment related deaths or clinical signs. One male at 3 mg/kg/day was killed prematurely due to problems with the mini-osmotic pump surgical implantation.

There were no treatment-related effects on bodyweights or food consumption.

As shown in Table B.6.5.3-15, there was a treatment-related increase in liver weight at the highest dose level in the main group, by 19% (absolute weight) and 15% (bodyweight adjusted) in comparison with controls. Liver weights for the recovery groups were unaffected by XDE-729 Methyl treatment.

Table B.6.5.3–15: Group mean liver weights

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)				
	0	3	10	52	261
Main group, day 29 termination					
Final bodyweight (g)	303	304	300	299	304
Liver weight (g) [% increase vs. control]	11.3	10.9	11.2	11.6	13.5* [19%]
Liver weight (% bodyweight) [% increase vs. control]	3.74	3.60	3.75	3.88	4.45* [15%]
4 day recovery group					
Final bodyweight (g)	310	-	312		-
Liver weight (g)	11.0	-	11.3	-	-
Liver weight (% bodyweight)	3.54	-	3.62	-	-
28 day recovery group					
Final bodyweight (g)	335	-	-	345	331
Liver weight (g)	12.2	-	-	12.7	12.4
Liver weight (% bodyweight)	3.65	-	-	3.70	3.75

*significantly different from control, p≤0.05

The microscopic findings for the liver for animals of the main group are presented in Table B.6.5.3-16. At 52 and 261 mg/kg/day very slight or slight hepatocellular hypertrophy with altered tinctorial properties and increased numbers of mitotic figures were present, together with an increased incidence of very slight vacuolisation (consistent with fatty change). There were no treatment-related microscopic findings in the liver for the 4 day and 28 d recovery group animals.

Table B.6.5.3–16 Microscopic pathology findings for liver: no. of affected animals: main group

Finding		Target dose level of XDE-729 Methyl (mg/kg/day)				
		0	3	10	52	261
No. examined		5	5	5	5	5
Liver						
Hypertrophy, with altered tinctorial properties, centrilobular/midzonal	v slight	0	0	0	5	0
	slight	0	0	0	0	5
Increased number of mitotic figures	v slight	0	0	0	1	3
Vacuolization, consistent with fatty change, hepatocytes, multifocal	v slight	1	0	0	4	5

*significantly different from control, p≤0.05

The results of the BrdU hepatocellular proliferation analysis conducted on the main and 28 day recovery groups, using a manual method of counting BrdU positive nuclei, are presented in Table 6.5.3-17. There was evidence of treatment-related, though modest, hepatocyte proliferative response only in the main group at 261 mg/kg/day, seen as significantly increased labelling indices for all hepatolobular zones. In the main group the labelling indices were also significantly increased at 3 mg/kg/day for the panlobular and periportal zones and at 52 mg/kg/day for the periportal zone; however, in the absence of a dose-dependent pattern these differences were regarded as being unrelated to XDE-729 Methyl treatment.

The interpretation of the proliferation analysis results for the 28 day recovery group is less straightforward. Statistically significant and dose-dependent increases in the labeling indices were observed at both 52 and 261 mg/kg/day for all hepatolobular zones. The Applicant argues that these differences were not treatment-related and can be attributed to unusually low labelling indices in the recovery group controls, below that observed for the main group controls. The Applicant states that studies published in literature (but no references are presented) demonstrate that hepatic labelling index responses are stable in rats between 10 and 20 weeks of age, a range which encompasses the age of the animals for this study, and therefore it is appropriate to compare the results of the 28 day recovery 52 and 261 mg/kg/day groups with the main group controls. Such a comparison, in the opinion of the Applicant, shows that the response of the 28 day recovery group was within the background range at 52 mg/kg/day and close to background at 261 mg/kg/day; the high dose group findings indicate a reversibility of the of the modest increase seen at this dose level in the main group. The Applicant's interpretation is supported by the reversal of the treatment-related liver weight increases and microscopic pathology changes, observed at 261 mg/kg/day main group animals, in the 28 day recovery group. However, the RMS considers that a comparison with concurrent controls must be given priority, and it must be concluded that reversibility has not been convincingly demonstrated for hepatocellular proliferation as measured by BrdU labelling, although the RMS can agree that the absence of liver weight increases and of histopathological changes in the 28 day recovery group provide strong evidence that the hepatocellular proliferation is reversible.

Automated BrdU positive nuclei counting was also conducted, which showed a similar pattern of results to the manual counting, though the response of the main group at 261 mg/kg/day less than seen in the manual count.

Table B.6.5.3-17 Mean hepatocellular BrdU labelling index values (%)

Group	Hepatolobular zone	Target dose level of XDE-729 Methyl (mg/kg/day)				
		0	3	10	52	261
Main group, day 29 termination	Panlobular	3.69	5.26*	3.09	4.95	8.15*
	Periportal	4.78	7.28*	3.84	7.52*	12.82*
	Midzoneal	4.04	5.38	3.40	4.32	7.80*
	Centrilobular	2.24	3.13	2.04	3.00	3.84*
28 day recovery group	Panlobular	2.32	-	-	4.99*	6.61*
	Periportal	3.24	-	-	6.88*	8.32*
	Midzoneal	2.46	-	-	5.00*	6.40*
	Centrilobular	1.26	-	-	3.10*	5.12*

*significantly different from control, $p \leq 0.05$

The hepatic gene expression analysis results are summarised in Table 6.5.3-18. Gene expression changes were seen only in the main group. *Cyp1a1* and *Cyp1a2* expression was increased in a dose-dependent manner at 52 and 261 mg/kg/day, a profile consistent with ligand-induced activation of the AhR signalling pathway. These responses correlate with liver weight increases seen at 261 mg/kg/day and microscopic pathology changes seen at 52 and 261 mg/kg/day. A modest increase in *Cyp1a1* was also present at 10 mg/kg/day in the main group. Similarly, the response and magnitude of the response for *Ugt1a6* at 261 mg/kg/day in the main group is consistent with activation of the AhR signalling pathway with previously reported alterations in thyroid weights and microscopic pathology. *Cyp2b1* expression was modestly increased (7.9-fold) in the main group at 261 mg/kg/day. However the magnitude of induction is not consistent with a ligand-specific activation of the CAR pathway resulting in hepatic phenotypic alterations

as observed for prototypical CAR ligands and, furthermore, absence of a *Cyp2b1* response at 52 mg/kg/day is inconsistent with the presence of a hepatic hypertrophic response at this dose level; therefore these differences should not be regarded as evidence of activation of the CAR pathway by XDE-729 Methyl. *Cyp3a23* and *Cyp4a22* expression was not increased, indicating that the PXR and PPAR-α signalling pathways were not activated by XDE-729 Methyl. Similarly, no gene expression changes were noted for the phase II glucuronidation-associated *Ugt1a1* or *Ugt2b1*. The absence of gene expression responses in the recovery groups demonstrates that the XDE-729 Methyl induced activation of the AhR signalling pathway was transient.

Table B.6.5.3-18 Gene expression analysis in liver, shown as fold-change compared with controls

Group		Target dose level of XDE-729 Methyl (mg/kg/day)				
		0	3	10	52	261
Main group, day 29 termination	*Cyp1a1* AhR responsive	1	1.2	7.7	684	12180
	Cyp1a2 AhR responsive	1	1.1	1.7	4.2	21.9
	Cyp2b1 CAR responsive	1	1.5	1.1	0.8	7.9
	Cyp3a23 PXR responsive	1	0.8	0.8	0.9	1.1
	Cyp4a22 PPAR-α responsive	1	0.7	0.7	0.8	1.1
	Ugt1a1 Thyroid hormone metab	1	0.9	0.8	0.9	1.1
	Ugt1a6 Thyroid hormone metab.	1	1.2	1.2	1.4	10.8
	Ugt2b1 Thyroid hormone metab.	1	1.0	1.0	1.1	1.8
4 day recovery group	*Cyp1a1* AhR responsive	1	-	1.0	-	-
	Cyp1a2 AhR responsive	1	-	1.0	-	-
	Cyp2b1 CAR responsive	1	-	3.0	-	-
	Cyp3a23 PXR responsive	1	-	0.9	-	-
	Cyp4a22 PPAR-α responsive	1	-	0.5	-	-
	Ugt1a1 Thyroid hormone metab	1	-	0.8	-	-
	Ugt1a6 Thyroid hormone metab.	1	-	2.3	-	-
	Ugt2b1 Thyroid hormone metab.	1	-	1.8	-	-
28 day recovery group	*Cyp1a1* AhR responsive	1	-	-	1.5	1.5
	Cyp1a2 AhR responsive	1	-	-	1.0	0.9
	Cyp2b1 CAR responsive	1	-	-	1.2	1.9
	Cyp3a23 PXR responsive	1	-	-	0.9	0.9
	Cyp4a22 PPAR-α responsive	1	-	-	1.0	0.9
	Ugt1a1 Thyroid hormone metab	1	-	-	1.1	1.2
	Ugt1a6 Thyroid hormone metab.	1	-	-	1.0	1.5
	Ugt2b1 Thyroid hormone metab.	1	-	-	0.8	0.9

The results of the toxicokinetic investigations are summarised in Table B.6.5.3–19.

In blood, XDE-729 Methyl was not present in quantifiable amounts. In contrast, XDE-729 Acid was present in quantifiable amounts in the blood of the treated groups, in amounts that were approximately dose-proportional. In the liver, XDE-729 Methyl was quantifiable, at low levels, and dose proportional at doses of 10, 52, and 261 mg/kg/day. The primary metabolite, XDE-729 Acid, was by far the most abundant metabolite in the liver; this was present in all treated groups in quantities that increased with XDE-729 Methyl dose, though the increase was less than dose proportional at 261 mg/kg/day. The five additional metabolites investigated, which were demethylated and/or conjugated XDE-729 methyl and/or acid, were generally present at

quantifiable levels in the liver at all dose levels; all were present at relatively low levels (less than 18% of those of XDE-729 Acid) and all showed approximate dose-proportionality.

In the 4 and 28 day recovery groups, no XDE-729 Methyl or its metabolites was detected in the blood, liver or urine.

Table B.6.5.3-19 Blood, liver and urine kinetics of XDE-729 Methyl and its major metabolites, main group

Analyte	Target dose level of XDE-729 Methyl (mg/kg/day)				
	0	3	10	52	261
Blood, mean concentration of analyte as µg-eq/g					
XDE-729 Methyl	NQ	NQ	NQ	NQ	NQ
XDE-729 Acid	NQ	0.059	0.222	1.143	4.341
Liver, mean concentration of analyte as µg-eq/g					
XDE-729 Methyl	NQ	NQ	0.076	0.358	3.011
Glucuronide conj. of O-demethyl XDE-729 Methyl	NQ	0.019	0.057	0.341	2.452
XDE-729 Acid	NQ	0.343	1.273	4.567	13.447
O-demethyl XDE-729 Acid	NQ	0.003	0.010	0.040	0.145
Sulphate conjugate of O-demethyl XDE-729 Acid	NQ	NQ	0.021	0.106	0.741
Glucuronide conjugate of O-demethyl XDE-729 Acid	NQ	0.007	0.018	0.108	1.088
Acyl glucuronide conjugate of O-demethyl XDE-729 Acid	NQ	0.008	0.018	0.107	1.083
Urine, total amount excreted in 24 h as mg-eq/kg bw [as % of dose]					
XDE-729 Methyl	NQ	0.003 [0.1%]	0.005 [0.04%]	0.036 [0.07%]	0.185 [0.07%]
XDE-729 Acid	NQ	1.34 [43%]	5.27 [51%]	28.05 [52%]	131.8 [49%]

CONCLUSION

Dietary administration of XDE-729 Methyl to the male rat for 4 weeks elicited changes in liver, observed as increased organ weight at 261 mg/kg/day and microscopic pathology findings at 52 and 261 mg/kg/day (very slight or slight hepatocellular hypertrophy with altered tinctorial properties, increased numbers of mitotic figures, and increased incidence of very slight vacuolisation which was consistent with fatty change). These liver changes were not present in a 28 day recovery group. BrdU hepatocellular proliferation analysis demonstrated a modest proliferative response at 28 days in the 261 mg/kg/day group and also possibly at 52 mg/kg/day, though reversibility of this effect was not convincingly demonstrated.

Hepatic gene expression investigations provided evidence of activation of the AhR signalling pathway by XDE-729 Methyl, based on increased expression of *Cyp1a*, *Cyp1a2* and *Ugt1a6* genes after 4 weeks exposure. The dose-response relationships for these changes in gene expression correlates with the liver weight and microscopic pathology changes described above. Gene expression changes were not present in 4 or 28 day recovery groups.

The toxicokinetic investigations show that administered XDE-729 Methyl is extensively converted to XDE-729 Acid. About 50% of the administered dose is excreted in the urine as the XDE-729 Acid metabolite. The glucuronide conjugate of O-demethyl XDE-729 Methyl and O-

demethyl XDE-729 Acid and its sulphate, glucuronide and acyl glucuronide conjugate were confirmed as metabolites of XDE-729 Methyl. XDE-729 Methyl or its metabolites were not detected in the blood, liver or urine of 4 and 28 day recovery groups.

[redacted] (2012)

XDE-729 Acid & XDE-729 Methyl	
Study	IIA 5.5.4/04 Evaluation of AhR activation potential via luciferase reporter and ligand binding assays
Reference	[redacted] (2012)
Date performed	Completed December 2011
Test facility	[redacted]
Report reference	Laboratory study ID NS000063
Guideline(s)	None
Deviations from the guideline	N/A
GLP	No
Test material	XDE-729 Acid (RSMP# 1077053), XDE-729 Methyl technical (97.2%) (RSMP# 1076831), XDE-729 Methyl (99.1%) (RSMP#1076831), and XDE-729 Methyl ultrapure (99.7%) (RSMP#1082768)
Study acceptable	Yes, as a study providing supporting information

METHODS

Cell-based luciferase reporter assay

XDE-729 Acid, XDE-729 Methyl technical (97.2%), XDE-729 Methyl high purity (99.1%), and XDE-729 Methyl ultrapure (99.7%) were tested for AhR agonist activity in mouse (Hepa 1.1 cells) and human (Hep G2 40/6 cells) stable cell lines containing a luciferase reporter vector driven by dioxin-responsive elements that are responsive to AhR agonists. 2-(1′Hindole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE), a potent AhR agonist, was included as a positive control and a reference for relative potency. Based on the solubility of the test substances in the cell culture media, the concentrations tested were 0.001, 0.01, 0.1, 1, 10 or 50 µM. Duplicate testing was conducted.

Competition ligand-binding assay

To determine if XDE-729 Methyl ultrapure (99.7%) is an actual AhR ligand that binds directly to the ligand-binding pocket of the AhR, a photoaffinity ligand competition-binding assay was performed. The AhR photoaffinity ligand was 2-azido-3-[^{125}I]iodo-7,8-dibromodibenzo-*p*-dioxin (PAL). The source of the AhR was a hepatic cytosol preparation derived from homogenised male Sprague Dawley rat liver. ITE served as a positive control and a reference for relative potency. The concentrations of test substance and positive control tested in this assay were 0.01, 0.1, 1, 10, 50 µM. Duplicate testing was conducted. Protein samples were subject to SDS-PAGE analysis and transferred to membrane, the membrane was stained with Ponceau Red S to demonstrate the quality of the protein transfer. The membrane was subjected to autoradiography and the radioactive Ah receptor band detected and the bands excised and the number of cpm determined. Results were expressed as percent competition with the photoaffinity ligand.

RESULTS

Cell-based luciferase reporter assay

The results are summarised in Table B.6.5.3-20.

XDE-729 Acid did not exhibit any agonist activity in either the mouse or human reporter cell lines.

In contrast XDE-729 Methyl technical (97.2%), XDE-729 Methyl high purity (99.1%) and XDE-729 Methyl ultrapure (99.7%) all exhibited significant and dose-dependent agonist activity at 10 and 50 μM in the mouse reporter cell line. The agonist activity of the XDE-729 Methyl samples was lower in than for ITE, and not quantitatively influenced by the level of purity. None of the XDE-729 Methyl samples exhibited significant agonist activity in the human reporter cell line.

At 0.001 μM XDE-729 Methyl high purity (99.1%) in the mouse cell reporter line, luciferase activity appeared to be increased, an observation that did not correlate with the dose and was inconsistent with the observations for XDE-729 Methyl technical (97.2%). The reason for this anomalous result is not known.

Table B.6.5.3-20 Cell-based luciferase reporter assay (measuring AhR activation): mean luciferase activity (relative units)

Concentration of test substance (μM)	Test substance					
	ITE (+ve control)	XDE-729 Acid	XDE-729 Methyl (97.2%)	XDE-729 Methyl (99.1%)	ITE (+ve control)	XDE-729 Methyl (99.7%)
Mouse Hepa 1.1 cells						
0 (vehicle con.)	0.4	0.4	0.4	0.4	0.03	0.06
0.001	19	0.3	0.4	4.9		
0.01	31	0.4	0.4	0.3	7.9	0.1
0.1	50	0.3	0.4	0.4	12	0.2
1	52	0.3	0.5	0.9	13	0.2
10	62	0.4	4.3	4.4	16	1.2
50	103	0.4	19	18	24	7.3
Human Hep G2 40/6 cells						
0 (vehicle con.)	7.1	7.1	7.1	7.1	1.7	1.7
0.001	5.7	6.4	6.7	6.5		
0.01	96	6.7	6.0	6.7	39	2.6
0.1	112	6.6	6.0	6.2	46	2.2
1	122	6.6	6.1	6.3	49	2.5
10	170	6.2	6.5	6.9	67	2.5
50	158	7.0	10.2	10.3	31	4.0

This table shows the results of the first assays; the results of the duplicate assays were very similar
blank cell = that test substance concentration was not tested

Comment from France coRMS: it is unfortunate that XDE-729 Methyl was not tested in a cell-based luciferase reporter assay using rat reporter cell line, which would have allowed a comparison of the AhR transactivation potential between rat (the most sensitive species) and human. The Applicant has responded by stating the luciferase reporter assays should be regarded as preliminary MoA investigations, with the hepatocyte study using mouse, rat, and human

primary hepatocytes to investigate species sensitivity in *Cyp1a1* induction (Laethem; Murphy 2012, IIA 5.5.4/05) being the definitive study. Furthermore, it appears that rat reporter cell lines were not available in the testing laboratory.

Competition ligand-binding assay

The results are summarised in Table B.6.5.3-21.

A comparison of the EC_{50} values for ITE and XDE-729 Methyl ultrapure (99.7%), 8.4×10^{-7} M and (1.5×10^{-5} M) respectively, indicate that XDE-729 methyl ultrapure (99.7%) is an AhR ligand that weakly competes with the photoaffinity ligand for the rat Ah receptor. This result is consistent with the observation of AhR agonist activity for the XDE-729 Methyl samples in the luciferase reporter assay using a mouse cell line.

The source of the AhR was Sprague-Dawley rats, although the majority of the studies showing that XDE-729 Methyl targets the liver were conducted in the F344 rat. However, the XDE-729 Methyl rat developmental toxicity study (IIA 5.6.10/03) showed that the liver is also a target for XDE-729 Methyl in the Sprague-Dawley rat with similar potency to that observed in F334 rats, although the microscopic pathology findings differed[1]. Thus, it is reasonable to use the results of this competition ligand-binding assay to support the hypothesis that the XDE-729 Methyl induced liver toxicity observed in the F334 rat studies is AhR-mediated. Furthermore, both Sprague Dawley and Fischer 344 rat livers have shown induction of Cyp1a1 mRNA in response to the prototypical AhR activator, dioxin, following a single oral dose of 40 ng/kg body weight, with F344 rats displaying a higher induction of Cyp1a1 mRNA than the Sprague Dawley rats (Jana, NR, et al. 1998). Although direct ligand binding of XDE-729 methyl to Fischer 344 rat AhR was not conducted, gene expression results from 7-day, 28-day, and 90-day repeated dose toxicity studies demonstrated that there is a dose-responsive increase in the Cyp1a1 mRNA levels following exposure to XDE-729 methyl. Cyp1a1is a well-characterized target gene for regulation by AhR and serves as a sensitive biomarker for AhR activation (Nebert, et al 2000; Schwarz and Appel, 2005; Whitlock, J.P. 1999).

References

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Nebert DW, Roe A L, Dieter WA, Solis YY, Dalton T P (2000) Role of the Aromatic Hydrocarbon Receptor and [Ah] Gene Battery in the Oxidative Stress Response, Cell Cycle Control, and Apoptosis. *Biochemical Pharmacology* 59: 65-85.

Schwarz M, Appel KE (2005) Carcinogenic risks of dioxin: Mechanistic Considerations. *Regulatory Toxicology and Pharmacology*. 43: 19-34.
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[1]The liver microscopic pathology findings were: altered cytoplasmic homogeneity of centrilobular/midzonal hepatocytes in the Sprague-Dawley rat developmental toxicity study (IIA 5.6.10/03); hypertrophy of centrilobular/midzonal hepatocytes, increased number of mitotic figures, hepatocyte vacuolisation consistent with fatty change in the F344 rat 28 day study (IIA 5.3.1/02)

Table B.6.5.3-21 Competition ligand-binding assay (assessing ability to directly bind to AhR): % photo-affinity labelling

Concentration of test substance (µM)	Test substance			
	ITE (+ve control)		XDE-729 Methyl ultrapure (99.7%)	
	1st assay	2nd assay	1st assay	2nd assay
0 (vehicle control)	83.8	83.0	82.8	79.3
0.001	78.8	74.0	72.1	66.9
0.01	54.3	54.5	77.4	66.2
0.1	41.5	40.5	67.0	51.9
1	28.9	27.0	50.4	40.1
50	2.9	8.1	27.9	32.6
EC_{50}	8.4×10^{-7} M		1.5×10^{-5} M	

CONCLUSION

XDE-729 Methyl is a weak AhR agonist in a mouse reporter cell line and essentially has no activity in a human reporter cell line. The agonist activity of the XDE-729 Methyl samples was not quantitatively influenced by the level of purity. XDE-729 Acid has no AhR agonist activity in either the mouse or human reporter cell lines. A cell-free *in vitro* photoaffinity ligand competition-binding assay, using AhR sourced from Sprague Dawley male rat liver cytosol, demonstrated that XDE-729 Methyl is an AhR ligand in this species.

[redacted] (2012)

XDE-729 Methyl	
Study	HA 5.5.4/05 *In vitro* assessment of AhR nuclear receptor activation and Cyp1A and CYP1A induction potential of XDE-729 Methyl in primary hepatocyte cultures
Reference	[redacted]
Date performed	Completed August 2012
Test facility	[redacted] (TRL), P.O. Box 12137 Research [redacted]
Report reference	Laboratory study ID SP0014001
Guideline(s)	None
Deviations from the guideline	N/A
GLP	No
Test material	XDE-729 Methyl, Lot # E2837-51, TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

The objective of this study was to assess the potential of XDE-729 methyl to induce Cyp1a1 and Cyp1a2 in primary cultures of human, mouse and rat hepatocytes.

Evaluation of the degree of CYP induction was performed by measuring and comparing mRNA levels between the various treatment groups by RT-PCR (TaqMan). Freshly isolated human hepatocytes from six separate donors were cultured on collagen-coated plates and incubated with control inducers, vehicle controls, or test article for varying amounts of time up to 24 hrs. The same was done for mouse and rat hepatocytes using 3 separate pools of 3 mice and 3 rats each. Samples at 6 and 24 hours were also taken to assay for Lactate Dehydrogenase (LDH) release as a marker of toxicity using the Promega CytoTox-ONE™ Homogeneous Membrane Integrity System. Gene expression of Cyp1a1 and Cyp1a2 were analyzed using RNA prepared at the specified time points. Control induction experiments were conducted using a dose response of 3-methylcholanthrene (3-MC) and 1 mM phenobarbital (PB, used as an additional control to monitor the induction of a different CYP isoforms). The 3-MC controls were quantitated for CYP1A or Cyp1a expression levels and the PB samples were assayed for CYP2B or Cyp2b expression levels.

Cultures were treated with XDE-729 Methyl at seven concentrations: 0, 0.01, 0.1, 1, 10, 30, and 100 µM. mRNA expression measurements were made at 0, 2, 4, 6, 8, 12, and 24 hours of incubation.

RESULTS

The results of the Cyp1/CYP1 induction investigations in mouse, rat and human hepatocytes are summarised in Table B.6.5.3-22 and Figures B.6.5.3-1, B.6.5.3-2 and B.6.5.3-3.

XDE-729 Methyl elicited inductive Cyp1/CYP1 responses in hepatocytes from all species generally only at the two highest concentrations tested, 30 and 100 µM. For Cyp1a1, the rat was the most responsive, with human being the least responsive. For Cyp1b, the rat was again the most responsive; the mouse was the least responsive for Cyp1b, showing only a marginal (2-fold) response. For both enzymes, all species were much more sensitive to 3-MC than to XDE-729 Methyl. Temporally, maximum responses occurred in the mouse at 4-6 h, in the rat at 2-8 hours and in human at 2-8 h (CYP1A1) or 8-24 h (CYP1A2).

For the positive control, 3-MC, a dose responsive increase in Cyp1a1 and Cyp1a2 was observed in mouse, rat and human hepatocytes. Cyp1a1 was more responsive compared to Cyp1a2 in the mouse and human, but Cyp1a2 was induced to a greater extent in the rat. For Cyp1a1 induction, mouse was the most sensitive to 3-MC, followed by the human. The rat was most responsive to 3-MC with respect to Cyp1a2 induction, with the mouse being the least responsive. Rat was most responsive to 3-MC with respect to Cyp1a2 induction

The second positive control, PB, induced robust induction responses by Cyp2b in the rat and by CYP2B in human. However, the Cyp2b response in the mouse was very marginal (1.3-fold, rather than an anticipated ~10-fold response); the reason for this is unknown, but the fact that the mouse hepatocytes demonstrated strong Cyp1 responses to 3-MC indicated that biological functioning of the hepatocytes was probably not compromised.

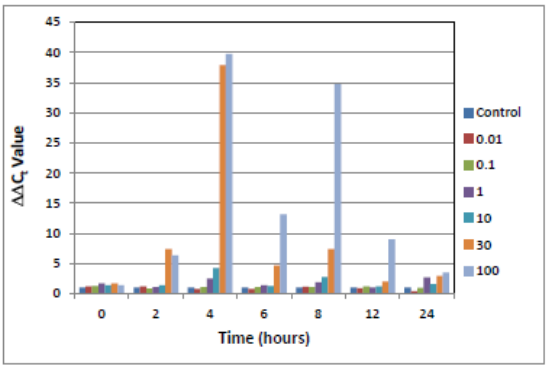
No significant cytotoxic effect was observed in any species with any treatment, which was demonstrated by the absence of LDH leakage (results not shown in this summary).

Table B.6.5.3-22 Maximum fold mRNA induction and EC_{50} values

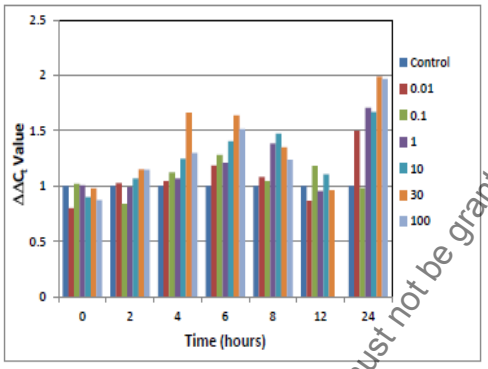
Hepatocyte culture	Maximum fold induction					EC_{50} (µM)			
	XDE-729 Methyl		3-MC		PB	XDE-729 Methyl		3-MC	
	Cyp1a1	Cyp1a2	Cyp1a1	Cyp1a2	Cyp2b	Cyp1a1	Cyp1a2	Cyp1a1	Cyp1a2
Mouse	37.8	2.0	1770	9.9	1.3	11.6	0.6	1.7	1.9
Rat	147.7	47.2	115.8	146.8	8.6	29.7	28.7	1.4	1.8
	CYP1A1	CYP1A2	CYP1A1	CYP1A2	CYP2B	CYP1A1	CYP1A2	CYP1A1	CYP1A2
Human 1	6.2	3.2	201.8	27.3	15.3	19.3	ND	0.8	0.7
Human 2	4.5	3.2	122.9	46.2	47.8	ND	ND	ND	ND
Human 3	34.0	10.4	232.4	44.7	20.3	19.1	11.7	2.1	0.4
Human 4	8.7	4.4	266.0	11.7	29.4	10.7	ND	0.6	0.01
Human 5	2.9	3.4	254.0	29.6	2.9	9.3	30.1	2.1	2.5
Human 6	10.3	1.1	108.7	12.7	10.1	64.8	20.3	1.3	1.9

Figure B.6.5.3-1 Mean $\Delta\Delta C_t$ values in mouse hepatocytes

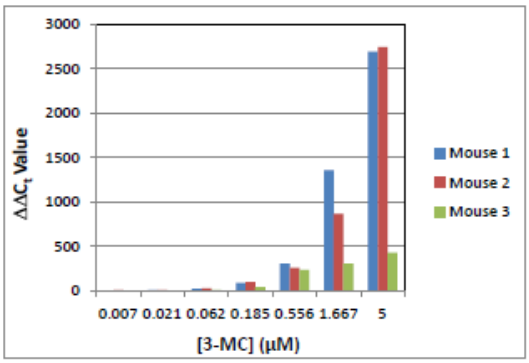
Cyp1a1 induction by XDE-729 Methyl



Cyp1a2 induction by XDE-729 Methyl



Cyp1a1 induction by 3-MC



Cyp1a2 induction by 3-MC

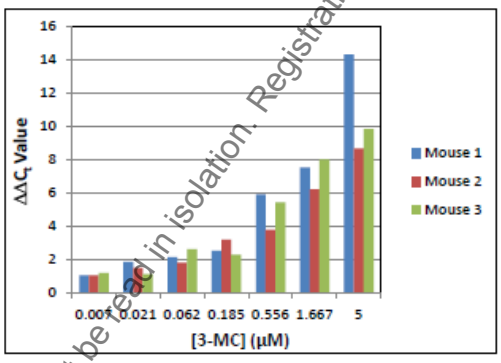
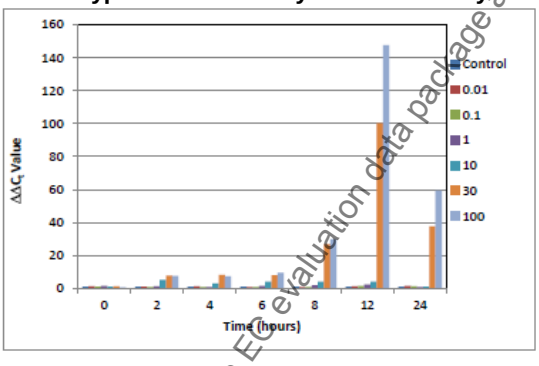
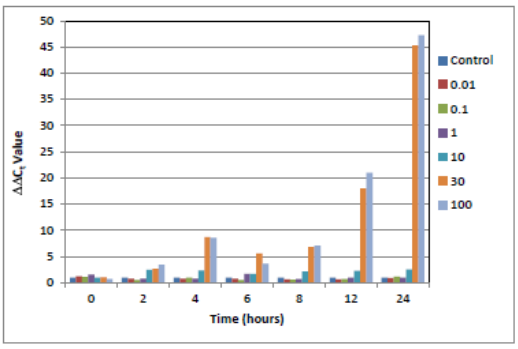


Figure B.6.5.3-2 Mean $\Delta\Delta C_t$ values in rat hepatocytes

Cyp1a1 induction by XDE-729 Methyl



Cyp1a2 induction by XDE-729 Methyl



Cyp1a1 induction by 3-MC

Cyp1a2 induction by 3-MC

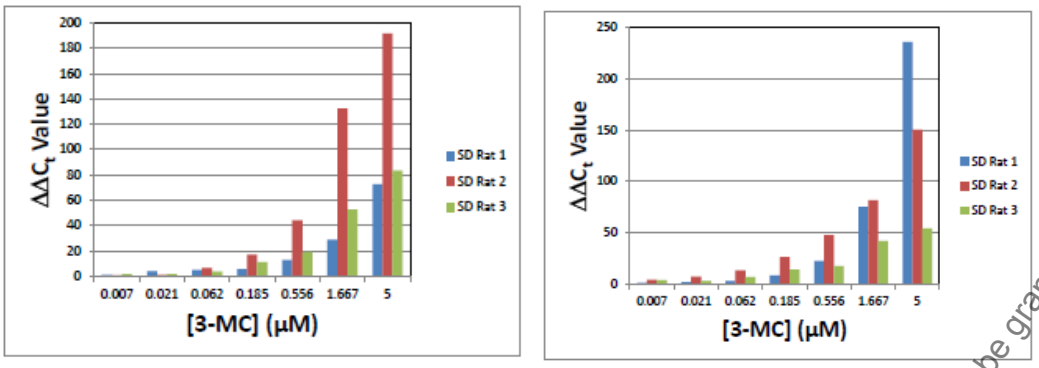
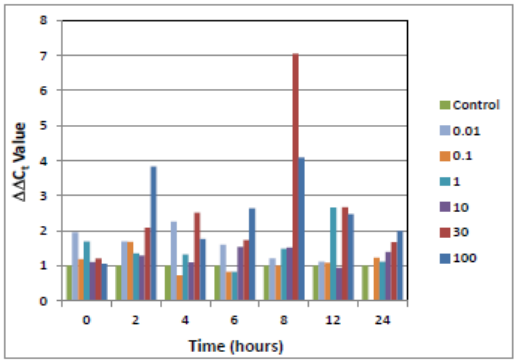
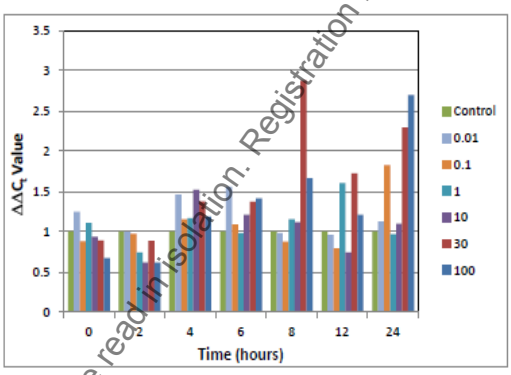


Figure B.6.5.3-3 Mean $\Delta\Delta C_t$ values in human hepatocytes

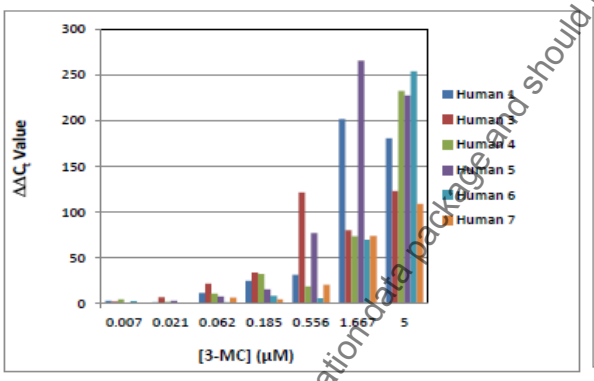
CYP1A1 induction by XDE-729 Methyl



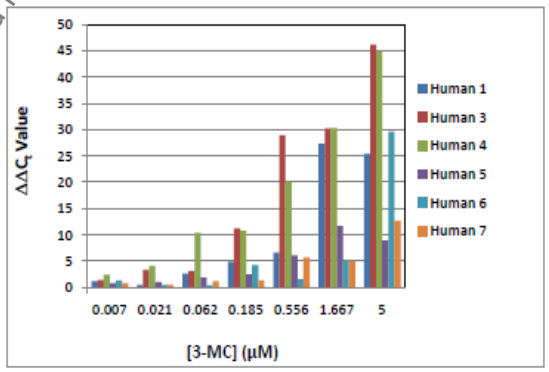
CYP1A2 induction by XDE-729 Methyl



CYP1A1 induction by 3-MC



CYP1A2 induction by 3-MC



CONCLUSION

XDE-729 Methyl induced Cyp1a1/CYP1A1 in rat, mouse and human hepatocytes, with the rat being the most sensitive and human being the least. XDE-729 Methyl induced Cyp1a2/CYP1A2 in rat, mouse and human hepatocytes, with the rat being the most sensitive and the mouse being the least. For both enzymes, all species were much more sensitive to induction by the positive control, 3-MC, than by XDE-729 Methyl.

The UK RMS notes that there is a lack of correlation between the results of this study, which showed some induction of CYP1A1/CYP1A2 in human hepatocytes by XDE-729 Methyl, and the cell-based luciferase reporter assay (Purdew 2012, IIA 5.5.4/06) in which XDE-729 Methyl had essentially no AhR agonist activity in a human reporter cell line. However, it should be

noted that induction of CYP1A1/CYP1A2 in human hepatocytes by XDE-729 Methyl appeared minimal when compared to the response to XDE-729 Methyl in the rat and the response to a prototypical AhR activator 3-MC by human hepatocytes. Also, the validity of a direct comparison between the transactivation data to the gene expression data is uncertain because the transactivation assays were conducted in a stably transfected continuously cultured cell line while the gene expression analysis was conducted in six different human primary hepatocyte donors.

[redacted] (author of report); [redacted] (author of MII summary) (2012)

XDE-729 Acid & XDE-729 Methyl	
Study	IIA 5.5.4/06 XDE 729 Methyl: Determination of *in vitro* hydrolysis rates in liver S9, blood and synthetic gastric fluid of mouse, rat and human, and physiologically-based pharmacokinetic simulations of systemic exposure in rats and humans
Reference	[redacted] (2012)
Date performed	Completed August 2012, revised report issued 28 June 2013
Test facility	[redacted]
Report reference	Laboratory study ID 110199
Guideline(s)	None
Deviations from the guideline	N/A
GLP	No
Test material	XDE-729 Methyl, Lot # E2837-51, TSN031117-0004, 97.2% purity, XDE-729, Lot # E2837-52, TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

In this study rates of metabolism of XDE-729 Methyl to XDE-729 Acid were determined by conducting *in vitro* incubations of XDE-729 Methyl in key physiological matrices (liver S9 cell fraction, whole blood, and synthetic gastric fluid (SGF)) of mouse, rat, and human. These rates were then incorporated into a whole-body, physiologically-based pharmacokinetic (PBPK) model to predict systemic exposures to XDE-729 Methyl and XDE-729 Acid in the blood and liver of rats and humans.

In vitro incubation with liver S9 fractions

XDE-729 Methyl was incubated with S9 cell fractions prepared from livers of mice (strain Crl:CD1(ICR)), rats (strain F344/DuCrl) and humans. Liver S9 was shipped from the vendor (Xenotech, LLC) on packages containing dry ice. Upon receipt, the liver S9 was transferred to freezers maintained at -80 °C. The *in vitro* incubations were conducted, at 37 °C in an incubating water bath. Incubations with rat liver S9 and human liver S9, were conducted at XDE-729 Methyl concentrations were 0.5, 1.0, 2.0, 5.0, and 10.0 µM. Incubations with mouse liver S9 were conducted at XDE-729 methyl concentrations of 0.25, 0.5, 1.0, 5.0, and 10.0 µM. Samples were taken from each incubation vessel at 0, 3, 8, 15, and 30 min (rat and human liver) or 0, 3, 8, 15, and 30, 60, and 120 min (mouse liver) and processed for analysis by HPLC/ESI-MS/MS for XDE-729 Methyl (substrate) and the primary metabolite XDE-729 Acid.

In vitro incubation with whole blood

XDE-729 Methyl was incubated with whole blood of mouse, rat, and human. The blood was received from the vendor (Bioreclamation, LLC) via overnight shipment, chilled on wet ice, one day after the blood was drawn and shipped by the vendor. Upon receipt, the blood was placed in a refrigerator, in the TERC facilities, where it was kept overnight pending the following day's scheduled incubation. Incubations with whole blood were conducted at 37 °C in an incubating water bath, in two sub-sets per species. In the first sub-set, whole blood (of each species) was incubated with XDE-729 Methyl at concentrations of 1 and 100 µM and duplicate time course samples were taken at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hours. In the second sub-set, whole blood was incubated with XDE-729 Methyl at concentrations of 0.25, 0.5, 1, 5, and 10 µM to allow mathematical derivations of 'saturable' (Michaelis-Menten) kinetics, if applicable. Time course samples were taken from the second series of whole blood incubations at 0, 0.05, 0.13, 0.25, 0.5, 1, and 2 hours. The samples were processed for analysis by HPLC/ESI-MS/MS for XDE-729 methyl (substrate) and the primary metabolite XDE-729 Acid.

In vitro incubations with SGF

Incubations with SGF prepared at pHs of 1.2, 3.0, and 4.0 were conducted at 37 °C in an incubating water bath at XDE-729 Methyl concentrations of 1 µM and 100 µM. The acidity of the SGF preparations were intended to approximate the pH of gastrointestinal fluids of human, rat and mouse, respectively, according to values reported in literature. Samples were taken from the incubations at 0, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hours and processed for analysis by HPLC/ESI-MS/MS for XDE-729 Methyl (substrate) and the primary metabolite XDE-729 Acid.

Computer simulations with PBPK model

The rates of conversion of XDE-729 Methyl to XDE-729 Acid determined from *in vitro* experiments with liver S9, whole blood and SGF were incorporated into a physiologically-based pharmacokinetic (PBPK) model that was used to generate predictive simulations of XDE-729 Methyl and XDE-729 Acid in tissues of interest (liver and blood) in rats and humans exposed to XDE-729 Methyl. The PBPK model is written in the acslX modeling language (AEgis Technologies, Huntsville, Alabama, USA) with the details of the model construct (PBPK flowchart, model code, etc.) described in a publication by Bartels *et al*., (2012[2]).

After all of the activities needed to adequately inform the PBPK model were completed (i.e. derivation of *in vitro*-defined rates of metabolism/hydrolysis, physiological allometric scaling, parameter estimation/optimisation, proofing of model against experimental dataset), the model was adapted to simulate various hypothetical exposure scenarios. The PBPK model runs were executed to simulate 28 days of dietary exposure of XDE-729 Methyl to rat and human at intakes of 0.001 (a predicted human exposure level according to the Applicant), 0.1 (an intake slightly higher than the proposed ADI) and 10 mg/kg/day (a proposed NOAEL for repeat dose toxicity). The model assumes alternating blocks of 12 hours feeding and 12 hours non-feeding. The primary model output variables from the model were concentrations over time of XDE-729 Methyl and XDE-729 Acid in liver and blood.

[2] Bartels et al. (2012) Development of PK- and PBPK-based modeling tools for derivation of biomonitoring guidance values. *Comput Methods Programs Biomed*, **108**: 773-788

RESULTS

In vitro incubations of XDE-729 Methyl in liver S9, blood and SGF

The results of the *in vitro* investigations of the metabolism of XDE-729 Methyl to XDE-729 Acid in liver, whole blood and SGF are summarised in Table B.6.5.3-23, which shows K_m and V_{max} values in liver and half lifes in blood and SGF.

In liver S9, XDE-729 Methyl was rapidly hydrolysed to XDE-729 Acid in all species, with the relative rates being human >> rat > mouse. The rate of hydrolysis was so rapid that XDE-729 Methyl was not detected after 8 minutes of incubation. The metabolism of XDE-729 Methyl in incubations with liver S9 appeared saturable (i.e. followed Michaelis-Menten kinetics), which is in contrast to the *in vitro* findings for blood and SGF which followed simple 1st order kinetics. A good mass balance was obtained from analyses of liver S9 incubation media for the parent XDE-729 Methyl and the hydrolysis product XDE-729 Acid in that the sum of the molar concentrations of the ester and acid yielded values that were approximately equivalent (within ± 10%) to the concentration of the XDE-729 Methyl substrate in incubation media at the start of the incubations. This indicates that XDE-729 Acid was the primary metabolite of XDE-729 Methyl, and furthermore, that there was minimal downstream metabolism by liver S9 of XDE-729 Acid.

In whole blood, hydrolysis of XDE-729 Methyl to XDE-729 Acid followed 1st order kinetics (i.e. rates were proportional to substrate concentration) and the rates did not appear to be saturable (plateau) over the range of concentrations tested. There was a clear difference observed in rates of hydrolysis of XDE-729 Methyl in blood of the three species, with the fastest rate occurring in blood of rat > mouse >> human. As was the case for incubations with liver S9, a good mass balance was obtained from analyses of whole blood for the parent XDE-729 Methyl and the metabolic product XDE-729 Acid indicating that XDE-729 Acid was the primary metabolite of XDE-729 Methyl.

The rate of hydrolysis of XDE-729 Methyl by SGF was the slowest of all test media evaluated. As for blood, 1st order kinetics were followed. The fastest rate of conversion occurred in SGF at pH 1.2 (representing the pH of human GI tract), with a half-life of approximately 100 hours, but much slower than the rates of metabolism determined for whole blood and liver S9.

Following appropriate scaling from *in vitro*- to *in vivo*-specific values and adjustments based on parameter estimation, these species-specific kinetic parameters were incorporated into the PBPK model described in the next section.

Table B.6.5.3-23 Summary of results of *in vitro* investigations of metabolism of XDE-729 Methyl to XDE-729 Acid in liver, whole blood and SGF

Parameter	Species		
	Rat	Mouse	Human
Liver			
Km (µM)	16..1	5.60	118
V_{max} (µmol/hr/mg S9 protein)	0.147	0.0214	3.12
Blood			
Mean half life in blood (hours)	0.26 ± 0.08	1.32 ± 0.38	35.7 ± 22.1
Synthetic gastric fluid			
Mean half life in SGF (hour)	725 at pH 3	2573 at pH 4	92.9 at pH 1.2

Computer simulations with PBPK model

The primary outputs of the simulations, which are concentrations over time of XDE-729 Methyl and XDE-729 Acid in liver and blood, are shown in Figures B.6.5.3-3, B.6.5.3-4 and B.6.5.3-5.

The simulated results indicate that XDE-729 Methyl and XDE-729 Acid achieve their steady state concentrations soon after exposure is begun, and that neither XDE-729 Methyl nor XDE-729 Acid would be expected to accumulate in either liver or blood following 28 days of dietary intake of XDE-729 Methyl. This is due to the rapid metabolism of XDE-729 Methyl (to XDE-729 Acid) and to the rapid elimination (via urine) of XDE-729 Acid. The peak concentrations of XDE-729 Methyl from a simulated 28-day exposure to XDE-729 Methyl at 0.0001 mg/kg/day were predicted to be 10000- to 15000-times lower than the limits of quantitation in humans; and at 0.1 mg/kg/day, predicted levels of XDE-729 Methyl in humans that are 10- to 15-times lower than the limits of quantitation (LOQs: in blood ~0.01 µg XDE-729 Methyl/g; in liver ~0.04 µg XDE-729 Methyl/g).

Figure B.6.5.3-3: PBPK model simulation of 28-day repeated dietary intake by rats and humans of 10 mg/kg/day XDE-729 Methyl

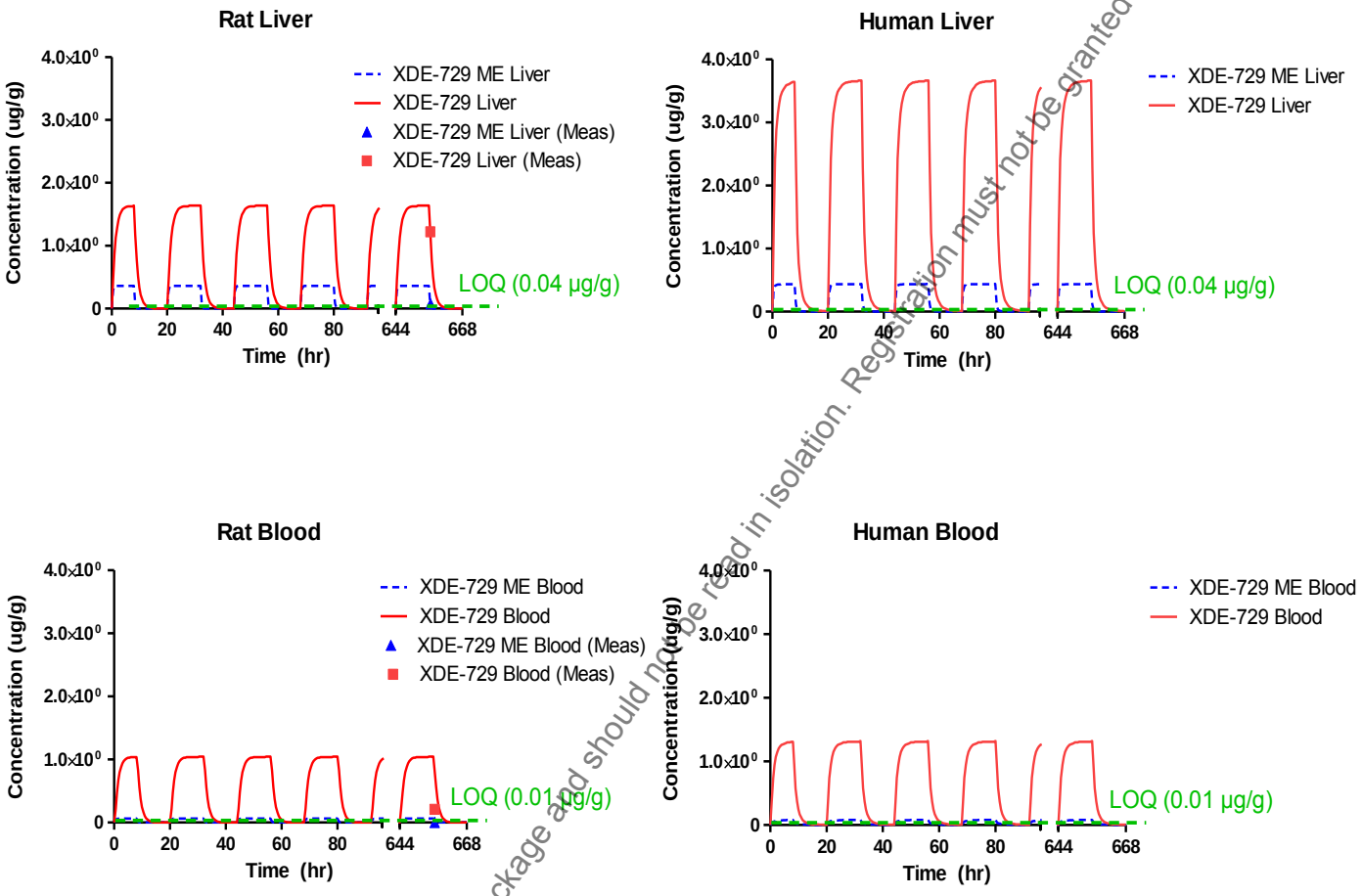


Figure B.6.5.3-4: PBPK model simulation of 28-day repeated dietary intake by rats and humans of 0.1 mg/kg/day XDE-729 Methyl

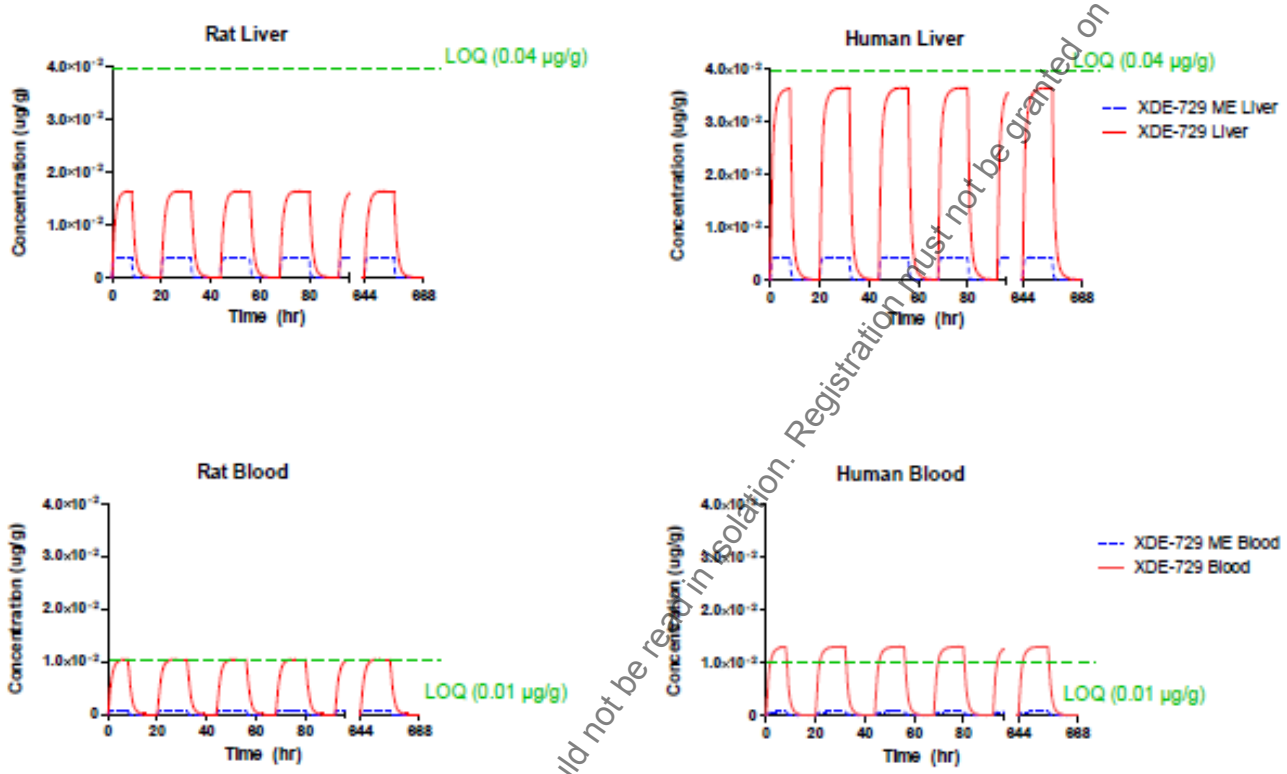
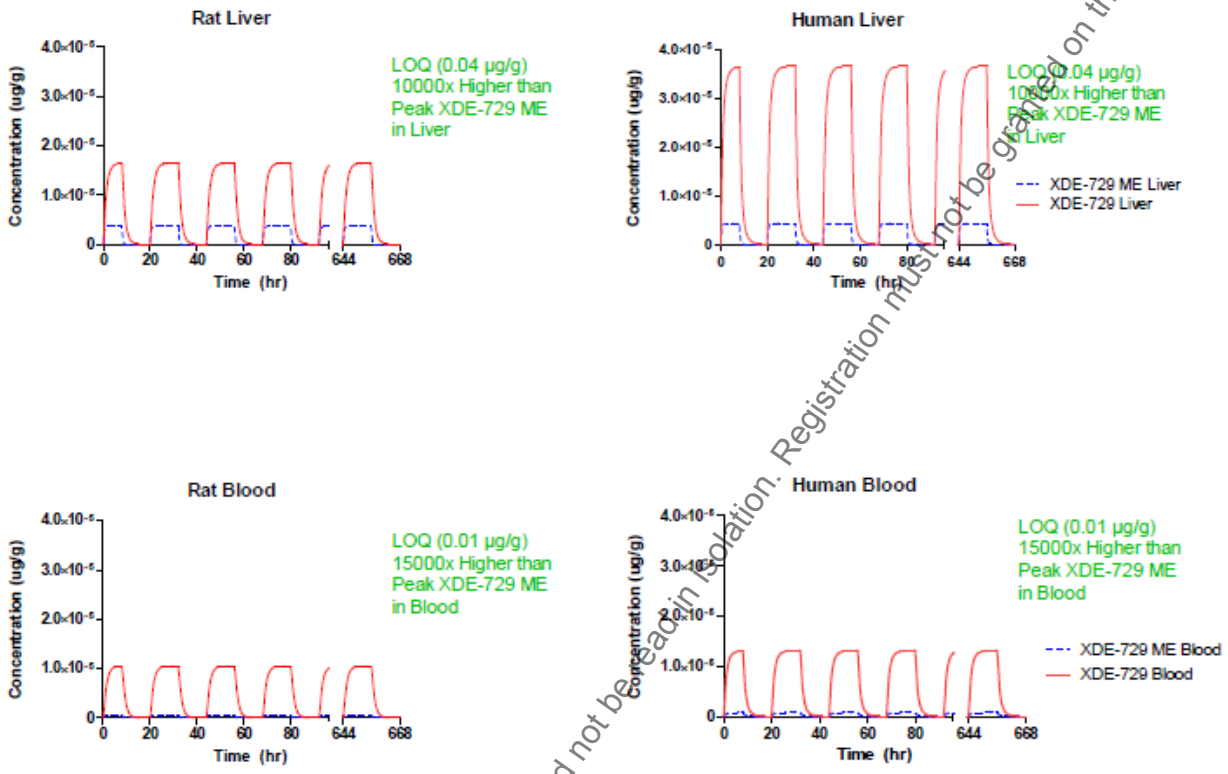


Figure B.6.5.3-5: PBPK model simulation of 28-day repeated dietary intake by rats and humans of 0.001 mg/kg/day XDE-729 Methyl



Based on the *in vitro*-derived and rate constant for 1st order metabolism of XDE-729 Methyl in blood, and Michaelis-Menten values (K_m and V_{max}) for liver metabolism, calculations were made to assess 'instantaneous' rates of metabolism in liver and blood, which are shown in Table B.6.5.3-24. Those calculations show that the liver is the primary organ responsible for the *in vivo* hydrolysis of XDE-729 Methyl. The rates of hydrolysis are ~120-fold and ~25000-fold faster in liver than in blood of rats and humans, respectively.

Table B.6.5.3-24 Calculations of 'instantaneous' rates of metabolism of XDE-729 Methyl in liver and blood, based on in vitro-derived metabolic rate constants outputs of simulations

Species	Dietary dose level (mg/kg/day)	Est. peak conc. XDE-729 Methyl		Int. rate of metab. at peak conc.		Liver metabolism rate X-times faster than blood
		Liver (µg/g)	Blood (µg/g)	Liver (mg/hr)[a]	Blood (mg/hr)[b]	
Rat	0.0001	0.00000358	0.000000631	0.00000340	0.0000000270	126
	0.1	0.00358	0.000637	0.00340	0.000027	126
	10	0.361	0.0637	0.328	0.00272	121
Human	0.0001	0.00000439	0.000000789	0.00237	0.0000000940	25193
	0.1	0.00440	0.000810	2.37	0.0000965	24597
	10	0.439	0.0790	235	0.00941	25002

[a]Instantaneous rate of hydrolysis in liver = [conc. of XDE-729 Methyl in liver/(conc. of XDE-729 Methyl in liver + Km)] x Vmax
For rat: Km = 7.841 mg/L; Vmax = 7.457 mg/hr. For human: Km = 57. 434 mg/L; Vmax = 31001 mg/hr

[b]Instantaneous rate of hydrolysis in blood = 1st order rate of hydrolysis (k_1 order) x conc. of XDE-729 in blood x blood volume
For rat: k_1 order = 3.166 hr-1; blood volume = 0.0135 L For human: k_1 order = 0.0229 hr-1; blood volume = 5.2 L

To further assess the relative impact among the various sites of metabolism (liver, blood, GIT) on the overall metabolism of XDE-729 Methyl *in vivo*, a series of simulations were conducted with the various sites of metabolism interchangeably 'de-activated'. Those simulations suggested that the liver accounts for about 60% of the total metabolism of XDE-729 Methyl in rats (data not shown). In humans, the liver was shown to be the primary organ responsible for metabolism of XDE-729 Methyl, accounting for >95% of the total metabolism.

AUCs were calculated from simulations for XDE-729 Methyl and XDE-729 Acid in liver and blood, at the 28th day of simulated dietary exposures to 0.0001, 0.01 and 10 mg/kg/day, as shown in Table B.6.5.3-25. The comparison of AUCs for rats and humans corroborate the graphical presentation of simulation results in that AUCs are comparable for all four model output variables. The proportionality observed between AUC values calculated from simulations of dietary exposures to the three levels of XDE-729 Methyl (for a given species/tissue) suggests that there would be probably be no appreciable saturation of metabolic processes within the exposure range 0.0001 to 10 mg/kg/day.

Table B.6.5.3-25 24-hr AUCs for liver and blood following XDE-729 Methyl exposure, calculated from PBPK simulations

Species	Dietary dose level for 28 days (mg/kg/day)	24 hour AUCs (µg-hr/g)			
		XDE-729 Methyl		XDE-729 Acid	
		Liver	Blood	Liver	Blood
Rat	0.0001	4.294E-05	7.567E-06	1.966E-04	1.255E-04
	0.1	4.295E-02	7.568E-03	1.966E-01	1.255E-01
	10	4.335E+00	7.638E-01	1.965E+01	1.254E+01
Human	0.0001	5.288E-05	9.885E-06	4.402E-04	1.583E-04
	0.1	5.288E-02	9.885E-03	4.402E-01	1.583E-01
	10	5.297E+00	9.901E-01	4.401E+01	1.583E+01

CONCLUSION

In vitro, XDE-729 Methyl is rapidly hydrolysed to XDE-729 Acid in liver S9 for all three tested species, with the relative rates being human >> rat > mouse. Metabolism of XDE-729 Methyl by liver S9 appears to follow (saturable) Michaelis-Menten kinetics; thus, K_m and V_{max} terms can be derived to mathematically define the metabolism of XDE-729 Methyl in liver tissue. In blood, XDE-729 Methyl is hydrolysed according to 1st order kinetics, with the relative rates between species being rat>mouse>>human. The rate of metabolism of XDE-729 Methyl in SGF also follows 1st order kinetics, but is the slowest of all test media evaluated; the fastest rate of conversion occurred in SGF at pH 1.2 (representing the pH of human GIT), with a half-life of approximately 100 hours.

PBPK modelling, which incorporated metabolic rates determined in the *in vitro* incubations, predicted the liver and blood concentrations of XDE-729 Methyl and XDE-729 Acid following 28 days dietary exposure of rats and humans to XDE-729 Methyl. The predicted hydrolysis of XDE-729 Methyl to XDE-729 Acid was most dependent on the rate of metabolism in liver tissue for both species, accounting for ~60% of total hydrolysis in rat and >95% in human. The model simulations indicate that XDE-729 Methyl and XDE-729 Acid achieve their steady-state concentrations soon after exposure is begun, the levels clear rapidly from blood and liver following the cessation of exposure, and that neither XDE-729 Methyl nor XDE-729 Acid would be expected to accumulate in either liver or blood following exposures to XDE-729 Methyl. Model-derived peak concentrations and 24-hr AUCs of XDE-729 Methyl in liver and blood are comparable between rats and humans at XDE-729 Methyl dietary exposure levels of 0.0001, 0.1 and 10 mg/kg/day. The peak concentrations from a simulated 28-day exposure to XDE-729 Methyl at 0.0001 mg/kg/day, a relevant exposure level based on residue data, resulted in predicted levels of XDE-729 Methyl in humans that are 10000- to 15000-times lower than the limits of quantitation; at 0.1 mg/kg/day, an exposure level slightly higher than the proposed ADI, predicted levels of XDE-729 Methyl in humans that are 10- to 15-times lower than the limits of quantitation (LOQs: in blood ~0.01 µg XDE-729 Methyl/g; in liver ~0.04 µg XDE-729 Methyl/g).

[redacted] 2012

Study	IIA 5.5.4/07 XDE 729 Methyl: Mode of action and human relevance framework analysis for XDE-729-induced rodent liver effects
Reference	[redacted] (2012)
Date performed	Completed August 2012
Test facility	[redacted]
Report reference	Laboratory study ID 120247
Guideline(s)	None
Deviations from the guideline	N/A
GLP	N/A
Test material	N/A
Study acceptable	Yes

1. Mode of action hypothesis

XDE-729 Methyl induces rodent liver effects via a proposed aryl hydrocarbon receptor-mediated mode-of-action (MoA) through the following key events (1) presystemic liver exposure to XDE-729 Methyl, (2) aryl hydrocarbon receptor (AhR) activation with associated liver weight increase and hepatocyte hypertrophy, leading to (3) hepatocellular proliferation. These key events have been evaluated in a series of guideline toxicity and MoA studies aimed at characterising the MoA for XDE-729 Methyl-mediated liver effects. This document represents the weight of evidence approach used to evaluate the data based upon the Bradford-Hill criteria followed by subsequent application in a Human Relevance Framework (HRF).

Following dietary administration to rodents, XDE-729 Methyl is rapidly hydrolysed to the primary metabolite XDE-729 Acid. Although XDE-729 Methyl is an AhR activator, XDE-729 Acid has been shown through both *in vitro* and *in vivo* assays not to induce AhR activation and associated liver responses. Therefore, this MoA/HRF analysis is focused on XDE-729 Methyl induced AhR-mediated effects in the liver. Presystemic exposure of the rodent liver to XDE-729 Methyl results in a dose responsive increase in *Cyp1a1* transcript levels as a sensitive biomarker for AhR activation. In addition, corresponding increases in liver weight and associated hepatocellular hypertrophy were observed. Supporting events for an AhR-mediated MoA for hepatocellular effects include ligand binding to the receptor, and a series of *in vitro* assays, which have collectively indicated that XDE-729 Methyl is a weak AhR agonist. Presystemic liver exposure to XDE-729 Methyl, followed by activation of rat AhR, leads to liver weight increases with hepatocellular hypertrophy and increases in hepatocellular proliferation. Importantly, these effects (i.e. measureable molecular and apical end points) occur at doses ≥50 mg/kg/day XDE-729 Methyl with a clear threshold for liver effects at NOAEL of 10 mg/kg/day. The data from these evaluations provides a high level of confidence that XDE-729 Methyl induces rodent liver effects through an AhR-mediated MoA.

2. Background on AhR Activation

Activation of the AhR signalling pathway and downstream hepatic effects have been extensively characterised with prototypical AhR ligands which have high receptor binding affinity and are metabolically stable and persistent. For these prototypical AhR agonists, sustained, long-term activation of the AhR is required to elicit changes in hepatocellular proliferation that could result in liver tumour formation (Walker, et al. 1998).

It is important to note that humans are typically exposed to an array of AhR ligands on a daily basis, primarily through the diet, and that these ligands are metabolically labile. Flavonoids found in vegetables, fruits, and teas, represent the largest group of naturally occurring dietary AhR ligands, and concentrations in human blood, have been found to be in the low micromolar range that likely activate the AhR pathway (Denison and Nagy 2003 and references therein; Jeuken, *et al.* 2003). Other AhR ligands include the indoles (weak AhR ligands) found in brussels sprouts, broccoli, and cauliflower, which are converted in the digestive tract to more potent AhR ligands; interestingly, tryptophan can also be converted to indoles in the mammalian digestive tract (Denison and Nagy 2003). Importantly, these dietary AhR ligands do not result in adverse hepatic responses probably because of their rapid metabolism and lack of sustained activation of the AhR.

Comprehensive toxicokinetic analysis in the course of the studies evaluated in this MoA/HRF determined that XDE-729 Methyl is rapidly metabolised and excreted in the urine following

dietary administration. Low levels of XDE-729 Methyl were present in the liver following dietary administration; however, after a four or 28 day recovery period there was no XDE-729 Methyl present in the livers of rats. Therefore, based on the observed rapid metabolism and weak agonist activity on AhR, XDE-729-methyl may induce biological responses consistent with naturally occurring dietary ligands as opposed to the prototypical AhR ligands.

3. Human Relevance

A physiologically-based pharmacokinetic (PBPK) model for XDE-729 Methyl exposure predicted similar levels of XDE-729 Methyl in the livers of humans and rats at the overall NOAEL from the toxicity studies of 10 mg/kg/day, and also at exposure levels of 0.1 and 0.0001 mg/kg/day, exposure levels relevant to predicted human exposure and a proposed ADI respectively. Although PBPK model outputs indicate that human liver would be exposed to XDE-729 Methyl, at realistic predicted human exposure levels the concentrations are well below those that would cause hepatic effects based on rodent toxicity studies. Furthermore, human AhR binding affinity for AhR ligands is quantitatively lower (~10-fold) than rodent AhR (Connor and Aylward 2006).

Several *in vitro* studies for AhR receptor activation were also completed as part of the assessment of human relevance. A conclusion from the rodent MoA data and *in vitro* mechanistic studies is that humans would be significantly less sensitive than rat to XDE-729 Methyl based on the following: 1) kinetic analysis of XDE-729 Methyl indicated this molecule is rapidly hydrolyzed to XDE-729 Acid by human liver S9 and does not bioaccumulate in rat liver tissues, 2) *in vitro* transactivation and binding assays with XDE-729 Methyl indicated that this molecule was a relatively weak ligand for rodent AhR and had no transactivation activity for human AhR, 3) at concentrations of XDE-729 Methyl that elicited substantial induction of *Cyp1a1* transcript levels in the rat, primary human hepatocytes had minimal induction of *CYP1A1* and *CYP1A2.* In addition, a clear threshold for liver effects exists for XDE-729 Methyl with comprehensive MoA and mechanistic data indicating that molecular and apical endpoints (*Cyp1a1* induction, liver enlargement with associated hypertrophy, and hepatocellular proliferation) do not occur below the dose level of 10 mg/kg/day XDE-729 Methyl.

4. Conclusions

The data from these evaluations provide a high level of confidence that XDE-729 Methyl induces rodent liver effects through an AhR-mediated MoA. Although XDE-729 Methyl was able to activate the AhR, it is a weak agonist that is rapidly metabolised; therefore, XDE-729 Methyl would not result in sustained activation of the AhR signalling pathway. In addition, all hepatic molecular and apical end points were reversible after short recovery periods. Analysis of toxicity, toxicokinetic and molecular end points following XDE-729 Methyl administration indicated a clear threshold for liver effects. Also, *in vitro* transactivation studies and gene expression studies in human liver cells demonstrated minimal activation of the human AhR with exposure to XDE-729 Methyl, which is coupled by the fact that *in vitro* hydrolysis studies using human and rat liver S9 determined that hydrolysis of XDE-729 Methyl is faster in the human compared to the rat. Even though the human relevance of an AhR-mediated MoA conservatively cannot be excluded, a margin of exposure risk assessment utilizing the 10 mg/kg/day NOAEL from the 90-day subchronic toxicity study in the rat to derive a chronic reference dose/acceptable daily intake (cRfD/ADI) of 0.1 mg/kg/day for XDE-729 Methyl would be protective of human health.

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Denison, M. S. and Nagy, S. R. (2003). Activation of the Aryl Hydrocarbon Receptor By Structurally Diverse Exogenous and Endogenous Chemicals. *Annu. Rev. Pharmacol. Toxicol.* 43: 309-334.

Jeuken, A., Keser, B. J. G., Khan, E., Brouwer, A., Koeman, J., and Denison, M. S. (2003). Activation of the Ah Receptor by Extracts of Dietary Herbal Supplements, Vegetables, and Fruits. *J. Agric. Food Chem.* 51: 5478-5487.

Walker, N. J., Miller, B. D., Kohn, M. C., Lucier, G. W., and Tritscher, A. M. (1998). Differences in kinetics of induction and reversibility of TCDD-induced changes in cell proliferation and CYP1A1 expression in female Sprague-Dawley rat liver. *Carcinogenesis.* 19: 1427-1435.

UK RMS comments on mode of action and human relevance framework analysis

The UK RMS agrees that AhR MoA for the XDE-729 Methyl induced liver effects in rats has been convincingly demonstrated, and that this MoA must be regarded as relevant to humans although it is likely that humans will be less sensitive to AhR agonist activity. The UK RMS agrees that the NOAEL for XDE-729 Methyl AhR-mediated liver toxicity in the rat for the oral route is 10 mg/kg/day.

However, the UK RMS is not convinced of the validity of one of the Applicant's arguments in support of the proposition that humans will be less sensitive to XDE-729 Methyl mediated AhR agonist activity than rats. The Applicant proposes that proposes that the faster *in vitro* hydrolysis rates observed for XDE-729 Methyl in presence of human liver S9 as compared to rats and mice (Rick et al 2012; IIA 5.5.4/06) provides evidence that *in vivo* humans will have lower exposure of the liver to XDE-729 Methyl. However, the results of the *in vitro* hydrolysis investigations are contradicted by the PBPK simulations which predict comparable peak concentrations and 24-hr AUCs of XDE-729 Methyl in liver and blood in rats and humans at XDE-729 Methyl dietary exposure levels of 0.0001, 0.1 and 10 mg/kg/day. Nevertheless, the UK RMS agrees that there is persuasive evidence that humans are likely to be less sensitive to XDE-729 Methyl mediated AhR agonist activity, provided by the results of the *in vitro* transactivation studies and gene expression studies, as discussed in Section B.6.5.3.2 below.

Also, the UK RMS does not agree that the NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity is the appropriate starting point for deriving a dietary reference dose for human risk assessment because a lower NOAEL of 5.78 mg/kg/day was identified for XDE-729 Methyl mediated liver toxicity in the rabbit in a developmental toxicity study (Ellis-Hutchings et al 2012, IIA 5.6.11/04), as discussed Sections B.6.5.3.2 and B.6.10.1 below

France (coRMS) comments on mode of action

Based on the cell-free *in vitro* photoaffinity ligand competition-binding assay, with AhR sourced from rat liver cytosol (Perdew 2012, IIA 5.5.4/04), XDE-729 Methyl seems to be only a weak partial agonist of AhR in this species. This result does not match up with the very high level of CYP1A1 gene expression (23970-fold increase) observed in rat exposed to XDE-729 Methyl in diet for 7 days (LeBaron et al 2012, IIA 5.5.4/01). In addition, induction of CYP1A1/CYP1A2 was found in human hepatocyte by XDE-729 Methyl in a *in vitro* assay (Laethem; Murphy 2012, IIA 5.5.4/05) while no AhR agonist activity was found in a human reporter cell line (LeBaron et al 2012, IIA 5.5.4/01). France is of opinion that the MoA of XDE-729 Methyl is not clearly demonstrated as the partial AhR agonism cannot explain alone the strong CYP 1A1 gene

expression increase observed in rat after XDE-Methyl exposure. Other mechanisms could be involved such as stabilisation of CYP1A1 mRNA, activation of AhR via other signalising pathways (Delescluse et al. 2000).

Reference
Delescluse C, Lemaire G, de Sousa G, Rahmani R (2000) Is CYP1A1 induction always related to AHR signaling pathway? Toxicology 153:73-82.

The Applicant has responded to the French comments by pointing out that, in their opinion, the ligand binding and reporter cell line assays provided only preliminary data regarding the MoA for XDE-729 Methyl mediated liver effects. They consider that the primary hepatocyte study utilizing mouse, rat, and human primary hepatocytes to investigate species sensitivity in *Cyp1a1* induction (Laethem; Murphy 2012, IIA 5.5.4/05) is the definitive study, providing the most direct and relevant comparison. In this study, a concurrent positive control (3-Methylcholanthrene) was run in each species of primary hepatocytes to determine dynamic range for *Cyp1a1* induction and to provide a direct comparison of the magnitude of XDE-729 Methyl mediated *Cyp1a1* induction relative to a prototypical AhR Activator. Differences in metabolic capacity, nuclear receptor activation, and magnitude of *Cyp* induction are a limitation for direct *in vitro* to *in vivo* comparisons of AhR activation. Gene expression analyses in the seven day MoA (LeBaron et al 2012, IIA 5.5.4/01), 28-day MoA (Boverhof et al 2012, IIA 5.5.4/01) and 90-day studies (Stebbins et al 2012, IIA 5.3.2/02) were performed following constant, repeated dietary exposure to XDE-729 methyl which resulted in induction of liver metabolism as evidenced by detectable amounts of XDE-729 methyl in the liver along with treatment-related increases in *Cyp1a1* induction, increased liver weights, hepatocellular hypertrophy, and increased mitotic figures at doses ≥52 mg/kg/day.

The Applicant also believes that there is a recognised limitation in relation to *in vitro* ligand binding assay and subsequent correlation to *in vivo* results. It is suggested that there are data to indicating that competitive ligand binding assays in which a higher affinity ligand, such as dioxin (as was used in the XDE-729 competitive ligand binding assays, Perdew 2012, IIA 5.5.4/04) may under estimate binding affinity of a weaker ligand since the higher affinity ligand could preferentially and persistently bind (Bohonowych, *et al* 2007). Furthermore, although high-affinity AhR ligands strongly bind to the AhR and induce *Cyp1a1*, the correlation of binding affinity for structurally diverse ligands to *in vivo Cyp1a1* induction is not as well-characterized (Denison and Nagy 2003).

Overall, in the opinion of the Applicant, data from the MoA assessment strongly support that XDE-729 methyl is quickly eliminated from tissues and does not bioaccumulate and that observed liver effects are reversible following withdrawal of exposure to XDE-729 methyl. These results, taken together, with the *in vitro* binding data and gene expression analyses are indicative of an AhR-mediated MoA mediated by a metabolically labile ligand. This is in contrast to prototypical AhR ligands which are metabolically stable, resistant to degradation, have a high binding affinity for the AhR and elicit the full spectrum of AhR-mediated toxic effects.

References
Bohonowych JE, Denison MS (2007) Persistent binding of ligands to the aryl hydrocarbon receptor Toxicol Sci. 98:99-109.

Denison MS, Nagy SR (2003) Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 43:309-34.

B.6.5.3 Summary of chronic toxicity and carcinogenicity studies

Table B.6.5.3-1 Summary of chronic toxicity and carcinogenicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
Rat, 12 mo. chronic/2 year carcinogenicity, oral dietary XDE-729 Acid 0-20-100-400-625/750 mg/kg/day target	Non-neoplastic M&F: 100 mg/kg/day	M: 404mg/kg/day	M: ↓ bodyweight, kidney: ↑ organ weight, tubular degeneration & regeneration, necrosis of individual tubular epithelial cells, hyperplasia of pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in collecting duct epithelium, interstitial inflammation of medulla, vacuolization of collecting duct epithelium in papilla, ↑ urine volume	[redacted] 2012 (091121)
		F:407 mg/kg/day	F: kidney, ↑ organ weight, tubular degeneration & regeneration, necrosis of individual tubular epithelial cells, chronic progressive glomerulonephropathy , hyperplasia of pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in collecting duct epithelium, interstitial inflammation of medulla, and vacuolization of collecting duct epithelium in papilla; ↑ urine volume	
	Neoplastic: M: 625mg/kg/day F: 400 mg/kg/day	M&F: -	No evidence of carcinogenicity	
Mouse, 18 mo. carcinogenicity, oral dietary XDE-729 Acid 0-50-250-750/1000 mg/kg/day target	Non-neoplastic: M: 50 mg/kg/day	M: 251mg/kg/day	M: Urinary bladder: inflammation, microscopic calculi in lumen	[redacted] (101021)
	F:1004mg/kg/day	F: -	[F: hypertrophy of kidney intercalated cells present at 251 mg/kg//day and above, considered to be an adaptive non-adverse effect]	
	Neoplastic: M: 751mg/kg/day F:1004mg/kg/day	M&F: -	No evidence of carcinogenicity	
Mode of action and human relevance analysis	UK RMS: There is a high level of confidence that XDE-729 Methyl induces liver effects in rat through an AhR-mediated MoA. Although human relevance of this MoA cannot be excluded, humans are expected to be significantly less sensitive than rat to activation of AhR by XDE-729 Methyl and human exposure of the liver to XDE-729 Methyl is likely to be lower relative to rats. The Applicant argues that an ADI for XDE-729 Methyl can justifiably be developed by the application of an uncertainty factor to the NOAEL of 10 mg/kg/day from the 90-day dietary rat study for XDE-729 Methyl. France coRMS: While it is clearly demonstrated that XDE-729 Methyl causes liver toxicity, hepatocellular proliferation and hepatic CYP1A gene expression, the MoA for the liver effects is not clearly demonstrated. This is because partial AhR agonism alone cannot account for the strong CYP 1A1 gene expression increase observed in rats in response to XDE-729 Methyl. Other mechanisms could be involved such as stabilisation of CYP1A1 mRNA, activation of AhR via other signalising pathways.			[redacted] 2012 (120247)

B.6.5.3.1 Carcinogenicity and chronic toxicity conclusion

A standard carcinogenicity study in the rat and one in the mouse demonstrate that XDE-729 Acid is not carcinogenic. As discussed in section B.6.5.3.2 below, these finding can be extrapolated to XDE-729 Methyl. Toxicokinetic investigations demonstrated that post-hepatic systemic exposure following oral administration of XDE-729 Methyl is mainly to XDE-729 Acid, so it can be also be concluded that XDE-729 Methyl will not be carcinogenic to organs other than the gastrointestinal tract (local effects) and liver. Concerning the liver, it can be concluded that XDE-729 Methyl is not carcinogenic to this organ at oral dose levels of up 10 mg/kg/day, which is the

NOAEL for for AhR-mediated liver toxicity in the rat (the potential carcinogenicity to the level at dose levels above 10 mg/kg/day cannot be assessed). Concerning the gastrointestinal tract, XDE-729 Methyl does not cause irritation in this organ system in 28 and 90 day dietary studies in the rat, and it is not genotoxic, so there are no concerns for gastrointestinal carcinogenicity.

Regarding the long-term non-neoplastic toxicity, the kidneys are identified at the principal target for XDE-729 Acid in the rat, as is the case for short-term exposure (see section B.6.3). Kidney changes occur at exposure levels of 400 mg/kg/day and above. The kidney effects seen at 12 months are chronic progressive glomerulonephropathy, hyperplasia of the pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in the collecting duct epithelium, chronic interstitial inflammation of the medulla, necrosis of collecting duct epithelium and vacuolization of collecting duct epithelium in the papilla, accompanied by increase kidney weight. Additional pathology changes in the kidney are observed at 24 months, namely tubular degeneration with regeneration and necrosis of individual tubular epithelial cells and, in occasional males, the presence of calculi in the pelvis. At 24 months there are also changes in the urinary bladder, characterised by hyperplasia of the transitional epithelium and inflammation in the submucosa beneath the hyperplastic epithelium and, in one animal, the presence of microscopic calculi in the lumen. Secondary to the kidney toxicity, slight hypertrophy and vacuolisation of the adrenal zona glomerulosa is seen at 24 months. The kidney toxicity is accompanied by reduced bodyweight gain from 400 mg/kg/day and increased urinary volume and, at the highest dose level, increased blood urea nitrogen and reduced survival. A NOAEL of 102 mg/kg/day (target 100 mg/kg/day) is identified for the long-term toxicity of XDE-729 Acid in the rat.

In mice the urinary bladder and kidneys are identified as the principal toxicity targets of XDE-729 Acid, affecting only males. At 250 mg/kg/day and above inflammation of the urinary bladder and microscopic calculi are present in the urinary bladder lumen of males. At 750 mg/kg/day the effects on the urinary bladder are additionally characterised by hyperplasia, increased numbers of mitotic figures, necrosis of individual cells and ulceration of the transitional epithelium; also, kidney tubular degeneration with regeneration and dilatation were present. The urinary bladder and kidney toxicity is associated with increased mortality among males at 750 mg/kg/day. Treatment-related hypertrophy of the intercalated cells of the kidney was present in males at 750 mg/kg/day and females at 250 and 1000 mg/kg/day; this is interpreted as being an adaptive response, possibly related to maintaining acid-base homeostasis. NOAELs of 50 mg/kg/day in males and 1004 mg/kg/day (target 1000 mg/kg/day) in females are identified for the long-term toxicity of XDE-729 Acid in the mouse.

B.6.5.3.2 Integrating the results of the MoA investigations with information from core and bridging toxicokinetic and toxicity studies.

Table B.6.5.3-2 Summary of mode of action studies

Main endpoints investigated	Test substance, exposure conditions	Results	Reference
Liver toxicity, hepatic gene expression and toxicokinetics Male F344 rats	7 d oral dietary XDE-729 Methyl 0, 782 mg/kg/day	↓ bodyweight gain, food consumption, ↑ liver weight, microscopic liver changes ↑ expression of *Cyp1a1* and *Ugt1a6* genes, consistent with activation of AhR pathway Extensively converted to XDE-729 Acid & excreted mainly as either XDE-729 Acid or its O-demethyl conjugates	[redacted] 2012 111088
	XDE-729 Acid 0, 750 mg/kg/day	No evidence of general or liver toxicity. Extent of formation of O-demethyl conjugates for XDE-729 Acid lower than for XDE-729 Methyl.	
Liver toxicity, hepatic gene expression and toxicokinetics CD1 mice	7 d oral dietary XDE-729 Methyl 0, 2612, 782 mg/kg/day	Slight ↑ liver weight, considered an adaptive response, at 782 mg/kg/day ↑ expression of *Cyp1a1*gene, consistent with activation of AhR pathway Most of parent XDE-729 Methyl rapidly converted to XDE-729 Acid	[redacted] 2010 110177
Liver toxicity, hepatic gene expression, hepatocellular proliferation, toxicokinetics Male F344 rats	28 d oral dietary 4, 28 d recovery groups XDE-729 Methyl 0, 3, 10, 52, 261 mg/kg/day	↑ liver weight at 261 mg/kg/day; hepatocellular proliferation, measured by BrdU labelling, at 261 mg/kg/day and possibly at 52 mg/kg/day; microscopic liver changes (hypertrophy) from 52 mg/kg/day; ↑ expression of *Cyp1a1*and *Cyp1a2* from 52 mg/kg/day & *Ugt1a6* at 261 mg/kg/day, consistent with activation of AhR pathway. Except for BrdU labelling, these changes were shown in recovery groups to be reversible. XDE-729 Methyl extensively converted to XDE-729 Acid. ~50% of administered dose excreted in the urine as XDE-729 Acid. A conjugate of O-demethyl XDE-729 Methyl and conjugates of O-demethyl XDE-729 Acid confirmed as metabolites of XDE-729 Methyl. Parent or metabolites not detected in blood, liver or urine of recovery groups.	[redacted] 2012 120037
Luciferase reporter assay for AhR agonist activity in mouse & human reporter cell lines	XDE-729 Acid	No AhR agonist activity in mouse or human cell lines	[redacted] 2012 NS000063
	XDE-729 Methyl technical (97.2%), high purity (99.1%) &, ultrapure (99.7%)	Significant, dose-related AhR agonist activity in mouse cell line; activity not influenced by purity. Agonist activity of +ve control (ITE) much higher. No AhR agonist activity in human cell line.	
AhR ligand competition binding assay using AhR from SD rat liver cytosol	XDE-729 Methyl ultrapure (99.7%)	XDE-729 Methyl is a weak AhR ligand	
In vitro assessment of Cyp1a1 and Cyp1a2 induction in human, mouse & rat hepatocyte cultures by measuring mRNA levels	XDE-729 Methyl	Cyp1a1 induced in rat, mouse and human hepatocytes; rat most sensitive, human least. Cyp1a2 induced in rat, mouse and human hepatocytes; rat most sensitive, mouse least. All species much more sensitive to induction by +ve control (3-MC)	[redacted] 2012 [redacted] 2012 SP0014001
In vitro assessment of hydrolysis rates in liver S9, blood and synthetic gastric fluid of mouse,	XDE-729 Methyl	Liver S9: rapid hydrolysis to XDE-729 Acid with the relative rates human >> rat > mouse. Hydrolysis saturable, i.e. following Michaelis-Menten kinetics. Blood: hydrolysis to XDE-729 Acid follows 1st order	[redacted] 2012 110199

rat and human		kinetics, with the relative rates rat>mouse>>human. Gastric fluid: hydrolysis to the Acid follows 1st order kinetics, but very much slower than liver or blood; fastest rate at pH of 1.2, representing human GIT	
PBPK simulations of systemic exposure in rats and humans	Simulated XDE-729 Methyl exposure	Hydrolysis to XDE-729 Acid is most dependent on the rates of metabolism in liver, accounting for ~60% of total hydrolysis in rat and >95% in human. Predicted concentrations XDE-729 Methyl in liver and blood comparable between rats and humans at XDE-729 Methyl dietary exposure levels of 0.0001, 0.1 and 10 mg/kg/day. Human liver/systemic exposure to XDE-729 Methyl is predicted to be negligible (i.e. considerably below the LOQ) at 0.0001 and 0.1 mg/kg/day, exposure levels relevant to predicted human exposure and a proposed ADI, respectively	

The core registration toxicological studies were conducted with XDE-729 Acid. Bridging studies have been conducted with XDE-729 Methyl with the aim of demonstrating toxicological equivalence between XDE-729 Acid and XDE-729 Methyl. The bridging studies included a toxicokinetics study in rats, genotoxicity studies, acute toxicity studies, a 28-day and 90 day repeat exposure (oral dietary) studies in the rat, and a developmental toxicity study in both the rat and rabbit.

Toxicokinetic investigations in rats, mice and rabbits demonstrated that post-hepatic systemic exposure following oral administration of XDE-729 Methyl is mainly to XDE-729 Acid, and that the systemic levels are quantitatively similar following oral administration of equivalent doses of XDE-729 Methyl and XDE-729 Acid (i.e. XDE-729 Methyl and XDE-729 Acid have 'toxicokinetic equivalence'). XDE-729 Methyl and XDE-729 Acid have also been shown to be toxicologically equivalent for acute toxicity and genotoxicity endpoints, and both do not cause specific developmental toxicity in rats and rabbits. However, on 28 and 90 day repeated oral dietary administration in rats XDE-729 Methyl and XDE-729 Acid have different main toxicity target organs; for XDE-729 Methyl it is the liver (short-term NOAEL 10 mg/kg/day) and for XDE-729 Acid it is the kidney (short-term NOAEL 250 mg/kg/day in rat).

MoA studies with XDE-729 Methyl demonstrated that the liver effects were mediated through activation of the aryl hydrocarbon receptor (AhR). The Applicant conducted additional MoA studies to investigate the AhR-mediated MoA and hydrolysis of XDE-729 Methyl to XDE-729 Acid both *in vivo* and *in vitro* studies across multiple species.

Integrating the results of the MoA studies, the bridging studies and core registration studies, it can be concluded that:

1. Systemic (post-hepatic) exposure following dietary intake of XDE-729 Methyl is to XDE-729 Acid

Additional toxicokinetic investigations consistently confirm the presence of negligible levels of XDE-729 Methyl in blood or urine of rats, mice and rabbits in a wide range of studies at various oral dose levels of XDE-729 Methyl, ranging from 10 to 782 mg/kg/day. These data demonstrate that post-hepatic systemic exposure following the administration of XDE-729 Methyl is mainly to its hydrolysis product XDE-729 Acid. Evidence of systemic exposure to a direct metabolite of the parent, namely a glucuronide conjugate of O-demethyl XDE-729 Methyl, has been seen in some XDE-729 Methyl rat studies, but this conjugate is present in blood only at dose levels

above the rat NOAEL of 10 mg/kg/day and no free O-demethyl XDE-729 Methyl has been detected in blood, liver or urine at any exposure level of XDE-729 Methyl, indicating low toxicological concern for this direct metabolite, certainly at the rat NOAEL for liver toxicity of 10 mg/kg/day. Overall, in relation to potential effects in the organs other than the gastrointestinal tract (local effects) and liver, the toxicity studies on XDE-729 Acid in rats, mice and rabbits can be regarded as predictive for XDE-729 Methyl for the oral route, especially at dose levels of 10 mg/kg/day and below.

PBPK modelling predicted comparable peak concentrations and 24-hr AUCs of XDE-729 Methyl in liver and blood between rats and humans at XDE-729 Methyl dietary exposure levels of 0.0001, 0.1 and 10 mg/kg/day, providing evidence that in humans systemic exposure is also mainly to the hydrolysis product. The PBPK modelling also predicted negligible (i.e. considerably below the LOQ) liver and post-hepatic systemic exposure to XDE-729 Methyl at a human exposure levels of 0.0001 and 0.1 mg/kg/day, exposure levels relevant to predicted human exposure and a proposed ADI, respectively.

2. Hepatic exposure to XDE-729 Methyl causes AhR-mediated liver toxicity and the 90-day dietary rat study defined the NOAEL for these effects as 10 mg/kg/day in the rat

In a XDE-729 Methyl 90 day dietary study conducted at dose levels 3, 10, 52, 261 and 500 mg/kg/day, a NOAEL of 10 mg/kg/day was identified. At dose levels of 52 mg/kg/day and above, the induction of hepatic *Cyp1a1* gene expression was notable and liver toxicity (increased organ weight, hepatocyte vacuolation) was observed. The severity of the hepatic changes correlated with the levels of XDE-729 Methyl detected in the liver. Thyroid changes were observed in the 90-day rat study, from 261 mg/kg/day, interpreted as being secondary to increased glucuronidation in the liver because markedly increased hepatic *Ugt1a6* gene expression was seen from 261 mg/kg/day. The induction of hepatic *Cyp1a1* and *Ugt1a6* gene expression, which correlated with the observed liver and thyroid changes, provides evidence of activation of the AhR signalling pathway. A 28-day dietary MoA study in rats showed that XDE-729 Methyl induced liver toxicity, hepatocellular proliferation, and hepatic *Cyp1a1* gene expression which are reversible after a short recovery period.

Comparison of the results of 7 day dietary toxicity in rats and mice indicate that mice are less sensitive than rats to XDE-729 Methyl induced liver toxicity and hepatic *Cyp1a1* gene expression in relation to a short exposure period.

3. XDE-729 Methyl is the AhR agonist and not an impurity, metabolite or XDE-729 acid

AhR transactivation assays demonstrate that XDE-729 Methyl is a weak AhR agonist in a mouse reporter cell line and essentially has no activity in a human reporter cell line. The agonist activity of the XDE-729 Methyl samples is not quantitatively influenced by the level of purity, indicating that it is unlikely to be an impurity that is responsible for the observed AhR agonist activity. XDE-729 Acid has no AhR agonist activity in either the mouse or human reporter cell lines. A cell-free *in vitro* photoaffinity ligand competition-binding assay, using AhR sourced from Sprague Dawley male rat liver cytosol, demonstrates that XDE-729 Methyl is an AhR ligand in this species.

4. Rats are significantly more sensitive to AhR activation by XDE-729 Methyl than humans

In vitro Cyp1a1 and Cyp1a2 gene expression studies in primary cultures of freshly isolated human, mouse and rat hepatocytes provide evidence of rats being more sensitive to AhR activation by XDE-729 Methyl than humans. For expression of Cyp1a1/CYP1A1 by XDE-729 Methyl, the rat was the most sensitive and human being the least. For Cyp1a2/CYP1A2 expression, the rat was also the most sensitive, and the mouse being the least. For both enzymes, all tested species were much more sensitive to induction by the positive control, 3-MC, than by XDE-729 Methyl.

As mentioned in point 3 above, AhR transactivation assays demonstrate that XDE-729 essentially has no AhR agonist activity in a human reporter cell line. This provides additional evidence that humans may have relatively low sensitivity to AhR activation by XDE-729 Methyl.

5. The NOAEL of 10 mg/kg/day established for AhR-mediated liver toxicity in the rat 90-day dietary study with XDE-729 Methyl is applicable to the human health risk assessment for longer-term exposures

The 90 day XDE-729 Methyl dietary study identified a NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity in the rat. A framework analysis shows that it is appropriate to make a conservative assumption that this MoA is relevant to humans. The 90 day NOAEL is considered to also be applicable for XDE-729 Methyl exposures of longer duration because there was no increase in severity of liver toxicity in the 90 day XDE-729 Methyl dietary study in rats as compared to the 28 day XDE-729 Methyl dietary study (both studies identified NOAELs and LOAELs of 10 and ~50 mg/kg/day, respectively). This NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity in rats is probably conservative in relation to humans because humans are likely to be less sensitive than rats to AhR activation and to subsequent induction of CYP1A1/CYP1A2 by XDE-729 Methyl.

Human post-hepatic systemic exposure following XDE-729 Methyl intake by the oral route will primarily be to the Acid, for which there is a full set of regulatory toxicity studies. Bridging studies are available for XDE-729 Methyl which show that XDE-729 Methyl and XDE-729 Acid are toxicologically equivalent in rats at dose levels of up to 10 mg/kg/day based on the absence of AhR-mediated effects in the liver at this dose level and below in the XDE-729 Methyl studies, although XDE-729 Methyl and XDE-729 Acid have different target organs and NOAELs at higher dose levels. Although a NOAEL of 10 mg/kg/day has been identified for AhR-mediated liver toxicity in the rat, a lower NOAEL of 5.78 mg/kg/day was identified for XDE-729 Methyl mediated liver toxicity in the rabbit in a developmental toxicity study (Ellis-Hutchings et al 2012, IIA 5.6.11/04). This lower NOAEL will be used as the starting point for deriving a dietary reference dose for the human risk assessment.

Regarding the dog, the toxicokinetics of XDE-729 Methyl have not been investigated in this species. Therefore, it must be assumed that the toxicokinetics of XDE-729 Methyl in the dog will be similar to the rat (i.e. post hepatic systemic exposure in the dog following XDE-729 Methyl is mainly to its hydrolysis product XDE-729 Acid) for the XDE-729 Acid dog studies to be regarded as predictive of the post-hepatic systemic toxicity of XDE-729 Methyl; in the opinion of the RMS, this is a reasonable assumption especially for dose levels up to the rat NOAEL of 10 mg/kg/day. To consider the liver as the target organ of XDE-729 Methyl, it is likely that the dog will be less sensitive than the rat to XDE-729 Methyl AhR-mediated liver toxicity, based on the following arguments provided by the Applicant:

1. The peer-reviewed literature indicates that dogs would likely be less sensitive than rat to XDE-729 Methyl mediated liver effects. Existing literature for AhR activation focuses primarily on dioxin and receptor binding affinity in rat, mouse, and human. The binding affinity of human AhR for AhR ligands (i.e. dioxin) is substantially lower with binding affinities that are ≤ 1/10 AhR binding affinities in species such as rat and mouse (Connor and Aylward, 2006). A previous study (Sandoz *et al.*, 1999) investigated the binding affinity of beagle dog AhR for dioxin, a potent AhR activator, using liver S9. This paper utilized competitive binding with TCDF as well as charcoal stripping to determine that beagle dog AhR has relatively low binding affinity for dioxin which is similar to that of human AhR. Previously conducted ligand binding assays with XDE-729 Methyl in Sprague Dawley rat liver cytosol indicate that although XDE-729 methyl binds to the ligand binding pocket of rat AhR, overall it has weak AhR binding potential (IIA 5.5.4/04). Collectively, these data indicates that dogs would likely be less sensitive than rat to XDE-729 methyl mediated liver effects based on species differences in AhR binding affinities for AhR ligands.

2. In further support of species differences in AhR activation, 28- and 90-day toxicity studies were conducted in the rat and dog using Indole-3-Carbinol (I3C), which forms ICZ, a potent AhR activator, through acidic condensation in the digestive tract. Liver was identified as a target organ in the rat on the 28- and 90-day studies and effects included increased liver weights with accompanying histopathological effects which were indicative of hepatic enzyme induction. Kidney and GI tract were identified as target organs for the dog in studies of the same duration of exposure (NCI, DCPC, 1996)

References

Connor KT, Aylward LL (2006). Human rsponse to dioxin: aryl hydrocarbon receptor (AhR) molecular structure, function, and dose-response data for enzyme induction indicate an impaired human AhR. *Journal of Toxicology and Environmental Health.* 9: 147-171.

NCI, DCPC (1996). Clinical Development Plan: Indole-3-Carbinol. Chemoprevention Branch and Agent Development Committee. Journal of Cellular Biochemistry 265, 127-136.

Sandoz C, Lesca P, Narbonne JF (1999). Hepatic Ah receptor binding affinity for 2, 3, 7, 8-tetrachlordibenzo-*p*-dioxin: similarity between beagle dog and cynomolgus monkey. *Toxicology Letters*. 109: 115-121.

For the dermal route of exposure to XDE-729 Methyl, systemic exposure is shown also to be primarily to XDE-729 Acid following the single application of concentrations up to 7.5 g/L for 10 hours (IIIA1 7.6.1/01; De Bie and Grossouw, 2011). Therefore, the results of the oral XDE-729 Acid studies can be extrapolated to the dermal route.

No information is available on the toxicokinetics for the inhalation route. However, human exposure is likely to be to material with particle size greater that 10 µm and so inhaled particles will be cleared to the stomach rather than penetrating the deep lung. Thus, oral XDE-729 Acid studies are relevant for the inhalation route of exposure.

France (coRMS) comments on integrating the results of the MoA investigations with information from core and bridging toxicokinetic and toxicity studies.

France agrees that it has been clearly demonstrated that XDE-729 Methyl causes liver toxicity, hepatocellular proliferation and hepatic CYP1A gene expression. However, France is of opinion that the MoA of XDE-729 Methyl has not been not clearly demonstrated because the partial AhR agonism cannot explain alone the strong CYP 1A1 gene expression increase observed in rat after XDE-729 Methyl exposure. Nevertheless, a NOAEL can be established for the liver effects and the coRMS is agrees with the NOAEL of 5.78 mg/kg/day proposed by the UK RMS (based on liver toxicity observed in the rabbit developmental toxicity study after XDE-729 Methyl exposure). Also, France agrees that the bridging strategy based on the demonstration of the saturation of hydrolysis of hydrolysis of XDE-729 Methyl may be acceptable.

B.6.6 Reproductive and developmental toxicity studies (IIA 5.6)

A two-generation reproductive toxicity (and probe study) on XDE-279 Acid has been conducted. A rat developmental toxicity study (plus probe study) and a rabbit toxicity study (plus probe study) have been conducted on both XDE-729 Acid and XDE-729-Methyl. All these studies used dietary administration.

B.6.6.1 Multi-generation study in the rat

XDE-729 Acid	
	IIA 5.6.1/01 A reproductive/developmental toxicity probe study in Crl:CD(SD) rats
Reference	[redacted] (2010)
Date performed	September – November 2009
Test facility	[redacted]
Report reference	Laboratory study 091061
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Acid, Lot #E2978-05-01 TSN030751-0005, 96.5% purity
Study acceptable	Yes

METHODS

Crl:CD(SD) rats, about 8 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.6.1–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of parental animals	
		Males	Females
1	0	12	12
2	50	12	12
3	250	12	12
4	750/500[a]	12	12
5	1000[b]	12	12

[a]the target dose reduced to 500 mg/kg/day on study day 16 because of excessive toxicity
[b]the 1000 mg/kg/day group was terminated on study day 8 because of bodyweight loss and reduced food consumption

The test substance was administered by incorporation in the diet. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 9% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The parental animals were exposed to the test substance for 4 weeks prior to pairing for mating, through the mating period, gestation and until termination on study day 47 for parental males or PND 14 for parental females.

For the parental animals, clinical signs of toxicity, bodyweights, and food consumption were recorded. Vaginal smears were examined daily during the mating period. Parameters assessed in

the offspring during lactation were litter size, sex, presence of gross abnormalities, clinical signs and weekly bodyweights. All litters were standardised on PND 4 to contain, as near as possible, 4 males and 4 females by culling of excess pups. All surviving pups were killed on PND 14.

A necropsy was conducted on all parental animals, during which macroscopic abnormalities were recorded. Uteri were stained with 10% sodium sulphide and examined for presence of implantation sites. Liver and kidney weight were recorded. Liver and kidneys from control and high dose parents, and gross lesions from animals from all groups were subject to microscopic (light) examination. At termination the offspring were subjected only to a external macroscopic examination.

Toxicokinetic investigations were conducted at part of this study. Blood samples were taken from the jugular vein of 4 parental rats/sex/group for determination of plasma XDE-729 Acid levels. Samples were taken from males at ~09.00 h on study days 15 and 28 and from females at ~09.00 h on PND 4. Additionally, in the offspring, terminal blood samples were taken from the left ventricle of the heart from one randomly selected culled pup/sex from the litters of the dams sampled on PND 4.

RESULTS

Parental generation

Doses received by the parents were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values for the pre-mating period are shown below:

Table B.6.6.1–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	50	250	750/500
Males	53.4	266	818/520
Females	53.9	269	754/535

At 1000 mg/kg/day, a number of animals (4/12 males, 4/12 females) lost weight and had reduced food consumption during the first week of the study. On study day 8 this group was removed from the study, and is not discussed hereafter in this summary.

Treatment-related effects on clinical condition were observed in females at 750 mg/kg/day. Decreased faecal output was reported in 5/12 females, which resolved for all but one female when the dietary concentration was reduced to 500 mg/kg/day. The one female that continued to be affected was killed prematurely on study day 26 because of poor general condition.

Parental bodyweights and food consumption are summarised in Tables B.6.6.1-3 and B.6.6.1-4. The mean bodyweights of males and females at 750 mg/kg/day were lower than controls from study days 1-15, mainly due to bodyweight losses in several individual animals, although the group mean changes did not achieve statistical significance. Bodyweights for this group were similar to those of the other groups after the target dose level was reduced to 500 mg/kg/day. Food consumption was reduced in this group for the period when the target dose level was 750 mg/kg/day, notably among females.

Table B.6.6.1–3 Group mean parental bodyweights, selected data (g)

Day	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750/500	0	50	250	750/500
Study day 1	261	259	261	261	192	193	191	194
Study day 15	370	360	371	355	233	235	232	221
Study day 29	425	413	422	416	247	248	242	245
Study day 43	471	455	474	456	-	-	-	-
Gestational day 0	-	-	-	-	255	252	244	248
Gestational day 7	-	-	-	-	290	291	284	282
Gestational day 14	-	-	-	-	325	323	316	311
Gestational day 20	-	-	-	-	396	398	391	377
Post natal day 1	-	-	-	-	299	294	294	288
Post natal day 14	-	-	-	-	332	355	325	323

Table B.6.6.1-4 Group mean parental food consumption, selected data (g/animal/day)

Day	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750/500	0	50	250	750/500
Study days 1-4	25.9	24.9	24.6	25.5	16.7	16.9	16.8	15.6
Study day 8-15	26.3	25.3	25.4	24.1	18.5	18.2	17.7	16.1
Study day 19-22	26.1	24.4	24.8	25.1	18.4	17.6	17.8	15.9
Study day 26-29	25.8	25.4	24.9	24.6	17.6	18.0	16.3	17.5
Gestational day 0-7	-	-	-	-	22.1	22.5	22.3	22.2
Gestational day 7-14	-	-	-	-	23.3	23.3	23.6	22.0
Gestational day 14-20	-	-	-	-	23.3	22.2	22.8	22.8
Post natal day 1-4	-	-	-	-	31.7	33.0	29.1	28.6
Post natal day 11-14	-	-	-	-	57.8	56.4	55.4	56.1

Mating performance and fertility was not affected by XDE-729 Acid treatment.

There were no treatment related kidney or liver weight changes, or macroscopic necropsy findings.

The findings of the microscopic examination, which identified the kidney as the target organ for XDE-729 acid treatment, are summarised in Table B.6.6.1-5. The kidney changes, observed only in the 500/750 mg/kg/day group, consisted of (a) multifocal hypertrophy and hyperplasia of the epithelial cell lining of the kidney collecting ducts with accompanying nuclear karyomegally; (b) necrosis of individual collecting duct epithelial cells; (c) increased mitotic figures (mitotic alteration) within the collecting ducts; and (d) tubular dilatation. The lesions were more prominent among females.

Table B.6.6.1–5 Selected microscopic pathology findings

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	500	0	50	250	500
Number examined	12	12	12	12	12	12	12	12
Kidneys								
Hypertrophy, epithelium, collecting duct, uni or bilateral, multifocal v slight	0	0	0	6	0	0	0	9
moderate	0	0	0	0	0	0	0	1
Hyperplasia, epithelium, collecting duct, bilateral v slight or slight	0	0	0	3	0	0	0	9
Mitotic alteration, increased, collecting duct, bilateral, multifocal slight	0	0	0	0	0	0	0	1
Necrosis, individual cell, epithelium, collecting tubule, uni or bilateral, multifocal, v slight	0	0	0	2	0	0	0	3
Dilatation, tubule, unilateral, focal or multifocal, v slight	2	1	2	1	1	1	1	1
Dilatation, tubule bilateral, multifocal slight	0	0	0	0	0	0	0	5
Inflammation, subacute to chronic, unilateral, focal v slight	0	0	0	2	0	0	0	2
Inflammation, subacute to chronic, unilateral, multifocal v slight	0	0	2	0	0	0	0	0
Inflammation, subacute to chronic, unilateral, multifocal v slight	0	0	0	0	0	0	0	3
Degeneration, tubule, focal or multifocal v slight	12	11	10	9	6	4	10	4
Degeneration, tubule, multifocal moderate	0	0	0	0	0	0	0	1
Hyperplasia, pelvic epithelium, uni or bilateral v slight or slight	0	0	0	2	0	0	0	4

F_1 offspring

There were no treatment-related clinical signs or malformations, or effects on litter size, pup survival or bodyweights.

Toxicokinetic investigation

Systemic exposures to XDE-729 Acid (as indicated by plasma concentrations) appeared to be approximately dose-proportional (linear) at all dose levels for both male and female parents and offspring (see Tables B.6.6.1-5 and B.6.6.1-6).

Table B.6.6.1–6 Toxicokinetic investigation: mean XDE-729 Acid dose and plasma concentration for male parents

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)			
	0	50	250	500
Actual dose of XDE-729 Acid at blood sampling on study day 15 (mg/kg/day)	0	49	266	788
XDE-729 Acid plasma concentration on day 15 (µg/g)	NQ	4.73	26.35	90.25
Actual dose of XDE-729 Acid at blood sampling on study day 28 (mg/kg/day)	0	50	264	483
XDE-729 Acid plasma concentration on day 28 (µg/g)	NQ	4.47	29.55	65.42

NQ = not quantifiable

Table B.6.6.1–7 Toxicokinetic investigation: mean XDE-729 Acid dose and plasma concentration for female parents and offspring

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)			
	0	50	250	500
Actual parental dose of XDE-729 Acid on PND 4 (mg/kg/day)	0	73	392	623
XDE-729 Acid plasma concentration on PND 4 (µg/g): mothers	NQ	11.3	72.4	109.8
XDE-729 Acid plasma concentration on PND 4 (µg/g): male pups	NQ	1.65	4.15	7.54
XDE-729 Acid plasma concentration on PND 4 (µg/g): female pups	NQ	1.53	6.50	10.57

NQ = not quantifiable

CONCLUSION

In a preliminary study, dietary administration of XDE-729 Acid for about 7 – 9 weeks to the rat at a target dose level of 500 mg/kg/day caused adverse effects in the kidney (hypertrophy, hyperplasia, necrosis and/or mitotic alteration of collecting duct epithelial cells, tubular dilatation) in the parental generation. At 750 mg/kg/day, reductions in bodyweight gain and food consumption were elicited. No evidence of reproductive toxicity was observed. Toxicokinetic investigations show that systemic exposures to XDE-729 Acid (as indicated by plasma concentrations) are approximately dose-proportional (linear) at all dose levels for both male and female parents and offspring. The study NOAEL for general parental toxicity is about 266 mg/kg/day (target 250 mg/kg/day). The study NOAEL for reproductive effects is 520 mg/kg/day (target 500 mg/kg/day), the highest dose level tested.

[redacted] (2010)

XDE-729 Acid	
Study	IIA 5.6.1/02 Two generation dietary reproductive toxicity study in Crl:CD(SD) rats
Reference	[redacted] (2011)
Date performed	March – November 2010
Test facility	[redacted]
Report reference	Laboratory study ID 091148
Guideline(s)	OECD 416
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Crl:CD(SD) rats, about 6 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.6.1–8: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals			
		F_0 parental males	F_0 parental females	F_1 parental males	F_1 parental females
1	0	27	27	27	27
2	20	27	27	27	27
3	100	27	27	27	27
4	450	27	27	27	27

The test substance was administered by incorporation in the diet. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 13% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

Exposure commenced with the F_0 generation and continued until study termination at the time of weaning of the F_2 generation. F_0 animals were mated within each dose group after 10 weeks exposure to produce the F_1 generation. The parental F_1 animals were selected at weaning and similarly mated after 10 weeks post-weaning exposure to produce the F_2 generation. Non-selected F_1 pups were killed at weaning on PND 21. F_2 pups were killed at weaning on PND 21. development event had occurred.

For the F_0 generation and parental F_1 animals, clinical signs of toxicity, bodyweights, and food consumption were recorded. Vaginal smears were examined daily for 3 weeks prior to mating and during the mating period and on the day of sacrifice for assessment of the oestrous cycle stage. A necropsy was conducted on all F_0 and F_1 parents. Sperm were sampled sperm motility, count (testicular and epididymal) and morphology assessments; counts and morphology assessments were conducted only in control and high dose animals. The number of uterine implantation sites were counted. The weights of major organs were recorded. Major organs, including reproductive organs, for control and high dose animals, together with gross lesions, kidney and aorta from the low and mid dose groups, were subjected to microscopic analysis. The microscopic examination of the testes included a qualitative assessment of stages of

spematogenesis. A quantitative evaluation of the ovarian primordial follicles was conducted on 15 F_1 parental females from the control and high dose groups.

Parameters assessed in the offspring during lactation were litter size, sex, presence of gross abnormalities, clinical signs and bodyweights. All litters were standardised on PND 4 to contain, as near as possible, 4 males and 4 females by culling of excess pups. The pups killed at culling were discarded. All litters were weaned on PND 21 and (when possible) and one pup/sex/litter was randomly selected for a necropsy which included the measurement of organ weights and two additional pups/sex/litter were randomly selected for gross necropsy only. The age at which vaginal opening and preputial separation occurred was recorded for the retained F_1 pups.

RESULTS

F_0 and F_1 parental generations

Mean actual dose levels, calculated in terms of mg XDE-729 Acid/kg body weight are shown in Table B.6.6.1-9 below:

Table B.6.6.1–9 Mean dose received during premating periods (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	20	100	450
F_0 parental males	20.7	104	465
F_0 parental females	20.5	103	465
F_1 parental males	20.3	101	459
F_1 parental females	19.5	98	443

There were no treatment related deaths.

The only clinical signs considered to be treatment related were seen only in two F_0 females at 450 mg/kg/day during the gestation and lactation periods. One female had decreased faecal production and another female and generally poor clinical appearance.

Treatment-related adverse effects on bodyweight and food consumption were limited to the observation of bodyweight loss, of 23 and 14 g respectively, and food consumption reductions, to 24 and 46% of the control value, during the last week of gestation in the two F_0 females at 450 mg/kg/day which also exhibited clinical signs.

As shown in Table B.6.6.1-10, there were no treatment related effects on mating performance or fertility of the F_0 and F_1 generations. F_1 post-implantation loss was increased in the treated groups, and greater than the historical control range at 200 and 450 mg/kg/day; however, in the absence of a clear dose-response and the absence of confirmatory observations in the F_0 generation, in the reproductive toxicity probe study (see IIA 5.6.1/01) and in the developmental toxicity studies (see IIA 5.6.10/01-03), these differences were regarded as chance observations.

Table B.6.6.1–10 F_0 and F_1 parental mating and fertility data

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	F_0 parents				F_1 parents			
	0	20	100	450	0	20	100	450
No. of M/F paired for mating	27/27	26/27	27/27	27/27	25/27	26/26	26/26	27/27
Male mating index (%)	96.3	100	100	100	92.0	96.2	92.3	100
Female mating index (%)	96.3	96.3	100	100	92.6	96.2	92.3	100
Male fertility index (%)	96.3	88.5	100	92.6	92.0	96.2	80.8	100
Female fertility index (%)	96.3	88.5	100	92.6	92.6	96.2	80.8	100
Gestation index (%)	100	100	96.3	100	100	100	100	100
Mean time to mating (days)	2.0	2.3	2.4	3.3	3.1	2.5	2.7	2.4
Mean gestation length (days)	21.8	21.3	21.5	21.6	21.6	21.6	21.7	21.7
Mean post implantation loss (%)	11.1	7.3	4.8	11.5	5.0	10.4	14.4	13.4
Historical control range[1]	5.1 – 10.7				6.8 – 10.8			

Mating index = % animals paired that mated
Fertility index = % of animals paired that sired a pregnancy or were pregnant
Gestation index = % of females with evidence of pregnancy that delivered a litter
[1]From 7 studies, conducted 2006-2010

There were no treatment related effects on oestrous cycling. There where no treatment-related effects on sperm motility, counts and morphology in either the F_0 or F_1 generation parental males. F_1 ovarian primordial follicle count was not affected.

Organ weight changes considered to be treatments related were observed in F_1 females at the highest dose level. As shown in Table B.6.6.1-11, kidney weights were significantly increased. This change is considered likely to be treatment related because it correlated with microscopic pathology changes which are summarised in Table B.6.6.1-12.

Table B.6.6.1–11 Selected group mean organ weights: F_1 generation parents

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males (F_1)				Females (F_1)			
	0	20	100	450	0	20	100	450
Final bodyweight (g)	604	630	647	620	313	313	313	322
Kidney weight (g)	4.100	4.242	4.363	4.324	2.236	2.319	2.336	2.469*
Kidney weight (% bodyweight)	0.680	0.678	0.675	0.700	0.714	0.744	0.748	0.767*

*significantly different from control $p<0.05$

The key macroscopic and microscopy pathology findings are presented in Tables B.6.6.1-12 (F_0 parents) and B.6.6.1-13 (F_1 parents). Treatment-related macroscopic necropsy findings were limited to the observation of aortic mineralization at 450 mg/kg/day in 2/27 F_0 females and 5/27 F_1 females. Treatment-related microscopic changes were also seen only at 450 mg/kg/day, which identified the kidney a primary target organ in both sexes and generations. Additionally, in F_0 and F_1 females at 450 mg/kg/day, mineralisation of the aorta, confirming the macroscopic findings, renal arteries and kidney tubular basal lamina, was observed in a small number of animals.

The adverse effects noted in the kidneys were characterised as (a) focal or multifocal, very slight to moderate, unilateral or bilateral multifocal hypertrophy of the epithelial cells lining the collecting ducts; (b) multifocal, very slight to moderate, unilateral or bilateral multifocal

hyperplasia of the epithelial cells lining the collecting ducts; (c) multifocal, very slight to moderate, unilateral or bilateral multifocal hyperplasia of the pelvic epithelium lining the papilla, or (d) multifocal, very slight or slight, unilateral or bilateral increase in the number of mitotic figures of the epithelial cells lining the collecting ducts. In addition, in F_0 and F_1 females, other changes included: (a) multifocal or diffuse, very slight to severe mineralisation of the aorta with or without accompanying inflammation, (b) multifocal, very slight mineralisation of the renal arteries and (c) multifocal, very slight or slight mineralisation of the basal lamina of renal tubules. The pathogenesis of the kidney findings is unclear. The study report authors speculated that the mineralisation within the aorta, renal arteries, and tubular basal lamina could be suggestive of systemic disturbances in calcium, phosphorus, and other electrolytes, possibly secondary to treatment-related renal effects and possible exacerbated by lactation stress.

Table B.6.6.1–12 Selected macroscopic and microscopic pathology findings: F_0 generation

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	20	100	450	0	20	100	450
Number examined	27	27	27	27	27	27	27	27
Macroscopic findings								
Aorta mineralisation	0	0	0	0	0	0	0	2
Microscopic findings								
Kidney								
Hypertrophy, epithelium, collecting duct, uni or bilateral, focal or multifocal: v slight	1	0	0	9	0	0	0	13
Hypertrophy, epithelium, collecting duct, uni or bilateral, multifocal: slight	0	0	0	1	0	0	0	3
moderate	0	0	0	0	0	0	0	1
Hyperplasia, epithelium, collecting duct, uni or bilateral, multifocal: v slight	0	0	0	9	0	0	0	5
slight	0	0	0	0	0	0	0	3
Hyperplasia, pelvic epithelium, uni or bilateral, multifocal: slight	1	2	1	4	2	0	0	4
Increased mitotic figures, collecting duct, uni or bilateral, multifocal: v slight	0	0	0	2	0	0	0	1
Mineralisation, basal lamina, tubule, multifocal: v slight	0	0	0	0	3	2	3	5
Mineralisation, artery: v slight	0	0	0	0	0	0	1	2
Aorta								
Mineralisation, aorta with or without inflammation, media, multifocal: v slight or slight	0	0	0	0	1	1	1	0
moderate	0	0	0	0	0	0	0	2

Table B.6.6.1–13 Selected macroscopic and microscopic pathology findings: F_1 generation

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	20	100	450	0	20	100	450
Number examined	27	27	27	27	27	27	27	27
Macroscopic findings								
Aorta mineralisation	0	0	0	0	0	0	0	5
Microscopic findings								
Kidney								
Hypertrophy, epithelium, collecting duct, uni or bilateral, focal or multifocal: v slight	1	0	1	7	0	0	0	19
Hypertrophy, epithelium, collecting duct, uni or bilateral, multifocal: slight	0	0	0	8	0	0	0	0
moderate	0	0	0	1	0	0	0	0
Hyperplasia, epithelium, collecting duct, un- or bilateral: v slight	1	0	1	5	0	0	0	8
slight	0	0	0	5	0	0	0	0
moderate	0	0	0	2	0	0	0	0
Hyperplasia, pelvic epithelium, uni- or bilateral: v slight	1	0	1	1	3	0	0	0
slight	1	2	2	10	1	1	2	6
Hyperplasia, pelvic epithelium, uni- or bilateral, multifocal: moderate	0	0	0	4	0	0	0	0
Increased mitotic figures, collecting duct, unilateral or bilateral, multifocal: v slight	0	0	0	6	0	0	0	0
Mineralisation, basal lamina, tubule, multifocal: v slight or slight	0	0	0	0	0	0	0	5
Mineralisation, artery: v slight	0	0	0	0	0	0	0	4
Aorta								
Mineralisation, aorta with or without inflammation, media, multifocal: v slight or slight	0	1	0	0	1	0	0	3
Mineralisation, aorta with or without inflammation, media, diffuse: moderate	0	0	0	0	0	0	0	2
Mineralisation, aorta with or without inflammation, media, multifocal: severe	0	0	0	0	0	0	0	3

F_1 and F_2 litter data

The key birth and lactation period data are summarised in Table B.6.6.1-14. There were no treatment-related adverse effects on litter size, sex ratio, pup survival or pup bodyweights.

Table B.6.6.1–14 Selected F_1 and F_2 birth and lactation data

Parameter	Target dose level of XDE-729 Acid (mg/kg/day)							
	F_1 generation offspring				F_2 generation offspring			
	0	20	100	450	0	20	100	450
Number of litters	26	23	26	25	25	25	20	26
Sex ratio PND 1 (M:F)	52:48	51:49	55:45	51:49	53:47	48:52	47:53	51:49
Mean live litter size PND 0	11.9	14.7*	14.5*	12.9	14.2	12.8	12.8	12.6
Survival index at birth (%)	99.4	99.4	99.7	97.0	100	97.0	98.9	95.5
PND 4 survival index (%)	98.1	98.5	98.4	94.1	99.2	97.2	97.8	93.8
PND 21 survival index (%)	100	98.9	100	99.4	100	100	100	99.0
Male pup weight (mean, g)								
- PND 1	7.5	6.9	7.1	7.1	6.8	7.0	7.1	6.9
- PND 7	16.8	15.6	16.0	16.0	15.7	15.8	16.8	15.9
- PND 21	55.9	53.5	54.5	54.0	54.7	53.0	55.7	55.0
Female pup weight (mean, g)								
- PND 1	7.1	6.5	6.7	6.6	6.5	6.8	6.7	6.6
- PND 7	16.3	15.0*	15.2	15.2	15.1	15.9	16.0	15.4
- PND 21	54.9	51.6	52.5	52.4	52.6	53.1	53.5	52.9

*significantly different from control, p≤0.05

There were no treatment-related macroscopic necropsy findings or effects on organ weights in the F_1 and F_2 weanlings. The age at which vaginal opening and preputial separation occurred in the retained F_1 offspring was not affected by XDE-729 Acid treatment.

CONCLUSION

Dietary administration of XDE-729 Acid over two generations caused general toxicity in the F_0 and F_1 parental generations at a target dose level of 450 mg/kg/day, the highest dose level tested, observed principally as microscopic changes in the kidney (hypertrophy, hyperplasia and/or mitotic alteration of collecting duct epithelial cells, mineralisation of tubule basal lamina and artery). No evidence of reproductive toxicity was observed. The study NOAEL for general parental toxicity is about 98 mg/kg/day (target 100 mg/kg/day). The study NOAEL for reproductive effects is 443 mg/kg/day (target 450 mg/kg/day), the highest dose level tested.

[redacted] (2011)

B.6.6.2 Developmental toxicity in the rat (IIA 5.6.10)

XDE-729 Acid	
Study	IIA 5.6.10/01 Dietary developmental toxicity study in Crl:CD(SD) rats
Reference	[redacted] (2010)
Date performed	January – March 2010
Test facility	[redacted]
Report reference	Laboratory study ID 091138
Guideline(s)	OECD 414
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Timed-mated Crl:CD(SD) strain rats, 10 – 11 weeks old, were randomly assigned to test groups as shown in the table below.

Table B.6.6.2–1: Study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of females
1	0	26
2	500	26
3	2000	26
4	8000	26

The test substance was administered by incorporation in the diet from gestational days (GD) 6 - 20. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within ~4% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 21 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver, kidney and intact uterus weight were recorded. Corpora lutea were counted and the uterine contents were examined. The foetuses were weighed and subjected to an external examination. About half of the foetuses were subjected to a detailed visceral examination and the remaining foetuses were double stained with Alcian Blue and Alizarin Red S for skeletal examination.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.6.2–2 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	500	2000	8000
Dams	34.1	140	526

Maternal toxicity

There were no treatment related deaths.

Adverse clinical reactions to treatment occurred at the highest dose level of 8000 ppm. Two females were killed prematurely GD 18 and 17, respectively, following the observation of excessive body weight loss and reduced food consumption. Reduced faecal output was observed in four females and two females had perinasal red soiling.

Maternal bodyweight gain and food consumption was lower than the controls at 8000 ppm, as shown in Tables B.6.6.2-3 and B.6.6.2-4. Although the differences were not statistically significant, this was considered to be a treatment related adverse effect.

Table B.6.6.2–3: Group mean maternal bodyweight gain: selected intervals (g)

Interval	Dietary concentration of XDE-729 Acid (ppm)			
	0	500	2000	8000
GD 6 - 9	15.6	16.2	18.1	16.2
GD 9 - 12	14.3	12.2	14.1	11.5
GD 12 - 15	24.2	21.7	23.8	19.7
GD 15 - 18	33.7	33.2	32.5	34.9
GD 18 - 21	55.1	54.3	57.4	34.9
GD 6 - 21	142.9	137.6	145.9	115.0

Table B.6.6.2–4: Group mean maternal food consumption maternal (g/animal/day)

Interval	Dietary concentration of XDE-729 Acid (ppm)			
	0	500	2000	8000
GD 6 - 9	22.7	22.6	23.4	22.4
GD 9 - 12	24.1	23.3	24.6	23.3
GD 12 - 15	25.0	23.7	25.1	23.3
GD 15 - 18	26.5	25.7	26.3	24.9
GD 18 - 21	26.5	26.0	27.7	21.8

At necropsy, there were no treatment-related macroscopic observations in any females, included to two killed prematurely. Maternal bodyweight-related kidney weight was significantly increased at 8000 ppm only, as shown in Table B.6.6.2–5.

Table B.6.6.2–5: Group mean maternal organ weights: kidney

Parameter	Dietary concentration of XDE-729 Acid (ppm)			
	0	500	2000	8000
Final bodyweight (g)	453	446	457	425
Kidney weight (g)	1.95	1.93	2.02	2.01
Kidney weight (% bodyweight)	0.43	0.43	0.44	0.48*

*significantly different from control, $p \leq 0.05$

Developmental toxicity

The key pregnancy and foetal data are summarised in Table B.6.6.2-6. XDE-729 Acid had no effect on the numbers of implantation sites and live foetuses, pre-and post-implantation loss or on sex ratio. The incidence of malformations was low, and similar, in all groups and therefore not influenced by XDE-729 Acid treatment. However, foetal weight was slightly reduced at 8000 ppm. Although statistical significance was not achieved, the report authors considered this to be a treatment related effect because the values at 8000 ppm were lower than the laboratory historical control range. There was an increased incidence of delayed ossification of centra of the thoracic vertebrae at 8000 ppm, in comparison with both the concurrent control group and the laboratory historical control range. The presence of slightly reduced foetal weights and an increased incidence of delayed ossification of the thoracic centra indicate a marginal retardation of development at 8000 ppm, which can be considered likely to be a secondary non-specific consequence of the slight maternal toxicity elicited in this dose group.

Table B.6.6.2–6: Key pregnancy and foetal data

Parameter	Dietary concentration of XDE-729 Acid (ppm)			
	0	**500**	**2000**	**8000**
Number pregnant females	26	26	26	24
Mean no. corpora lutea	14.5	13.7	13.7	14.2
Mean no. implantation sites	14.1	13.5	13.3	14.0
Pre-implantation loss (%)	2.2	1.2	4.3	1.4
Post-implantation loss (%)	2.2	3.6	4.5	6.2
Mean no. live foetuses/litter	13.8	13.0	12.7	13.1
Sex ratio, M:F	50:50	54:46	50:50	46:54
Mean foetal weight (g) -all	5.8	5.8	5.9	5.5
	Historical control range 5.6 – 5.9 g			
- males	5.9	6.0	6.1	5.7
	Historical control range 5.8 – 6.1 g			
- females	5.6	5.6	5.8	5.4
	Historical control range 5.5 – 5.7 g			
Total no. of foetuses examined	359	338	330	314
No. of malformed foetuses (litters)	2 (1)	2 (2)	1	3 (3)
Type of malformations	1 with missing ribs 1 with sternoschisis	1 with hydronephrosis 1 with sternoschisis	1 with hydrocephaly	1 with ectopic adrenal 1 with convoluted ureter 1 with missing ribs
Skeletal variants:				
Delayed ossification of thoracic centra				
No. of foetuses (%)	2/180 (1.1)	1/167 (0.6)	2/164 (1.2)	7/157 (4.5)*
	Historical control range 0 - 3.1%			
No. of litters (%)	1/26 (3.8)	1/26 (3.8)	1/25 (4.0)	7/24 (29.2)
	Historical control range 0 - 13.6%			

*significantly different from control, $p \leq 0.05$
Historical control data obtained from 6 or 7 rat studies conducted at the testing laboratory between 2005 and 2010 (method of administration is not described).

CONCLUSION

Oral (dietary) administration of XDE-729 Acid to the rat during pregnancy caused slight maternal toxicity only at the highest dose level tested, 8000 ppm, observed as a reduction in bodyweight gain and food consumption and increased kidney weight. There was evidence of a very marginal developmental delay at 8000 ppm, observed as a slight reduction in foetal weight and a slight increase in the incidence of delayed ossification of the thoracic centra. As the developmental changes were very minor, and observed in association with evidence of maternal toxicity, it can be concluded that XDE-729 Acid is not a specific developmental toxin. A study NOAEL of 2000 ppm (intake of about 140 mg/kg/day) is identified for both maternal and developmental toxicity.

[redacted] (2010)

XDE-729 Methyl	
Study	IIA 5.6.10/02 Dietary developmental toxicity probe study in Crl:CD(SD) rats
Reference	[redacted] (2012)
Date performed	May – June 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111070
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Timed-mated Crl:CD(SD) strain rats, sexually mature weighing 200-250 g, were randomly assigned to test groups as shown in the table below.

Table B.6.6.2–7: Study design

Test group	Dietary concentration of XDE-729 Methyl (ppm)	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of females
1	0	0	5
2	521	41	5
3	2083	162	5
4	4167	324	5
5[a]	8333	648	5

[a]group 5 was terminated on gd 9 because of excessive toxicity, and no further observations were recorded

The test substance was administered by incorporation in the diet from gestational days (GD) 6 - 21. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 9.5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The dose levels were selected to evaluate toxicological bioequivalency in relation to the rat developmental toxicity study for XDE-729 Acid (IIA 5.6.10/01). Assuming an equivalency factor of 95.93, the chosen dietary concentrations correspond to XDE-729 Acid concentrations of 0, 500, 2000, 4000 and 8000 ppm, covering the range of concentrations used in the Acid developmental toxicity study. Taking account of the results of the 28-day XDE-729 Methyl study in rats (IIA 5.3.1/02), these concentrations were expected to provide adequate data to establish a maximum tolerated dose and to provide dose-response data for any toxicity observed.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 21 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver and kidney weight were recorded. Corpora lutea were counted and the

uterine contents were examined. The foetuses were discarded without a detailed foetal examination being conducted.

Toxicokinetic investigations were conducted as part of this study. Firstly, to determine diurnal variations in systemic dose at steady state, a blood sample was collected from the jugular vein of all dams (non-fasted) at 06.00 h, 09.00 h and 17.00 h on gd 20. Secondly, at the time of necropsy (around 08.00 h on GD 21), a blood sample was taken from the jugular vein of all dams, and from the umbilical cord of all foetuses from their respective litters. The blood samples were analysed for concentration of XDE-729 Acid and XDE-729 Methyl. The blood samples from foetuses of the same litter were pooled for analysis.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.6.2–8 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Methyl (ppm)	521	2083	4167	8333
Dams	40.4	169	303	490

Maternal toxicity

There were no treatment related deaths or clinical signs of toxicity.

Group mean maternal bodyweights and food consumption are shown in Tables B.6.6.2-9 and B.6.6.2-10. Animals of the 8333 ppm group exhibited markedly reduced bodyweight gain and food consumption at the start of the dosing period. This group was terminated on GD 9 with no further observations being recorded. At 4167 ppm, maternal bodyweight gain was slightly reduced, being about 10% lower than controls for the dosing period (GD 6-21). Also, food consumption at 4167 ppm was slightly reduced throughout the dosing period. There were no effects on maternal bodyweight or food consumption at 521 or 2083 ppm.

Table B.6.6.2–9 Group mean maternal bodyweights and bodyweight gain: selected intervals (g)

Interval/parameter	Dietary concentration of XDE-729 Methyl (ppm)				
	0	521	2083	4167	8333
Bodyweight GD 6	267	272	274	263	264
Bodyweight GD 9	286	291	297	279	266
Bodyweight GD 15	331	346	341	318	-
Bodyweight GD 21	410	436	428	392	-
Gain GD 6-9	19.0	19.6	23.0	15.1	2.0
Gain GD 6-21	144	164	154	129	-

Table B.6.6.2–10 Group mean maternal food consumption (g/animal/day)

Interval (GD)	Dietary concentration of XDE-729 Methyl (ppm)				
	0	521	2083	4167	8333
6-9	23.3	22.7	24.9	20.2	15.6
9-12	25.6	26.1	26.5	21.7	-
12-15	26.5	26.8	28.0	23.5	-
15-18	27.7	28.0	28.3	25.3	-
18-20	25.3	26.3	27.3	22.8	-

There were no treatment-related maternal macroscopic necropsy findings or liver and kidney weight differences.

Developmental toxicity

Pregnancy rate, number of corpora lutea and implantation sites and pre- and post-implantation loss were not affected by treatment.

Toxicokinetics

The toxicokinetic findings are summarised in Table B.6.6.2.11.

On GD 20, XDE-729 Methyl was present in maternal samples at 06.00 h, but was decreased or absent at the remaining sampling times of 09.00 and 17.00 h. On GD 21, sampling time ~08.00 h, XDE-729 Methyl was not detected in any maternal blood samples. The presence of XDE-729 Methyl in blood samples taken on GD 20 was inconsistent with the absence of XDE-729 Methyl on GD 21 in the current study, and with the fact that XDE-729 Methyl has not been routinely detected in blood in other repeat dose XDE-729 Methyl rat studies, namely the 28-day and 90-day repeat dose studies (IIA 5.3.1/02 and IIA 5.3.2/02), the 28-day MoA study (IIA 5.5.4/03) and the developmental toxicity study (IIA 5.6.10/03). XDE-729 Acid was present in maternal blood samples at all time points, with highest levels being observed at 06.00 h though diurnal variation was modest.

Concentrations of XDE-729 Acid were very much higher than for XDE-729 Methyl, at about two orders of magnitude higher for the time points at which XDE-729 Methyl was present. Systemic exposure to both XDE-729 Methyl and XDE-729 Acid appeared to be approximately dose-proportional (linear) at all exposure levels.

In the foetuses, XDE-729 Methyl was not present in quantifiable amounts. In contrast, XDE-729 Acid was present in the foetuses, and in quantities that were approximately dose-proportional (linear). Foetal blood concentrations of XDE-729 Acid were about 70% of maternal concentrations.

Table B.6.6.2-11: Group mean amount of XDE-729 Methyl and XDE-729 Acid in maternal and foetal blood on GD 20 and 21

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Calculated intake of XDE-729 Methyl GD20-21 (mg/kg/day)	0	32	134	246
Maternal blood conc. XDE-729 Methyl (µg/g)				
GD 20, 06.00 h	NQ	0.028 ± 0.014	0.077 ± 0.021	0.157 ± 0.034
GD 20, 09.00 h	NQ	NQ	0.057 ± 0.031	0.106 ± 0.014
GD 20, 17.00 h	NQ	NQ	NQ	0.043 ± 0.026
GD 21 at necropsy, 08.00 h	NQ	NQ	NQ	NQ
Maternal AUC_{24h} for XDE-729 Methyl (µg h/ml)	-	-	-	-
Foetal blood conc. XDE-729 Methyl (µg/g) GD 21 at necropsy	NQ	NQ	NQ	NQ
Maternal blood conc. XDE-729 Acid (µg/g)				
GD 20, 06.00 h	NQ	2.878 ± 0.904	8.988 ± 2.473	13.944 ± 0.994
GD 20, 09.00 h	NQ	1.665 ± 0.920	5.507 ± 1.572	7.692 ±1.906
GD 20, 17.00 h	NQ	1.442 ± 0.710	4.823 ± 2.610	8.409 ± 4.059
GD 21 at necropsy, 08.00 h	NQ	1.735 ± 0.895	4.069 ± 1.813	6.507 ± 1.204
Maternal AUC_{24h} for XDE-729 Acid (µg h/ml)	-	47.321 ±17.737	152.836 ± 36.704	242.157 ± 51.225
Foetal blood conc. XDE-729 Acid (µg/g) GD 21 at necropsy	NQ	0.972 ± 0.303	2.887 ± 1.517	5.0151 ± 1.433

NQ = not quantifiable - not possible to calculate

CONCLUSION

Oral (dietary) administration of XDE-729 Methyl to the rat during pregnancy caused maternal toxicity at 4167 and 8333 ppm, observed as a reduction in bodyweight gain and food consumption. The effects at 8333 ppm were severe, such that this group was terminated on GD 9, with no further observations being made. There was no evidence of developmental toxicity at dietary concentrations of up to 4167 ppm. Study NOAELs of 2083 ppm (intake of about 169 mg/kg/day) for maternal toxicity and 4167 ppm (intake of about 303 mg/kg/day) for developmental toxicity were identified.

Toxicokinetic investigations demonstrate that maternal systemic exposure to the XDE-729 Acid metabolite is very much greater (~2 orders of magnitude) than to XDE-729 Methyl. Foetal exposure to XDE-729 Methyl is not quantifiable. Systemic exposure to the Acid metabolite in mothers and foetuses is dose-proportional. In mothers acid exposure is highest at 06.00 h, though diurnal variation was modest. Acid metabolite levels in the foetus are about 70% of those found in the mother.

[redacted] (2012)

XDE-729 Methyl	
Study	IIA 5.6.10/03 Dietary developmental toxicity study in Crl:CD(SD) rats
Reference	[redacted] 2012)
Date performed	July – August 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111071
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Timed-mated Crl:CD(SD) strain rats, sexually mature weighing 200-250 g, were randomly assigned to test groups as shown in the table below.

Table B.6.6.2–12: Study design

Test group	Dietary concentration of XDE-729 Methyl (ppm)	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of mated females
1	0	0	24
2	521	41	24
3	2083	162	24
4	4167	324	24

The test substance was administered by incorporation in the diet from gestational days (GD) 6 - 21. The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 8.5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The dose levels were selected taking account of the results of a probe study (IIA 5.6.10/02) and to permit an investigation of toxicological bioequivalency in relation to the rat developmental toxicity study for XDE-729 Acid (IIA 5.6.10/01). Assuming an equivalency factor of 95.93, the chosen dietary concentrations correspond to XDE-729 Acid concentrations of 0, 500, 2000, 4000 ppm. The highest concentration was expected to induce mild overt maternal toxicity and the lower concentrations were expected to provide dose-response data for any adverse effects that were induced.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 21 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver, kidney and intact uterus weight were recorded. Microscopic examination of maternal liver samples was conducted for all dose groups. Corpora lutea were counted and the uterine contents were examined. The foetuses were weighed and subjected to an external examination. About half of the foetuses were subjected to a detailed visceral

examination and the remaining foetuses were double stained with Alcian Blue and Alizarin Red S for skeletal examination.

A toxicokinetic investigation was conducted as part of this study. At the time of necropsy (about 09.00 h, on GD 21), a blood sample was taken from the jugular vein of 4 mothers per dose group, and from the umbilical cord of all foetuses from their respective litters, for analysis of the concentration of XDE-729 Methyl and XDE-729 Acid. The blood samples from foetuses of the same litter were pooled for analysis.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.6.2–13 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Methyl (ppm)	521	2083	4167
Dams	41.1	159	323

Maternal toxicity

There were no treatment related deaths or clinical signs of toxicity.

At 2083 and 4167 ppm, maternal bodyweight gain and food consumption were reduced during the first few days of the treatment period (GD 6-9) only, as shown in Table B.6.6.2-14. For the remainer of the study maternal bodyweights and food consumption were unaffected by treatment.

Table B.6.6.2-14: Group mean maternal bodyweight gain and food consumption: selected intervals

Interval	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Bodyweight gain (g)				
GD 6-9	19.7	20.7	17.7	15.1*
GD 9-12	21.9	22.1	22.1	22.9
GD6-21	152	158	149	156
Food consumption (g/animal/day)				
GD 6-9	21.5	22.2	19.9*	19.7*
GD 9-12	23.5	23.7	22.0	22.3
GD15-18	26.1	26.6	26.3	28.0*

*significantly different from control, p≤0.05

At necropsy, there were no treatment-related macroscopic observations in any females. Maternal absolute liver weight was significantly increased at 4197 ppm, and bodyweight-related liver weight was significantly increased at all treatment levels, as shown in Table B.6.6.2–15. Microscopic examination of maternal livers was conducted. As shown on Table B.6.6.2-16, a dose-related increased incidence of altered cytoplasmic homogeneity of centrilobular/midzonal hepatocytes, graded ‘very slight’ was seen at 2083 and 4197 ppm. The liver weight increases and microscopic changes at 2083 and 4197 ppm were considered to be adverse effects of XDE-729 Methyl treatment. The bodyweight-related increased liver weight at 521 ppm can be regarded as

being an adaptive response to treatment because increase was modest (i.e. much less than 10% of control value) and correlating microscopic changes were absent.

Table B.6.6.2–15: Group mean maternal organ weights: liver

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Final bodyweight (g)	408	414	402	412
Liver weight (g)	14.6	15.7	15.7	17.8*
Liver weight (% bodyweight)	3.57	3.78*	3.90*	4.32*
[% increase vs. control]		[5.9%]	[9.2%]	[21%]

*significantly different from control, p≤0.05

Table B.6.6.2–16: Maternal microscopic pathology findings: liver

Finding	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Number examined	24	24	24	24
Altered cytoplasmic homogeneity of centrilobular/midzonal hepatocytes, very slight	3	2	14	21

Developmental toxicity

The key pregnancy and foetal data are summarised in Table B.6.6.2-17.

XDE-729 Methyl had no effect on the numbers of implantation sites and live foetuses, pre-and post-implantation loss or on sex ratio. The incidence of malformations was very low and not influenced by XDE-729 Methyl treatment. The incidence of external, visceral and skeletal foetal variations was similar in all groups.

However, mean male foetal weight was statistically significantly lower at 4167 ppm. According to the Applicant, the statistically significant lower male foetal body weight should be deemed spurious and unrelated to treatment because: 1) the statistical significance was limited to only males analysed alone, and 2) the foetal body weights (males, females, combined) in the 4167 ppm group were only 0.1g below the recent historical control values with overlapping standard deviations. However, The RMS considers that the significant lower male foetal body weight should be conservatively regarded as a treatment-related adverse effect as the mean foetal weight is outside the historical control range and because foetal weights of females were also slightly reduced (although statistical significance was not achieved when compared to concurrent controls).

Table B.6.6.2–17: Key pregnancy and foetal data

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Number pregnant females	22	23	22	23
Mean no. corpora lutea	12.6	12.8	12.7	13.1
Mean no. implantation sites	12.0	12.6	11.9	12.6
Pre-implantation loss (%)	3.9	1.4	6.4	4.0
Post-implantation loss (%)	4.1	6.7	5.3	4.7
Mean no. live foetuses/litter	11.5	11.7	11.3	12.0
Sex ratio, M:F	48:52	51:49	48:52	53:47
Mean foetal weight (g) -all	5.8 ± 0.3	6.0 ± 0.3	5.9 ± 5.9	5.7 ± 5.7
	Historical control values: 5.8 ± 0.3, 5.9 ± 0.2, 5.8 ± 0.3, 5.8 ± 0.3; range 5.8 – 5.9 g			
- males	6.0 ± 0.3	6.1 ± 0.3	6.0 ± 0.2	5.8 ± 0.4*
	Historical control values: 6.0 ± 0.4, 6.1 ± 0.3, 5.9 ± 0.3, 5.9 ± 0.4; range 5.9 – 6.1 g			
- females	5.7 ± 0.3	5.8 ± 0.2	5.7 ± 0.3	5.5 ± 0.4
	Historical control values: 5.6 ± 0.3, 5.7 ± 0.2, 5.7 ± 0.3, 5.6 ± 0.3; range 5.6 – 5.7 g			
Total no. of foetuses examined	254	270	248	276
No. of malformed foetuses (litters)	2 (2)	0	1	0
Type of malformations	1 with dilated cerebral ventricles 1 with missing thoracic centra & vertebrae, missing ribs		1 with shortened ribs	

*significantly different from control, p≤0.05
Historical control data obtained from 4 rat studies conducted at the testing laboratory between 2009 and 2010 (method of administration is not described).

Toxicokinetics

Toxicokinetic analysis of maternal and foetal blood showed that XDE-729 Methyl was not present at quantifiable concentrations at all dose levels. In contrast, XDE-729 Acid was present in maternal and foetal blood, in quantities that were approximately dose-proportional (linear). Foetal blood concentrations of XDE-729 Acid were about 65% of maternal concentrations.

Table B.6.6.2–17: Mean amount of XDE-729 Methyl and XDE-729 Acid in maternal and foetal blood on GD 21

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Calculated intake of XDE-729 Methyl on GD 21 (mg/kg/day)	0	35.5	135	272
Maternal blood concentration of XDE-729 Methyl (µg/g)	NQ	NQ	NQ	NQ
Foetal plasma concentration of XDE-729 Methyl (µg/g)	NQ	NQ	NQ	NQ
Maternal blood concentration of XDE-729 Acid (µg/g)	NQ	2.23	5.97	13.1
Foetal blood concentration of XDE-729 Acid (µg/g	NQ	1.41	4.75	7.13

NQ = not quantifiable

CONCLUSION

Oral (dietary) administration of XDE-729 Methyl to the rat during pregnancy caused maternal toxicity at 2083 and 4167 ppm, observed as a reduction in bodyweight gain and food consumption at the start of the dosing period, increased liver weight and altered cytoplasmic homogeneity of centrilobular/midzonal hepatocytes. There was evidence of a very marginal developmental toxicity at 4167 ppm, observed as a slight reduction in foetal weight. As the observed developmental changes were very minor, and present in association with evidence of maternal toxicity, it can be concluded that XDE-729 Methyl is not a specific developmental toxin. Study NOAELs of 521 ppm (intake of about 41 mg/kg/day) for maternal toxicity and 2083 ppm (intake of about 159 mg/kg/day) for developmental toxicity are identified.

Toxicokinetic investigations demonstrate that there is negligible maternal and foetal systemic exposure to XDE-729 Methyl. Systemic exposure to the Acid metabolite occurs in both mothers and foetuses, which is dose-proportional. Acid metabolite levels in the foetus are about 65% of those found in the mother.

[redacted] (2012)

B.6.6.3 Developmental toxicity in the rabbit (IIA 5.6.11)

	XDE-729 Acid
Study	IIA 5.6.11/01 Dietary developmental toxicity probe study in New Zealand white rabbits
Reference	[redacted] (2011)
Date performed	April – May 2010
Test facility	[redacted]
Report reference	Laboratory study ID 091142
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Timed-mated female New Zealand white rabbits, about 5 to 6 months old, were randomly assigned to test groups as shown in the table below:

Table B.6.6.3–1: Study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of females
1	0	7
2	5000	7
3	10000	7
4	15000	7
5	23000	7

The test substance was administered by incorporation in the diet from gestational days (GD) 7 - 28. The test and control diets were prepared using Lab Diet 5325. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within ~5% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 28 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver, kidney and intact uterus weight were recorded. Corpora lutea were counted and the uterine contents were examined. The foetuses were not subjected to a detailed examination, although any external malformations were recorded.

A toxicokinetic investigation was conducted as part of this study. At the time of necropsy, a blood sample was taken from the jugular vein of 4 mothers per dose group, and from the umbilical cord of all foetuses from their respective litters, for analysis of plasma concentration of XDE-729 Acid. The blood samples from foetuses of the same litter were pooled for analysis.

A further toxicokinetic investigation was conducted to provide a comparison of systemic exposure following dietary and gavage administration. Five additional non-mated female New Zealand white rabbits were fed test diet containing 4000 ppm XDE-729 Acid for 24 hours and blood samples were taken via surgically implanted (by the animal breeding company) vascular access ports at 0.25, 0.5, 1, 2, 3, 6, 12, 24, 48 and 72 hours after the commencement of feeding the test diets. Following the withdrawal of the test diet, the five females were fed control diet for 48 h and then each was administered a gavage dose of 149-188 mg/kg XDE-729 Acid (matched to the estimated dietary intake of XDE-729 Acid 48 hours previously) and blood samples were taken 0.08, 0.15, 0.25, 0.5, 1, 2, 3, 6, 12, 24, 48 and 72 hours after dosing. The blood samples were analysed for plasma concentration of XDE-729 Acid.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.6.3-2: Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	5000	10000	15000	23000
Mothers	196	358	495	929

Maternal toxicity

There were no premature maternal deaths or treatment related clinical signs of toxicity. Maternal bodyweights and food consumption were not affected by XDE-729 Acid treatment. There were no treatment-related maternal macroscopic necropsy observations or organ weight changes.

Developmental toxicity

Pregnancy rate, number of corpora lutea and implantation sites and pre- and post-implantation loss were not affected by treatment.

Toxicokinetics

The results of the GD 28 maternal and foetal plasma XDE-729 Acid analyses showed that plasma concentrations were approximately dose proportional in both mothers and foetuses, and that foetal concentrations were about 40% of the maternal concentrations (see Table B.6.6.3-3).

Table B.6.6.3-3: Mean amount of XDE-729 Acid in maternal and foetal plasma on GD 28

Parameter	Dietary concentration of XDE-729 Acid (ppm)				
	0	5000	10000	15000	23000
Calculated intake of XDE-729 Acid on GD 28 (mg/kg/day)	0	171	261	562	900
Maternal plasma concentration of XDE-729 Acid (µg/g)	NQ	1.23 ± 1.87	2.34 ± 2.67	0.97 ± 0.95	5.75 ± 5.27
Foetal plasma concentration of XDE-729 Acid (µg/g)	NQ	0.34 ± 0.11	0.51 ± 0.18	0.94 ± 0.30	2.52 ± 2.18

NQ = not quantifiable

The comparison of the toxicokinetic profile following gavage and dietary administration, demonstrated similar systemic exposure over a 24h period, as measured by AUC, between the two methods of oral administration (AUC_{0-t} was 151 and 121 µg h g^{-1} for gavage vs. diet, respectively) . However, dietary administration resulted in far more consistent exposure over a 24h period relative to gavage with only a 6-fold vs. 368-fold fluctuation between C_{min} and C_{max} by the diet vs. gavage, respectively. Furthermore, plasma XDE-729 Acid was detectable only up to 12 h by gavage (elimination $t_{½}$ for gavage route was 1.08 h), but was present for up to 24 h by dietary administration, driven by continued dietary test material intake over a 24 h period. Considering that embryo/foetal development occurs continuously during gestation, the dietary route of oral administration is preferable to gavage in developmental toxicity studies as it produces a more stable and consistent systemic test material exposure, particularly for molecules with short elimination half-lives like XDE-729 Acid.

CONCLUSION

There is no evidence of maternal or developmental toxicity following oral (dietary) administration of XDE-729 Acid to the rabbit during pregnancy at exposure levels of up to 23000 ppm (intake of about 929 mg/kg/day) in this probe study. A toxicokinetic investigation showed that systemic bioavailability of XDE-729 Acid following dietary administration is approximately dose-proportional in both the mothers and foetuses. In the foetus, plasma concentrations of XDE-729 Acid are about 40% of maternal concentrations.

A comparison of the toxicokinetic profile in non-mated female rabbits following gavage and dietary administration showed the dietary method produces a more stable and consistent pattern of maternal systemic exposure to XDE-729 Acid.

[redacted] (2011)

XDE-729 Acid	
Study	IIA 5.6.11/02 Dietary developmental toxicity study in New Zealand white rabbits
Reference	[redacted] (2011)
Date performed	July – August 2010
Test facility	[redacted]
Report reference	Laboratory study ID 091143
Guideline(s)	OECD 414
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

Timed-mated female New Zealand white rabbits, about 5 to 6 months old, were randomly assigned to test groups as shown in the table below:

Table B.6.6.3–4: Study design

Test group	Dietary concentration of XDE-729 Acid (ppm)	Number of females
1	0	26
2	4000	26
3	10000	26
4	28292[a]	26

[a]analytically determined concentration is cited; the intended concentration was 24000 ppm

The test substance was administered by incorporation in the diet from gestational days (GD) 7 - 28. The test and control diets were prepared using Lab Diet 5325. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 7.5% of the declared concentrations, demonstrating satisfactory preparation of the dietary formulations.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 28 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver, kidney and intact uterus weight were recorded. Corpora lutea were counted and the uterine contents were examined. The foetuses were weighed and subjected to an external examination. All foetuses were subjected to a detailed visceral examination. About half of the foetuses were subjected to a craniofacial examination, involving serial sectioning of the head. All foetuses were then eviscerated and stained with Alizarin Red S for skeletal examination.

A toxicokinetic investigation was conducted as part of this study. At the time of necropsy, a blood sample was taken from the jugular vein of 4 mothers per dose group and from the umbilical cord of all foetuses from their respective litters, for analysis of plasma concentration of XDE-729 Acid. The blood samples from foetuses of the same litter were pooled for analysis.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.6.3–5 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Acid (ppm)	4000	10000	28292
Mothers	170	434	1094

Maternal toxicity

There were no treatment-related mortalities or clinical signs of toxicity. However, one mother from the low dose group was killed prematurely on GD 11 following the observation bodyweight loss, low food consumption decreased faecal production and diarrhoea.

At the highest dose level, there was a slight reduction in bodyweight gain from GD 7-28 and food consumption was slightly reduced from GD 10 to 28 in comparison with controls, although the intergroup differences did not achieve statistical significance.

Table B.6.6.3–6: Group mean maternal bodyweight gain and food consumption: selected intervals

Interval	Dietary concentration of XDE-729 Acid (ppm)			
	0	4000	10000	28292
Bodyweight gain (g)				
GD 7-28	+337	+349	+319	+291
Food consumption (g/animal/day)				
GD 10-13	133	132	137	119
GD 13-16	124	126	131	101
GD 16-20	149	141	144	129
GD 20-24	144	138	138	126
GD 24-28	132	127	124	115

The only noteworthy macroscopic maternal necropsy finding was a higher incidence of paraovarian cysts in the mid and high dose groups, as shown in Table B.6.6.3-7. However, these observations were considered to be unrelated to XDE-729 Acid treatment as the incidence was within the laboratory historical control range. Furthermore, these paraovarian cysts are small and clear, residing external to the ovary and oviduct, and do not involve any parenchymal tissue, which indicates that the cysts are very unlikely to have an adverse impact on reproductive function.

Table B.6.6.3–7: Incidence of paraovarian cysts in mothers

Finding	Dietary concentration of XDE-729 Acid (ppm)			
	0	4000	10000	28292
Number of dams	26	26	26	26
Paraovarian cysts (left, right and bilateral)	3 (11.5%)	4 (15.4%)	7 (26.9%)	9 (34.6%)
Historical control range 0 – 42.3% (from 9 studies, conducted 2005 – 2009)				

Developmental toxicity

The key pregnancy and foetal data are summarised in Table B.6.6.3-9.

XDE-729 Acid had no effect on the numbers of implantation sites and live foetuses, pre- and post-implantation loss, sex ratio or foetal weights. The incidence of malformations was relatively low in all dose groups, and lowest in the high dose group, and therefore was not influenced by XDE-729 Acid treatment. The incidence of visceral and skeletal variations was similar in all groups, and comparable to historical control ranges.

Table B.6.6.3–9: Key pregnancy and foetal data

Parameter	Dietary concentration of XDE-729 Acid (ppm)			
	0	4000	10000	28292
Number pregnant females	23	24	24	24
Number pregnant females alive GD 28	23	23	24	24
Mean no. corpora lutea	9.0	9.1	9.2	9.0
Mean no. implantation sites	8.5	9.0	8.4	8.4
Pre-implantation loss (%)	5.3	3.2	9.3	7.3
Post-implantation loss (%)	4.1	7.9	4.3	4.1
Mean no. live foetuses/litter	8.1	8.1	8.0	8.0
Sex ratio, M:F	50:50	43:57	49:51	51:49
Mean foetal weight (g) -all	34.3	34.4	34.7	33.8
- males	35.1	35.0	35.0	34.8
- females	33.4	34.0	34.3	32.7
Total no. of foetuses examined (litters)	187 (23)	187 (23)	193 (24)	193 (24)
No. of foetuses with malformations seen at external examination (litters)	2 (2)	1	1	0
Type of external malformations	1 with meningoencephalocele 1 with cleft lip, encephalocele & absent eyelid	1 with missing teeth, hypoplastic nose, cleft lip & micrognathia	1 with hypoplastic tail	
No. of foetuses with malformations seen at visceral examination (litters)	3 (3)	6 (6)	6(5)	2 (2)
Type of visceral malformations	1 with persistent truncus arteriosis 2 with bifurcated renal artery	1 with missing gall bladder 1 with missing ventricular septum & persistent truncus arteriosis 1 with retrooesophageal subclavian artery 1 with anencephaly 2 with bifurcated renal artery	3 with missing gall bladder (from 2 litters) 1 with missing gall bladder, dilated brain ventricles & hydronephrosis 1 with missing inominate artery 1 with misshapen heart, ventricular septal defect, dilated pulmonary artery & diaphragmatic hernia	1 with missing inominate artery 1 with bifurcated renal artery
No. of foetuses with malformations seen at skeletal examination (litters)	1	1	2 (2)	1
Type of skeletal malformations	1 with fused thoracic centra and thoracic hemivertebrae	1 with fused frontal, maxilla & nasal	1 with forked ribs & lumbar hemivertebrae 1 with misaligned sacral centra & caudal vertebrae	1 with fused thoracic centra & thoracic hemivertebrae

Toxicokinetics

The GD 28 toxicokinetic investigation maternal and foetal plasma XDE-729 Acid analyses demonstrated the presence of XDE-729 Acid in all treated females and litters, with the exception of one litter at 4000 ppm and one litter at 26292 ppm. Systemic bioavailability of XDE-729 Acid to mothers and foetuses appeared to be rather less than dose proportional (see Table B.6.6.3-8), but the high variability between animals in each group precluded the conduct of a reliable assessment of dose proportionality. The report authors speculate that the high intra-group variability was due to the short plasma half-life in the rabbit (an elimination $t_{½}$ for gavage route of 1.08 h was reported in the probe study IIA 5.6.11/01) and erratic feeding patterns in the mothers at the end of pregnancy. Foetal plasma concentrations of XDE-729 Acid were on average about 35% of maternal concentrations.

Table B.6.6.3-8: Mean amount of XDE-729 Acid in maternal and foetal plasma on GD 28

Parameter	Dietary concentration of XDE-729 Acid (ppm)			
	0	4000	10000	28292
Calculated intake of XDE-729 Acid on GD 28 (mg/kg/day)	0	161 ± 50	380 ± 50	1029 ± 215
Maternal plasma concentration of XDE-729 Acid (µg/g)	NQ	3.84 ± 6.36	4.58 ± 4.05	17.68 ± 9.21
Foetal plasma concentration of XDE-729 Acid (µg/g)	NQ	1.81 ± 2.81	1.21 ± 0.81	5.51 ± 6.69

NQ = not quantifiable

CONCLUSION

Oral (dietary) administration of by XDE-729 Acid during pregnancy to the rabbit caused slight maternal toxicity only at the highest dose level tested, 28292 ppm, observed as a marginal reduction in bodyweight gain and food consumption. There was no evidence of developmental toxicity. Study NOAELs of 10000 ppm (intake of about 434 mg/kg/day) for maternal toxicity and 28292 ppm (intake of about 1094 mg/kg/day) for developmental toxicity are identified.

A toxicokinetic investigation demonstrated maternal and foetal systemic exposure to XDE-729 Acid in all treated groups. Foetal plasma concentrations of XDE-729 Acid are on average about 35% of maternal concentrations.

[redacted] (2011)

XDE-729 Methyl

Study	IIA 5.6.11/03 Dietary developmental toxicity probe study in New Zealand white rabbits
Reference	[redacted] (2012)
Date performed	August 2011 – September 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111045
Guideline(s)	None
Deviations from the guideline	N/A
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Timed-mated female New Zealand white rabbits, about 5 to 7 months old and weighing 2500-3700 g, were randomly assigned to test groups as shown in the table below:

Table B.6.6.3–9: Study design

Test group	Dietary concentration of XDE-729 Methyl (ppm)	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of females
1	0	0	5 + 5*
2*	521	21	5
3*	2083	85	5
4*	4167	174	5
5*	8333	341	5
6	10417	426	5
7	15625	639	5
8	24500	1000	5

* the groups marked with an asterisk were added to the study following the premature termination of groups 6, 7 & 8 because of the presence of excessive toxicity .

The test substance was administered by incorporation in the diet from gestational days (GD) 7 - 28. The test and control diets were prepared using Lab Diet 5325. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within ~75% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The initial dose levels of 10417, 15625 and 24500 ppm were selected in part to evaluate toxicological bioequivalency in relation to the rabbit developmental toxicity study for XDE-729 Acid (IIA 5.6.11/02). Assuming an equivalency factor of 95.93, the chosen low and mid dose dietary concentrations correspond to XDE-729 Acid concentrations of 10000 and 15000 ppm and the high dose concentration of 24500 ppm was aimed at producing the test guideline limit dose of 1000 mg/kg/day.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 28 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver and kidney weight were recorded. Corpora lutea were counted and the uterine contents were examined. The foetuses were weighed, but were not subjected to a detailed examination.

A toxicokinetic investigation was conducted as part of this study. In order to determine diurnal variations in systemic dose at steady state, blood samples were collected from all mothers (without fasting) at two time points on GD 27 (12.00 and 15.00 hours and at termination on GD 28 (07.00 h). The blood samples were analysed for concentration of XDE-729 Methyl and XDE-729 Acid.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.6.3-10: Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Methyl (ppm)	521	2083	4167	8333	10417	15625	24500
Mothers	21.5	83.3	157	244	371	426	727

Maternal toxicity

There were no treatment related mortalities or clinical signs of toxicity.

In the 8333, 10417, 15625 and 24500 ppm groups minimal bodyweight gain or bodyweight losses and decrease/absent food consumption occurred in most animals between GD 5 and 15. As the maximum tolerated dose had clearly been exceeded al animals in these groups were terminated prematurely, on or prior to GD 15. No macroscopic abnormalities were observed at necropsy.

To consider the groups taken through to the scheduled termination on GD 28, at 2083 and 4167 ppm treatment related reductions on bodyweight gain occurred between GD 7 and 28, which correlated with slight reductions in food consumption, as shown in Table 6.3.3-11.

Table B.6.6.3-11: Group mean maternal bodyweight gain and food consumption: selected intervals

Interval	Dietary concentration of XDE-729 Methyl (ppm)			
	0	4000	10000	28292
Bodyweight gain (g)				
GD 7-28	+367	+372	+278	+295
Food consumption (g/animal/day)				
GD 7-8	150	150	145	150
GD 11-12	148	150	148	147
GD 15-16	150	150	131	123
GD 19-20	150	150	149	143
GD 23-24	150	149	118	124
GD 27-28	115	116	124	109

At necropsy no treatment related macroscopic abnormalities were observed on the 521, 2083 and 4167 ppm groups. However, absolute liver weight and bodyweight-related lever weight were increased in a dose-related manner in all XDE-729 Methyl treated groups, as shown in Table B6.6.3-12.

Table B.6.6.3–12: Group mean maternal organ weights: liver

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Final bodyweight (g)	3763	3730	3616	3697
Liver weight (g)	78.0	88.4	105.4	116.0
[% increase vs. control]		[13.3]	[35.1]	[48.7]
Liver weight (% bodyweight)	2.07	2.37	2.92	3.15
[% increase vs. control]		[14.5]	[41.1]	[52.2]

*significantly different from control, p≤0.05

Developmental toxicity

Pregnancy rate, number of corpora lutea and implantation sites and pre- and post-implantation loss were not affected by treatment. However, mean foetal weight was lower at 2083 and 4167 ppm, as shown in Table B.6.6.3-13.

Table B.6.6.3–13: Group mean foetal weights (g): males and females combined

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Foetal weights (g)	35.5	36.2	32.9	31.9

Toxicokinetics

Toxicokinetic analysis of maternal blood on GD 27 and GD 28 showed that XDE-729 Methyl was not present at quantifiable concentrations at all dose levels. In contrast, XDE-729 Acid was present in maternal blood, and in quantities that were approximately dose-proportional (linear). Acid concentrations were highest at 15.00 h, though diurnal variation did not appear to be great.

In the XDE-729 Acid rabbit developmental toxicity study (IIA 5.6.11/02) the mean maternal plasma level of the Acid on GD 28 at the dietary dose level of 4167 ppm was 3.84 µg/g (see Table B.6.6.3-8, approximately similar to the amount of the XDE-729 Acid found in mothers on GD 28 at 4000 ppm (2.03 µg/g) in the current XDE-729 Methyl study. This suggests equivalence in terms of maternal systemic exposure to XDE-729 Acid following dietary exposure to XDE-729 Methyl and to XDE-729 Acid at dietary exposure levels of about 4000 ppm. Because the relationship between dietary exposure to both the Acid and Methyl forms and maternal systemic exposure XDE-729 Acid is consistently dose-proportional it can be anticipated that this equivalence applies to all the dietary exposure concentrations used in the XDE-729 rabbit developmental toxicity studies.

Table B.6.6.3-14: Group mean amount of XDE-729 Methyl and XDE-729 Acid in maternal blood on GD 27 and 28

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	521	2083	4167
Calculated intake of XDE-729 Methyl GD27-28 (mg/kg/day)	0	16.2	72.1	122
Maternal blood conc. XDE-729 Methyl (µg/g)				
GD 27, 12.00 h	NQ	NQ	NQ	NQ
GD 27, 15.00 h	NQ	NQ	NQ	NQ
GD 28, 07.00 h	NQ	NQ	NQ	NQ
Maternal AUC_{24h} for XDE-729 Methyl (µg h/ml)	-	-	-	-
Maternal blood conc. XDE-729 Acid (µg/g)				
GD 27, 12.00 h	NQ	0.40 ± 0.15	0.99 ± 0.90	2.92 ± 1.67
GD 27, 15.00 h	NQ	0.54 ± 0.19	1.70 ± 1.38	3.42 ± 1.53
GD 28, 07.00 h	NQ	0.29 ± 0.21	1.04 ± 1.03	2.03 ± 1.45
Maternal AUC_{24h} for XDE-729 Acid (µg h/ml)	-	9.81 ±4.15	31.05 ± 26.71	65.55 ± 23.04

NQ = not quantifiable

CONCLUSION

Oral (dietary) administration of XDE-729 Methyl to the rabbit during pregnancy caused maternal toxicity at 521 ppm and above, observed as increased liver weight. At higher dose levels, reductions in maternal bodyweight gain and food consumption are induced. There was evidence of developmental toxicity at 2038 and 4167 ppm, observed as decreased foetal weight. A study NOAEL for maternal toxicity was not identified as adverse effects occurred at the lowest dose level; therefore, a LOAEL of 521 ppm (intake of about 21.5 mg/kg/day) is identified. The study NOAEL for developmental toxicity is 521 ppm (intake of about 21.5 mg/kg/day).

Toxicokinetic investigations demonstrate that there is negligible maternal systemic exposure to XDE-729 Methyl. Maternal systemic exposure to Acid metabolite occurs, which is dose-proportional and shows little diurnal variation. A comparison with maternal systemic exposure to the Acid reported in the XDE-729 Acid rabbit developmental toxicity study (IIA 5.6.11/02) indicates equivalence in terms of maternal systemic exposure to the Acid following dietary exposure to XDE-729-Methyl and to XDE-729-Acid in the rabbit.

[redacted] (2012)

XDE-729 Methyl	
Study	IIA 5.6.11/04 Dietary developmental toxicity study in New Zealand white rabbits
Reference	[redacted] (2012)
Date performed	October – December 2011
Test facility	[redacted]
Report reference	Laboratory study ID 111137
Guideline(s)	OECD 414
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Timed-mated female New Zealand white rabbits, about 5 to 6 months old, were randomly assigned to test groups as shown in the table below:

Table B.6.6.3–15: Study design

Test group	Dietary concentration of XDE-729 Methyl (ppm)	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of females
1	0	0	24
2	122	5	24
3	391	16	24
4	1539	63	24

The test substance was administered by incorporation in the diet from gestational days (GD) 7 - 28. The test and control diets were prepared using Lab Diet 5325. The stability of the test substance in the diet for the period of use was established prior to study commencement. The

achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 4% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The dose levels were selected taking account of the results of a probe study (IIA 5.6.11/03) and to permit an investigation of toxicological bioequivalency in relation to the rabbit developmental toxicity study for XDE-729 Acid (IIA 5.6.11/02). Assuming an equivalency factor of 95.93, the chosen dietary concentrations correspond to XDE-729 Acid concentrations of 0, 117, 366, 1466 ppm. The highest concentration was expected to induce mild overt maternal toxicity and the lower concentrations were expected to provide dose-response data for any adverse effects that were induced.

Clinical signs, bodyweight and food consumption were recorded. The females were killed on GD 28 and a necropsy was conducted. Macroscopic abnormalities of maternal visceral organs were recorded. Maternal liver, kidney and intact uterus weight were recorded. Corpora lutea were counted and the uterine contents were examined. Liver samples were subjected to microscopic examination. The foetuses were weighed and subjected to an external examination. All foetuses were subjected to a detailed visceral examination. About half of the foetuses were subjected to a craniofacial examination, involving serial sectioning of the head. All foetuses were then eviscerated and stained with Alizarin Red S for skeletal examination.

A toxicokinetic investigation was conducted as part of this study. At the time of necropsy (GD 28) a blood sample was taken from the jugular vein of 4 mothers per dose group and from the umbilical cord of all foetuses from their respective litters, for analysis of blood concentration of XDE-729 Methyl and XDE-729 Acid. The blood samples from foetuses of the same litter were pooled for analysis.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.6.3–16 Mean dose received (mg/kg/day)

Dietary concentration of XDE-729 Methyl (ppm)	122	391	1539
Mothers	5.78	18.5	71.6

Maternal toxicity

There were no treatment-related mortalities or clinical signs of toxicity.

Maternal bodyweights and food consumption were not affected by treatment.

There were no treatment related macroscopic necropsy findings. However, absolute liver weight and bodyweight-related liver weight were significantly increased in a dose-related manner at 391 and 1539 ppm, as shown in Table B6.6.3-17.

Table B.6.6.3–17: Group mean maternal organ weights: liver

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	122	391	1539
Final bodyweight (g)	3290	3240	3226	3269
Liver weight (g)	79.2	81.3	86.6*	100.4*
[% increase vs. control]		[2.7]	[9.3]	[26.8]
Liver weight (% bodyweight)	2.4	2.5	2.7*	3.1*
[% increase vs. control]		[4.2]	[12.5]	[29.2]

*significantly different from control, $p \leq 0.05$

Treatment-related microscopic changes were present in the liver (the only organ examined) at 391 and 1539 ppm, as shown in Table B.6.6.3-18. The most prominent finding was a dose-dependent increase in the incidence and severity of very slight or slight hypertrophy with altered tinctorial properties (increased cytoplasmic eosinophilia) of periportal hepatocytes. Also, there was a higher incidence of very slight increased numbers of mitotic figures of hepatocytes and slight altered cytoplasmic homogeneity of centrilobular/midzonal hepatocytes (consistent with increased amounts of cytoplasmic glycogen). At 1539 ppm only there was an increased incidence of slight panlobular vacuolization of hepatocytes, which was consistent with fatty change.

Table B.6.6.3–18: Maternal microscopic pathology findings: liver

Finding		Dietary concentration of XDE-729 Methyl (ppm)			
		0	**122**	**391**	**1539**
Number examined		21	19	21	19
Altered cytoplasmic homogeneity: hepatocyte, centrilobular/midzonal	-slight	1	0	7	12
Hypertrophy, with altered tinctorial properties: hepatocyte, periportal	-very slight	1	1	14	1
	-slight	1	0	0	18
Increased number of mitotic figures: hepatocyte	-very slight	0	0	3	7
Vacuolization, consistent with fatty change: panlobular,	-very slight	2	2	3	1
	-slight	2	0	1	8

Developmental toxicity

The key pregnancy and foetal weight findings are summarised in Table B.6.6.3-19. There was a treatment-related statistically significant reduction in mean foetal weight, by about 9% in comparison with controls, at the highest dose level only. XDE-729 Methyl had no effect on the numbers of implantation sites and live foetuses, pre-implantation loss and sex ratio. Post-implantation loss was statistically significantly increased at 391 and 1539 ppm; however, these differences are considered to be due to chance for several reasons: a dose response relationship was absent; post-implantation loss for the control group lower that recent historical control values; post-implantation loss was not increased at higher dose levels in the probe study (IIA 5.6.11/03); the post-implantation loss at 391 and 1539 ppm was mainly due to the presence of litters with single resorptions.

Table B.6.6.3–19: Key pregnancy and foetal weight findings

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	122	391	1539
Number females on study	24	24	24	24
Number pregnant	21	19	21	19
Mean no. corpora lutea	9.0	8.8	9.7	9.5
Mean no. implantation sites	8.7	8.4	8.8	8.6
Pre-implantation loss (%)	4.4	4.8	9.3	8.7
Post-implantation loss (%)	1.2	2.5	6.3*	5.1*
	Laboratory historical control range 1.8 – 4.7%, 8 studies conducted 2007-2011			
Mean no. live foetuses/litter	8.6	8.2	8.2	8.2
Sex ratio, M:F	45:55	53:47	49:51	46:54
Mean foetal weight (g) -all	33.8	33.5	32.9	30.7*
- males	33.7	33.7	33.3	31.5
- females	33.4	33.0	32.5	29.9*

*significantly different from control, p≤0.05

The key foetal examination data are summarised in Table B.6.6.3-20. The incidence of foetal malformations was relatively low in all dose groups, and was not influenced by XDE-729 Methyl treatment. The incidence of foetal visceral variations was similar in all groups, with exception of the presence of paraovarian cyst; however, the incidence of this variation was within the historical control range and was considered unrelated to treatment. The skeletal examination revealed a treatment-related increased incidence of delayed ossification of the pubis at 1539 ppm only, which correlated with the decreased foetal weights observed in this group. An increased incidence of delayed ossification of the hyoid was seen at 1539 ppm, but the incidence of this variation was well within the historical control range and so a relationship with treatment is less certain. The incidence of other skeletal variations in the treated groups was similar to concurrent controls.

Table B.6.6.3–19: Foetal examination data: summary of key findings

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	**122**	**391**	**1539**
Total no. of foetuses examined (litters)	180 (21)	156 (19)	173 (21)	156 (19)
No. of foetuses with malformations seen at external examination Type of external malformations	0	0	1 1 with anasarca	0
No. of foetuses with malformations seen at visceral examination (litters) Type of visceral malformations	1 1 with missing subclavian artery, persistent truncus arteriosus and ventricular septal defect	3 (2) 2 with missing gall bladder (from 1 litter) 1 with abnormal course of pulmonary artery, enlarged aorta and ventricular septal defect	0	3 (3) 1 with misshapen heat, persistent truncus arteriosus and ventricular septal defect 1 with persistent vena cava 1 with with missing gall bladder
No. of foetuses with malformations seen at skeletal examination (litters) Type of skeletal malformations	1 1 with lumbar hemivertebra	2(2) 1 with misaligned thoracic centra, fused thoracic centra and misshapen thoracic centra 1 with fused thoracic rib	0	1 1 with missing thoracic centra, missing thoracic vertebrae and lumbar hemivertebra
Selected visceral variations: No. [%] foetuses with paraovarian cyst (litters)	0	0	0	3 [3.5%] (3)
	Laboratory historical control range 0 - 4.3% of foetuses, 8 studies conducted 2007-2011			
Selected skeletal variations: No. [%] foetuses with delayed ossification of pubis (litters)	0	1 [0.6%]	6 [3.5%] (3)	11 [7.1%]* (8)
	Laboratory historical control range 0.5 - 4.6% of foetuses, 8 studies conducted 2007-2011			
No. [%] foetuses with delayed ossification of hyoid (litters)	27 [30.7%] (11)	24 [30.4] (11)	19 [22.1%] (12)	33 [42.9%] (15)
	Laboratory historical control range 35.4 – 54.4% of foetuses, 8 studies conducted 2007-11			

*significantly different from control, $p \leq 0.05$

Toxicokinetics

Toxicokinetic analysis of maternal and foetal blood at termination (GD 28) showed that XDE-729 Methyl was not present at quantifiable concentrations at all dose levels. In contrast, XDE-729 Acid was present in maternal blood at all dose levels, in quantities that were approximately dose-proportional (linear). The Acid metabolite was also detected in foetal blood, but only in the 391 and 1539 mg/kg/day groups in quantities that were not dose-proportional.

Table B.6.6.3–20: Mean amount of XDE-729 Methyl and XDE-729 Acid in maternal and foetal blood on GD 28

Parameter	Dietary concentration of XDE-729 Methyl (ppm)			
	0	**122**	**391**	**1539**
Calculated intake of XDE-729 Methyl on GD 28 (mg/kg/day)	0	5.63	16.5	72.5
Maternal blood concentration of XDE-729 Methyl (µg/g)	NQ	NQ	NQ	NQ
Foetal plasma concentration of XDE-729 Methyl (µg/g)	NQ	NQ	NQ	NQ
Maternal blood concentration of XDE-729 Acid (µg/g)	NQ	0.16	0.24	1.65
Foetal blood concentration of XDE-729 Acid (µg/g	NQ	NQ	0.10	0.06

NQ = not quantifiable

CONCLUSION

Oral (dietary) administration of XDE-729 Methyl to the rabbit during pregnancy caused maternal toxicity at 391 and 1539 ppm, which was restricted to changes in liver. The organ weight was increased and microscopic changes were observed, primarily an increase in the incidence of slight hypertrophy with altered tinctorial properties of periportal hepatocytes. There was evidence of developmental toxicity at the highest dose level of 1539 ppm, observed as reduced foetal weight and delayed ossification the pubis. Study NOAELs of 122 ppm (intake of about 5.78 mg/kg/day) for maternal toxicity and 391 ppm (intake of about 18.5 mg/kg/day) for developmental toxicity are identified.

A toxicokinetic investigation demonstrates that there is negligible systemic maternal or foetal exposure to XDE-729 Methyl. However, maternal systemic exposure to XDE-729 Acid occurs at all exposure levels and is approximately dose-proportional. Foetal exposure to XDE-729 Acid also occurs, but can only be demonstrated at 391 and 1239 ppm and dose-proportionality is not observed.

[redacted] (2012)

B.6.6.4 Summary of reproductive and developmental toxicity studies

Table B.6.6.4 Summary of reproductive and developmental toxicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
Rat reproductive probe dietary XDE-729 Acid 0-50-250-750/500 mg/kg/day target	Reproductive/fertility M: 520 mg/kg/day F: 535 mg/kg/day	M: - F: -	No adverse effects observed	[redacted] 2010 (091061)
	Adults: general tox (F_0): M: 266 mg/kg/day F: 269 mg/kg/day	M: 520 mg/kg/day F: 535 mg/kg/day	M&F: kidney: hypertrophy, hyperplasia, necrosis and/or mitotic alteration of collecting duct epithelial cells, tubular dilatation	
	Offspring (F_1) 535 mg/kg/day	-	No adverse effects observed	
Rat two-generation dietary XDE-729 Acid 0-20-100-450 mg/kg/day target	Reproductive/fertility M: 459 mg/kg/day F: 443 mg/kg/day	M: - F: -	No adverse effects observed	[redacted] 2011 (091148)
	Adults: general toxicity (F_0, F_1): M: 101 mg/kg/day F: 98 mg/kg/day	M: 459 mg/kg/day F: 443 mg/kg/day	M&F: kidney: hypertrophy, hyperplasia and/or mitotic alteration of collecting duct epithelial cells, mineralisation of tubule basal lamina and artery	
	Offspring (F_1, F_2) 443 mg/kg/day	-	No adverse effects observed	
Rat developmental dietary XDE-729 Acid 0-500-2000-8000 ppm	Maternal: 2000 ppm (140 mg/kg/day)	8000 ppm (526 mg/kg/day)	↓ bodyweight gain; ↓ food consumption; ↑ kidney weight	[redacted] 2010 (091138)
	Developmental: 2000 ppm (140 mg/kg/day)	8000 ppm (526 mg/kg/day)	Slight ↓ foetal weight; slight ↑ incidence of retarded ossification	
Rat developmental probe dietary XDE-729 Methyl 0-521-2083-4167-8333 ppm	Maternal: 2083 ppm (169 mg/kg/day)	4167 ppm (303 mg/kg/day)	↓ bodyweight gain; ↓ food consumption	[redacted] 2012 (111070)
	Developmental: 4167 ppm (303 mg/kg/day)	-	No adverse effects observed	
Rat developmental dietary XDE-729 Methyl 0-521-2083-4167-ppm	Maternal: 521 ppm (41 mg/kg/day)	2083 ppm (159 mg/kg/day)	↓ bodyweight gain; ↓ food consumption; ↑ liver weight, altered cytoplasmic homogeneity of hepatocytes	[redacted] 2012 (111071)
	Developmental: 2083 ppm (159 mg/kg/day)	4167 ppm (323 mg/kg/day	Slight ↓ foetal weight	
Rabbit developmental probe dietary XDE-729 Acid 0-5000-10000-15000-23000 ppm	Maternal: 23000 ppm (929 mg/kg/day)	-	No adverse effects observed	[redacted] 2011 (091142)
	Developmental: 23000 ppm (929 mg/kg/day)	-	No adverse effects observed	
Rabbit developmental dietary XDE-729 Acid 0-4000-10000-28292 ppm	Maternal: 10000 ppm (434 mg/kg/day)	28292 ppm (1094 mg/kg/day)	Slight ↓ bodyweight gain; slight ↓ food consumption	[redacted] 2011 (091143)
	Developmental: 28292 ppm (1094 mg/kg/day)	-	No adverse effects observed	
Rabbit developmental probe dietary XDE-729 Methyl 0-521-2083-4167-8333-10417-15625-	Maternal: -	521 ppm (21.5 mg/kg/day)	↑ liver weight	[redacted] 2012 (111045)
	Developmental: 521 ppm (21.5 mg/kg/day)	2038 ppm (83.3 mg/kg/day)	↓ foetal weight	

24500 ppm				
Rabbit developmental dietary XDE-729 Methyl 0-122-391-1539 ppm	Maternal: 122 ppm (5.78 mg/kg/day)	391 ppm (18.5 mg/kg/day)	Liver: ↑ weight, slight hypertrophy and altered tinctorial properties of periportal hepatocytes	[redacted] l 2012 (111137)
	Developmental: 391 ppm (18.5 mg/kg/day)	1539 ppm (71.6 mg/kg/day)	↓ foetal weight, delayed ossification of pubis	

The reproductive toxicity of XDE-279 Acid has been adequately investigated in a rat two generation study and in a rat developmental toxicity study and a rabbit developmental toxicity study. Also, the developmental toxicity of XDE-279 Methyl has been investigated in a rat and a rabbit study.

The two generation study demonstrates that XDE-729 Acid does not have an adverse effect on fertility or general reproductive performance and classification for this endpoint is not warranted. A NOAEL of 443 mg/kg/day, the highest dose level investigated, is identified for effects on fertility and general reproductive performance. This conclusion can be extrapolated to XDE-729 Methyl (see section B.6.5.3.2 for discussion on bridging from XDE-729 Acid studies).

In the rat there is evidence that both XDE-729 Acid and XDE-729 Methyl can cause marginal developmental effects, such as a slight ossification delay or slightly reduced foetal weight, but only in association with maternal toxicity. In the rabbit, XDE-729 Acid does not cause developmental toxicity; however, XDE-729 Methyl causes developmental toxicity, manifested as reduced foetal weight and a slight ossification delay, at an exposure level that also causes slight maternal liver toxicity. NOAELs for developmental toxicity of 159 mg/kg/day for XDE-729 Acid (based on the rat study) and 18.5 mg/kg/day for XDE-729 Methyl (based on the rabbit study) are identified. As the only developmental changes observed for both XDE-729 Acid and XDE-729 Methyl occurred in association with maternal toxicity, and were of the type that can be assumed to secondary non-specific consequences of maternal toxicity, the criteria for classification for developmental toxicity are not met.

The reproductive studies provide additional information on the general toxicity and toxicokinetics of XDE-729 Acid and XDE-729 Methyl.

Toxicokinetic investigations in the rat and rabbit developmental toxicity studies demonstrate that following XDE-729 Methyl administration there is both maternal systemic and foetal exposure to the Acid metabolite, but negligible maternal systemic and foetal exposure to the parent compound. In the rabbit it was shown that that maternal systemic exposure to XDE-729 Acid shows dose-equivalence for dietary exposure to XDE-729 Methyl and to the Acid (not investigated in the rat). In the rat, foetal plasma concentrations of XDE-729 Acid are about 70% of maternal levels following XDE-729 Methyl dietary exposure (not possible to assess ratio in rabbit because of the use of relatively low dietary exposure levels). In the rabbit, foetal plasma concentrations of XDE-729 Acid are about 40% of maternal levels following XDE-729 Acid dietary exposure (not determined in the rabbit).

The two generation study provides a NOAEL of 266 mg/kg/day for XDE-729 Acid short-term repeated dose general toxicity and confirmes that the kidney is the main target organ for this substance. The rat developmental toxicity studies confirm that the liver is a main target organ for XDE-729 Methyl. The liver was also identified as a target for XDE-729 Methyl in the rabbit. However, the rabbit is more sensitive to the toxicity of XDE-729 Methyl, since the maternal toxicity NOAEL is 5.78 mg/kg/day, compared with 41 mg/kg/day for the rat.

B.6.7 Neurotoxicity studies (IIA 5.7)

XDE-729 Acid	
Study	IIA 5.7.1/01 Acute neurotoxicity study in F334/DuCrl rats
Reference	[redacted] (2010)
Date performed	April 2010
Test facility	[redacted]
Report reference	Laboratory study ID 101016
Guideline(s)	OECD 424
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

F334/DuCrl strain rats, about 7 weeks old at dosing, were randomly assigned to the test groups as shown in the table below.

Table B.6.7.1–1: Study design

Test group	Dose level of XDE-729 Acid (mg/kg)	Number of animals	
		Males	Females
1	0	10	10
2	250	10	10
3	750	10	10
4	2000	10	10

The animals received a single gavage dose of XDE-729 Acid in aqueous 0.5% Methocel™ (day of dosing = study day 1). Analysed samples of dosing formulations used on the study were within 3% of nominal, with low relative standard deviations, demonstrating that the achieved concentrations and homogeneity were satisfactory.

General clinical observations were recorded daily and detailed clinical observations were made on days 2, 3 and 4. Bodyweights were determined weekly. A functional observational battery (home cage and open field observations, grip performance, landing foot splay, rectal temperature) and motor activity measurements were conducted 4-5 days prior to dosing and on days 1 (at about 5 hours after dosing) 8 and 15. The timing of the day 1 FOB assessments was chosen after a probe study suggested that there was no 'time-of-peak effect' for clinical changes.

On study day 16 all animals were killed. Five rats/sex/group were perfusion-fixed *in situ*. Major organs were examined macroscopically at a necropsy. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were dissected and stored in glutaraldehyde/formaldehyde fixative. Neurological tissues from only the control and high dose group were subjected to microscopic examination. A gross necropsy was performed on the remaining animals.

RESULTS

There were no unscheduled mortalities or treatment related clinical observations.

Statistical analysis of the bodyweights, when males and females were combined, indicated a slight treatment-related reduction in bodyweights at 750 and 2000 mg/kg/day (see Table B.6.7.1–2).

Table B.6.7.1–2 Group mean bodyweights (g)

Day	Dose level of XDE-729 Acid (mg/kg)							
	Males				Females			
	0	250	750	2000	0	250	750	2000
Pre-dose	114	113	113	112	94	97	96	96
1	127	125	125	123	101	103	100	101
8	148	145	142	139	113	113	109	110
15	163	162	158[a]	156[a]	122	122	119[a]	121[a]

[a]Repeat measurements analysis revealed a significant p value for the comparison of time x control vs. dose group, indicating differences between control and treated animals (males + females) at one or more time intervals.

There were no treatment-related effects on rectal temperature, grip performance, landing foot splay, or motor activity in males or females at any dose level tested. In the open field assessments there was one possible treatment related change; the level of activity in males only on day 1 at 750 and 2000 mg/kg was reduced, as shown in Table B.6.7.1-3. These activity observations were statistically significantly different from the concurrent controls and outside the historical control ranges. However, these observations are considered to represent equivocal evidence of a treatment-related effect because of the absence of a clear dose-response relationship, the magnitude of the change was slight, the more comprehensive motor activity assessments did not reveal differences and, finally, because there were no corroborative changes in other open field parameters or neuropathological observations.

Table B.6.7.1–3: Open field observations: activity levels in males on day 1

Observation	Dose level of XDE-729 Acid (mg/kg)			
	Males			
	0	250	750	2000
No. of males with minimal activity rank	1	3	7*	7*
	Historical control range: 0 – 3 males			
No. of males with moderate activity rank	9	7	3*	3*
	Historical control range: 7 – 10 males			

Historical control range based on 5 studies conducted by the testing laboratory from 2005

There were no treatment related macroscopic necropsy findings. The microscopic examination of the nervous system tissues of the animals from the high dose did not reveal any treatment related changes.

CONCLUSION

Single gavage doses of XDE-729 Acid at 750 and 2000 mg/kg/day elicited slight general systemic toxicity, observed as a marginal reduction in bodyweight gain. There was equivocal evidence of a treatment effect on neurological parameters, limited to a slight reduction in open field activity on the day of dosing in males at 750 and 2000 mg/kg/day. XDE-729 Acid caused no microscopic neurological lesions and it can be concluded that there was no evidence of specific irreversible neurotoxicity. A study NOAEL of 250 mg/kg/day is identified, based on the

observation of slight reduced bodyweights and equivocal evidence of reduced activity several hours after dosing.

[redacted] 2010)

XDE-729 Acid	
Study	IIA 5.7.4/01 90-day dietary neurotoxicity study in F334/DuCrl rats
Reference	[redacted] (2011)
Date performed	July – October 2010
Test facility	[redacted]
Report reference	Laboratory study ID 101006
Guideline(s)	OECD 424
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Acid, Lot #E2837-52 TSN030751-0006, 95.3% purity
Study acceptable	Yes

METHODS

F334/DuCrl strain rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.7.2–1: Study design

Test group	Target dose level of XDE-729 Acid (mg/kg/day)	Number of animals	
		Males	Females
1	0	10	10
2	50	10	10
3	250	10	10
4	750	10	10

The test and control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Acid in analysed samples of test diet used on the study were within 15% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

General clinical observations were made twice daily and more detailed clinical examinations were conducted weekly. Bodyweights and food consumption were determined weekly. A functional observational battery (home cage and open field observations, grip performance, landing foot splay, rectal temperature) and motor activity measurements were conducted before treatment commenced and during weeks 2, 4, 8 and 13. Ophthalmoscopy examinations were conducted before treatment and during week 12.

After 13 weeks exposure all animals were killed and subjected to a necropsy during which macroscopic abnormalities of major organs were recorded. Five rats/sex/group were perfusion-fixed *in situ*. Fixed brain weight was recorded. Various peripheral nerves, parts of the brain and brain-associated organs, parts of the spinal cord and muscles were dissected, processed; microscopic examined of the nervous system tissues was conduced for control and high dose animals.

RESULTS

Received doses were calculated in terms of mg XDE-729 Acid/kg body weight. Mean values are shown below:

Table B.6.7.2–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Acid (mg/kg/day)	50	250	750
Males	52.5	260	774
Females	51.2	256	772

There were no treatment-related unscheduled deaths or clinical signs of toxicity.

There was a treatment-related reduction in bodyweight gain throughout the study among males at 750 mg/kg/day, as shown in Table B.6.7.2-3. Mean bodyweight gain from days 1-85 for this group was about 10% lower than controls. The reduced bodyweight gain of high dose males correlated with observation of significantly lower food consumption in this group throughout much of the study (Table B.6.7.2-5). Statistically significant reductions in food consumption were also occasionally observed among males at 50 and 750 mg/kg/day, but these are considered to be chance observations in the absence of correlating bodyweight differences and treatment related adverse effects on food consumption at similar dose levels in the rat 90-day study (IIA 5.3.2/01, Yano et al 2010) and 2-generation study (IIA 5.6.1/02, Rasoulpour, 2011). Female bodyweight and food consumption was not affected by XDE-729 Acid treatment.

Table B.6.7.2–3 Group mean bodyweights, selected findings (g)

Day	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750[a]	0	50	250	750
1	150	148	147	147	102	101	102	101
15	195	198	193	186	126	127	130	127
29	230	232	227	221	145	146	150	146
57	272	275	270	262	162	164	165	160
85	313	314	309	293	181	181	183	180
Gain d 1-85	163	166	162	146	79	80	81	79

[a]inferential statistical analysis revealed a significant p value for the comparison of time x control vs. dose group for males, indicating that there were differences between control and treated males at one or more intervals

Table B.6.7.2–4 Group mean food consumption, selected findings (g/animal/day)

Day	Target dose level of XDE-729 Acid (mg/kg/day)							
	Males				Females			
	0	50	250	750	0	50	250	750
1-8	14.8	14.7	14.6	13.4*	11.0	10.6	11.3	10.8
15-22	15.1	15.3	14.9	15.3	10.7	10.4	10.9	10.6
57-64	15.4	14.7*	14.5*	14.4*	10.1	10.1	10.3	10.5
71-78	15.7	15.5	15.3	13.8*	10.4	10.5	10.6	10.4
85-90	15.3	14.9	14.5*	14.3*	9.9	9.7	10.0	10.3

* significantly different from control, p≤0.05

There were no treatment-related ophthalmoscopy findings.

There were no differences in the parameters measured in the FOB or on motor activity that were considered to be treatment-related.

There were no treatment-related macroscopic necropsy findings. The microscopic examination of the nervous system tissues did not reveal any treatment related changes.

CONCLUSION

Dietary administration of XDE-729 Acid for 90 days to the rat at a target dose level of 750 mg/kg/day elicited general toxicity in males at 750 mg/kg/day, observed as a reduction in bodyweight gain and food consumption. There was no evidence of general toxicity in females, or of neurotoxicity in either sex. The study NOAELs for general toxicity are a target dose of 250 mg/kg/day (actual mean dose 260 mg/kg/day) in males and a target of 750 mg/kg/day (actual dose 772 mg/kg/day) in females.

[redacted] (2011)

B.6.8 Further toxicological studies (IIA 5.8)

B.6.8.1 Immunotoxicity

	XDE-729 Methyl
Study	IIA 5.10.1/01 Assessment of immunotoxic potential using the sheep red blood cell assay after 28-day dietary exposure to female F344/DUCRL rats
Reference	[redacted] (2012)
Date performed	January – March 2012
Test facility	[redacted]
Report reference	Laboratory study ID 121004
Guideline(s)	US EPA, OPPTS 870.7800 (Immunotoxicity)
Deviations from the guideline	None
GLP	Yes
Test material	XDE-729 Methyl, Lot #E2837-51 TSN031117-0004, 97.2% purity
Study acceptable	Yes

METHODS

Female F334/DuCrl rats, about 7 weeks old at the start of treatment, were randomly assigned to the test groups as shown in the table below.

Table B.6.8.1–1: Study design

Test group	Target dose level of XDE-729 Methyl (mg/kg/day)	Number of females
1	0 (negative control)	10
2	10	10
3	52	10
4	500	10
5	Cyclophosphamide 20 mg/kg intraperitoneal route, days 24-28 (positive control)	10

The test (containing XDE-729 Methyl) and negative control diets were prepared using Lab Diet Certified Rodent Diet #5002. The stability of the test substance in the diet for the period of use was established prior to study commencement. The achieved concentrations of XDE-729 Methyl in analysed samples of test diet used on the study were within 12% of the nominal, demonstrating satisfactory preparation of the dietary formulations.

The highest dose level of 500 mg/kg/day was selected, based on the results of the 28- and 90-day rat XDE-729 Methyl studies (see IIA5.3.1/02, IIA5.3.2/02) to produce some measureable sign of toxicity (effects on liver and body weight) without producing significant stress, malnutrition or fatalities. Females were used as these 28- and 90-day studies did not show any gender differences and the female is the specified default sex in the test guideline.

General clinical observations were recorded at least once daily and a more detailed clinical examination was conducted weekly. Bodyweights and food consumption were measured at least weekly.

Five days prior to scheduled necropsy each rat was immunized with a single 0.5 ml i.v. injection of 4 x10^{8} SRBC/ml (in isotonic sterile saline, via the lateral tail vein

At the time of the scheduled necropsy, non-fasted blood samples were taken from the orbital sinus for the measurement of standard haematology parameters (not conducted for the positive controls) and evaluation of the primary antibody response to sheep red blood cells (SRBC). Anti-SRBC IgM was analysed using a commercially available ELISA kit, which utilised detergent solubilised SRBC ghosts for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat IgM antibodies for detection.

A necropsy was conducted on all animals the end of the 4 week treatment period. The weights of the liver, spleen and thymus were recorded. Macroscopic changes were recorded. For the vehicle control and XDE-Methyl groups, representative samples of liver, spleen, thymus, sternum, mesenteric lymph node, and Peyer's patch were preserved.

RESULTS

Received doses were calculated in terms of mg XDE-729 Methyl/kg body weight. Mean values are shown below:

Table B.6.8.1–2 Mean dose received (mg/kg/day)

Target dose level of XDE-729 Methyl (mg/kg/day)	10	52	500
Females	10.2	53.6	505

There were no deaths or clinical signs of toxicity. XDE-729 Methyl had no effect on bodyweights, food consumption.

The haematology analyses revealed treatment-related changes at 500 mg/kg/day in three red blood cell parameters, as shown in Table B.6.8.1-3. Haemoglobin concentration was significantly decreased and reticulocytes were significantly increased. Haematocrit was lower than the negative controls, though statistical significance was not achieved. Although these mean values were within background ranges it can be concluded that there was a treatment-related effect because similar changes were seen in the 28- and 90-day rat XDE-729 Methyl studies (see IIA5.3.1/02, IIA5.3.2/02).

Table B.6.8.1–3 Group mean red blood cell parameters

Parameter	Historical control[1]	Historical control[2]	Target dose level of XDE-729 Methyl (mg/kg/day)			
			0	10	52	500
Haemoglobin (g/dL)	15.0-15.6	14.3-16.7	15.7	15.5	15.3	15.1*
Haematocrit (%)	47.7-48.3	42.1-53.3	48.9	48.8	48.2	47.7
Reticulocytes (E^9/L)	110.8-112.2	136.9-264.2	141.1	157.3	153.8	174.5*

*significantly different from negative control, alpha=0.05
[1]Historical controls group means from two 28-day immunotoxicity dietary studies done in female F344/DuCrl rats since 2010.
[2]Historical controls group means from seven 28-day dietary studies done in female F344/DuCrl rats since 2009

There were no treatment related macroscopic necropsy findings. Treatment-related liver and thymus weight changes were observed, as shown in Table B.6.8.1-4. Absolute and relative liver weights were significantly increased at 52 mg/kg/day (by 7%) and 500 mg/kg/day (by~28%). Absolute and relative thymus weights were significantly deceased at 500 mg/kg/day (by 14%). Similar organ weight changes were observed in the 28- and 90-day rat XDE-729 Methyl studies (see IIA5.3.1/02, IIA5.3.2/02). The liver weight change at 52 mg/kg/day was less than 10% and therefore may be regarded as representing an adaptive, rather than adverse, response to treatment.

Table B.6.8.1–4 Group mean maternal organ weights: liver

Parameter	Historical control[1]	Historical control[2]	Target dose level of XDE-729 Methyl (mg/kg/day)			
			0	10	52	500
Final bodyweight (g)	153-155	127-136	151	151	152	150
Liver weight (g)	5.496	3.810-4.199	5.461	5.571	5.878*	6.953*
Liver weight (% bodyweight)	3.596	2.847-3.192	3.608	3.687	3.863*	4.637*
Thymus weight (g)	0.297-0.327	0.278-0.350	0.298	0.288	0.291	0.255*
Thymus (% bodyweight)	0.191-0.214	0.203-0.259	0.197	0.191	0.191	0.170*

*significantly different from negative control, p≤0.05
[1]Historical controls group means from two 28-day immunotoxicity dietary studies done in female F344/DuCrl rats since 2010.
[2]Historical controls group means from seven 28-day dietary studies done in female F344/DuCrl rats since 2009

The SRBC antibody response was not influenced by XDE-729 Methyl treatment, as shown in Table B.6.8.1-5. The positive control treatment (cyclophosphamide) elicited the expected marked reduction in the SRBC antibody response.

Table B.6.8.1–5 Group mean SRBC antibody response (± SD)

Parameter	Target dose level of XDE-729 Methyl (mg/kg/day)				Positive control
	0	10	52	500	
Anti-SRBC IgM [u/mL]	33204 ± 36590	22238 ± 18806	44042 ± 38158	43481 ± 36537	329 ± 154*

*significantly different from negative control, alpha= 0.05

CONCLUSION

Dietary administration of XDE-729 Methyl for 28 days to the female rat at target dose levels of up to 500 mg/kg/day did not elicit immunotoxicity, based on the absence of an influence the primary immune response to SRBCs. XDE-729 Methyl elicited general toxicity at a target dose of 500 mg/kg/day, observed as reduced haemoglobin concentration and haematocrit, increased reticulocyte count, increased liver weight and decreased thymus weight. The study NOAELs are a target of 500 mg/kg/day (actual mean dose 505 mg/kg/day) for immunotoxicity and a target of 52 mg/kg/day (actual mean dose 54 mg/kg/day) for general toxicity.

[redacted] (2012)

B.6.8.2 Relevant metabolites

X11449757 (O-demethyl XDE-729 Acid) is a metabolite of XDE-729 Methyl that is found in soil (aerobic and anaerobic compartments), groundwater, surface water, wheat crops and in rats goats and hens.

	X11449757
Study	IIA 5.8/01 Bacterial Reverse Mutation Test using *Salmonella typhimurium*
Reference	Nagane RM (2012)
Date performed	May – June 2012
Test facility	Jai Research Foundation, Gujarat, India
Report reference	Laboratory study 481-1-06-4565, Dow study ID 120592
Guideline(s)	OECD 471
Deviations from the guideline	None
GLP	Yes
Test material	X11449757, Lot YB1-100780-103, TSN031413-0003, 99% purity
Study acceptable	Yes

METHODS

X11449757 was evaluated in a bacterial mutagenicity assay using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA 100, TA 102), in the presence and absence of a Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

The plate incorporation method was used. An initial cytotoxicity assay was conducted, which established that no toxicity was present at the limit dose of 5000 µg/plate. A dose range of 156.25 to 5000 µg/plate was used in a first assay, and a range of 51.2 to 5000 µg/plate was used in a confirmatory assay. The study design included the testing of appropriate positive and solvent

(DMSO) controls. For all test substance, solvent control and positive control treatments, triplicate plates were used.

RESULTS

There were no increases in the number of revertants in the X11449757 plates. The expected responses were observed in the positive control plates.

There was evidence of toxicity to the bacteria. Precipitation of the test substance was not seen at any of the concentrations investigated.

CONCLUSION

X11449757 is non-mutagenic in this *Salmonella typhimurium* reverse mutation assay.

Nagane RM (2012)

X11449757	
Study	IIA 5.8/02 *In vitro* mammalian cell gene forward mutation test at the HGPRT locus of the Chinese hamster ovary cell (CHO)-K1 cell line
Reference	[redacted] (2012)
Date performed	May – July 2012
Test facility	[redacted]
Report reference	Laboratory study 481-1-06-4566, Dow study ID 120594
Guideline(s)	OECD 476
Deviations from the guideline	None
GLP	Yes
Test material	X11449757, Lot YB1-100780-103, TSN031413-0003, 99% purity
Study acceptable	Yes

METHODS

X11449757 was tested *in vitro* for its ability to induce forward mutations in mammalian cells by assessing the mutation of the HGPRT locus in Chinese hamster ovary cells.

Two independent sets of experiments were conducted in the presence and absence of a rat liver (Aroclor 1254 induced) derived metabolic activation system (S9-mix), using a 4 h X11449757 exposure period. Concentrations of 100 – 3210 µg/mL were tested. The highest concentration (equivalent to 10 mM) was selected as the limit concentration for this type of assay and because cytotoxicity (observed as a marked reduction in relative cloning efficiency) and precipitation of the test substance in the culture medium was reported at 1605 and 3210 µg/mL in preliminary experiments.

For each treatment, duplicate cultures were set up. The study design included the testing of appropriate positive controls (ethyl methanesulfonate, EMS, without S9; benzo(a)pyrene with S9) and solvent controls (DMSO). After the exposure period, treatment media were replaced by culture medium and the cells were incubated for 8 days for expression of mutant cells. This was followed by an 8 day incubation of cells in selection medium containing 6-thioguanine.

RESULTS

There were no increases in the numbers of mutant colonies in the X11449757 treated cultures. The expected responses were observed in the positive control cultures.

Evidence of toxicity, observed as a reduction in relative cloning efficiency, was present at 1605 and 3210 µg/mL in both main experiments. Whether or not precipitation of the test substance occurred in the main experiments was not reported.

CONCLUSION

X11449757 is not mutagenic in Chinese hamster ovary cells treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2012)

X11449757	
Study	IIA 5.8/03 *In vitro* chromosome aberration test in human peripheral blood lymphocytes
Reference	[redacted] (2012)
Date performed	May – July 2012
Test facility	[redacted]
Report reference	Laboratory study 488-1-06-4567, Dow study ID 120593
Guideline(s)	OECD 473
Deviations from the guideline	None
GLP	Yes
Test material	X11449757, Lot YB1-100780-103, TSN031413-0003, 99% purity
Study acceptable	Yes

METHODS

X11449757 was evaluated in an *in vitro* chromosomal aberration assay using human peripheral blood lymphocytes, in the presence and absence of Aroclor 1254 induced rat liver-derived metabolic activation system (S9).

Approximately 48 hours after the initiation of whole blood cultures, cells were treated in the presence or absence of S9 with X11449757 at concentrations ranging from 25.3 – 810 µg/ml. The concentration range was selected on the basis of a preliminary cytotoxicity study which showed that X11449757 caused a significant level of toxicity (close to 50% reduction in mitotic index) at a dose level of 802.5 µg/ml. In phase I of the study, cultures were treated for 3.5 h in the presence or absence of S9. In phase II, cultures were treated for 24 h in the absence of metabolic activation. In phase III, cultures were treated for 3.5 h in the presence of metabolic activation.

Solvent (DMSO) and positive controls (mitomycin C and cyclophosphamide) were included in the study.

The concentration selected for evaluation, by light microscopy, were 202.5, 405, and 810 µg/ml for Phase I and 101.3, 202.5 and 405 µg/ml for Phases II and III. Chromosomes of approximately 200 metaphases per concentration, i.e. 100 metaphases from each of two parallel cultures, were scored for chromosome aberrations.

RESULTS

The chromosome assessment did not reveal any relevant or statistically significant increases in numbers of metaphases with aberrations at any time points in the X11449757 treated cultures, either in the presence or absence of S9.

Toxicity, manifested as a close to 50% reduction in mitotic index, was present at 810 µg/ml in Phase I and at 405 µg/ml in Phases II and III.

The sensitivity of the system was demonstrated by significant increases in metaphases with aberrations in positive control cultures, both with and without S9 mix.

CONCLUSION

X11449757 is not clastogenic in peripheral blood lymphocytes treated *in vitro* in the presence or absence of metabolic activation.

[redacted] (2012)

B.6.9 Medical data and information (IIA 5.9)

B.6.9.1 Medical surveillance of manufacturing plant personnel (AII 5.9.1)

There are no data available because manufacture of the active substance has not yet started.

B.6.9.2 Clinical cases and poisoning incidents (IIA 5.9.2)

There are no data available because manufacture of the active substance has not yet started.

B.6.9.3 Exposure of the general population and epidemiological studies (IIA 5.9.3)

There have been no studies, reports or observations with regards to effects of XDE-729 exposure in humans. No studies on the exposure of the general population or epidemiological studies are known to the Applicant.

B.6.9.4 Clinical signs, symptoms of poisoning and details of clinical tests (IIA 5.9.4)

There are no data available from clinical cases or tests and poisoning incidents.

B.6.9.5 First aid measures (IIA 5.9.5)

The Applicant has provided the following advice:

First aid measures are aimed at prompt surface decontamination and appropriate medical follow-up.

In the event of eye exposure flush eyes thoroughly with water for several minutes. Remove contact lenses after initial 1-2 minutes and continue flushing for several additional minutes. If effects occur, consult a physician, preferably an ophthalmologist.

In the event of skin exposure immediately wash skin with soap and plenty of water. Remove contaminated clothing. Wash contaminated clothing before reuse.

In the event of ingestion never give fluids or induce vomiting if the patient is unconscious or is having convulsions. Do not induce vomiting; call a physician. The decision of whether to induce vomiting or not should be made by a physician.

In the event of inhalation exposure remove the patient to fresh air. Consult a physician.

Note to Physician: provide supportive care. The treatment should be based on the judgment of the physician in response to the symptoms of the patient.

B.6.9.6 Expected effects and duration of poisoning as a function of the type, level and duration of exposure or ingestion (IIA 5.9.6)

No specific effects are expected.

B.6.9.7 Summary of medical data

No medical data are available because manufacture of the active substance has not yet started.

Supportive first aid measures and medical follow-up are recommended in cases of overexposure to XDE-729 Methyl.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and MAC (drinking water limit) (IIA 5.10)

Table B.10-1 Summary of toxicity studies

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
Rat, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000/500 mg/kg/day target	M: 270 mg/kg/day	M: 732 mg/kg/day	M: ↓ bodyweight gain & food consumption; mild regenerative anemia; kidney: tubular degenerative changes, hypertrophy & vacuolation of collecting duct epithelium; spleen: extramedullary haematopoiesis; urine: ↑ volume & ↓ specific gravity	[redacted] 2009 (081115)
	F: 250 mg/kg/day	F: 982 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: tubular degenerative changes, hypertrophy & vacuolation of collecting duct epithelium; urine: ↑ volume & ↓ specific gravity	
Rat, 28 day oral dietary XDE-729 Methyl 0-10-52-261-782 mg/kg/day target	M&F: 10.7 mg/kg/day	M&F: 55.5 mg/kg/day	M: liver: ↑ weight, hepatocyte hypertrophy, hepatocytes in mitosis, slight hepatocellular vacuolization; thyroids: diffuse hypertrophy of follicular cells	[redacted] 2011 111005
			F: liver: ↑ weight	
Rat, 90 day oral dietary XDE-729 Acid 0-10-50-250-750 mg/kg/day target	M: 262 mg/kg/day	M: 782 mg/kg/day	M: ↓ bodyweight gain; kidney: ↑ weight, hypertrophy, vacuolation, necrosis of collecting duct epithelium, tubular dilatation; urine: ↑ volume & ↓ specific gravity	[redacted] 2010 (091016)
	F: 252 mg/kg/day	F: 758 mg/kg/day	F: kidney: ↑ weight, hypertrophy, vacuolation, necrosis of collecting duct epithelium, tubular dilatation; urine: ↑ volume & ↓ specific gravity	
Rat, 90 day oral dietary XDE-729 Methyl 0-3-10-52-261-500 mg/kg/day target	M: 10.3 mg/kg/day	M: 53.5 mg/kg/day	M: liver hepatocellular vacuolation, consistent with fatty change	[redacted] 2012 (111082)
	F: 10.1 mg/kg/day	F: 52.3 mg/kg/day	F: ↑ serum cholesterol concentration; liver: ↑ weight	
Mouse, 28 day oral dietary XDE-729 Acid 0-10-50-250-1000 mg/kg/day target	M: 268 mg/kg/day	M: 1025 mg/kg/day	M: slight ↓ RBC	[redacted] 2009 (081116)
	F: 958 mg/kg/day	F: -	F: no adverse effects observed	
Mouse, 90 day oral dietary XDE-729 Acid 0-50-250-500-1000 mg/kg/day target	M: 495 mg/kg/day	M: 989 mg/kg/day	M: urinary bladder: slight inflammation of mucosa or wall, submucosal oedema	[redacted], 2010 (091056)
	F: 1008 mg/kg/day	F: -	F: no adverse effects observed	
Dog, 28 day oral dietary XDE-729 Acid 0-300-3000-15000(F)-30000(M) ppm	M: 300 ppm 11 mg/kg/day	M: 3000 ppm 80 mg/kg/day	M: kidney: slight tubular degeneration & regeneration	[redacted] 2010 (081127)
	F: 3000 ppm 85 mg/kg/day	F: 15000 ppm 323 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: slight tubular degeneration & regeneration; thymus: lymphoid atrophy	
Dog, 90 day oral dietary XDE-729 Acid 0-500-2500-12500 ppm	M&F: 250 ppm 80 mg/kg/day	M: 12500 ppm 424 mg/kg/day	M: ↓ bodyweight gain & food consumption; kidney: tubular degeneration & regeneration, degeneration of collecting ducts, focal granuloma in cortex/medulla; liver: ↑ weight; thymus: lymphoid atrophy; bone marrow: hyperplasia	[redacted] 2011 (091070)
		F: 15000 ppm 415 mg/kg/day	F: ↓ bodyweight gain & food consumption; kidney: tubular degeneration & regeneration, degeneration of collecting ducts, focal granuloma in cortex/medulla; liver: ↑ weight, extramedullary haematopoiesis; thymus: lymphoid atrophy; bone marrow:	

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
			hyperplasia; spleen: extramedullary haematopoiesis	
Dog, 1 year day oral dietary XDE-729 Acid 0-500-2500-10000/5000 ppm	M: 2500 ppm 82 mg/kg/day	M: 10000 ppm 355 mg/kg/day	M: kidney: very slight tubular degeneration & regeneration, degeneration of collecting ducts, fibrosis or focal granuloma in cortex, glomerulosclerosis	[redacted] 2012 (101163)
	F: 500 ppm 16.7mg/kg/day	F: 2500 ppm 90mg/kg/day	F: kidney: very slight tubular degeneration & regeneration, degeneration of collecting ducts, glomerulosclerosis	
Rat, 28 day dermal XDE-729 Acid 0-100-300-1000 mg/kg/day	M&F: 1000 mg/kg/day	M&F: -	M&F: no adverse effects observed	[redacted] 2010 (101031)
Rat, 28 day dermal XDE-729 Methyl			*Study in progress*	
Rat, 12 mo. chronic/2 year carcinogenicity, oral dietary XDE-729 Acid 0-20-100-400-625/750 mg/kg/day target	Non-neoplastic M&F: 100 mg/kg/day	M: 404mg/kg/day	M: ↓ bodyweight; kidney: ↑ organ weight, tubular degeneration & regeneration, necrosis of individual tubular epithelial cells, hyperplasia of pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in collecting duct epithelium, interstitial inflammation of medulla, vacuolization of collecting duct epithelium in papilla; ↑ urine volume:	[redacted] 2012 (091121)
		F:407 mg/kg/day	F: kidney: ↑ organ weight, tubular degeneration & regeneration, necrosis of individual tubular epithelial cells, chronic progressive glomerulonephropathy , hyperplasia of pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in collecting duct epithelium, interstitial inflammation of medulla, and vacuolization of collecting duct epithelium in papilla; ↑ urine volume	
	Neoplastic: M: 625mg/kg/day F: 400 mg/kg/day	M&F: -	No evidence of carcinogenicity	
Mouse, 18 mo. carcinogenicity, oral dietary XDE-729 Acid 0-50-250-750/1000 mg/kg/day target	Non-neoplastic: M: 50 mg/kg/day	M: 251mg/kg/day	M: Urinary bladder: inflammation, microscopic calculi in lumen	[redacted] (101021)
	F:1004mg/kg/day	F: -	[F: hypertrophy of kidney intercalated cells present at 251 mg/kg//day and above, considered to be an adaptive non-adverse effect]	
	Neoplastic: M: 751mg/kg/day F:1004mg/kg/day	M&F: -	No evidence of carcinogenicity	
Rat reproductive probe, oral dietary XDE-729 Acid 0-50-250-750/500 mg/kg/day target	Reproductive/ fertility M: 520mg/kg/day F: 535mg/kg/day	M: - F: -	No adverse effects observed	[redacted] 2010 (091061)
	Adults: general tox (F_0): M: 266mg/kg/day F: 269 mg/kg/day	M: 520 mg/kg/day F: 535 mg/kg/day	M&F: kidney: hypertrophy, hyperplasia, necrosis and/or mitotic alteration of collecting duct epithelial cells, tubular dilatation	
	Offspring (F_1) 535 mg/kg/day	-	No adverse effects observed	
Rat two-generation, oral dietary	Reproductive/ fertility M: 459mg/kg/day F: 443 mg/kg/day	M: - F: -	No adverse effects observed	[redacted] 2011 (091148)

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
XDE-729 Acid 0-20-100-450 mg/kg/day target	Adults: general toxicity (F_0, F_1): M: 101mg/kg/day F: 98 mg/kg/day	M: 459mg/kg/day F: 443 mg/kg/day	M&F: kidney: hypertrophy, hyperplasia and/or mitotic alteration of collecting duct epithelial cells, mineralisation of tubule basal lamina and artery	
	Offspring (F_1, F_2) 443 mg/kg/day	-	No adverse effects observed	
Rat developmental dietary XDE-729 Acid 0-500-2000-8000 ppm	Maternal: 2000 ppm (140 mg/kg/day)	8000 ppm (526 mg/kg/day)	↓ bodyweight gain; ↓ food consumption; ↑ kidney weight	[redacted] 2010 (091138)
	Developmental: 2000 ppm (140 mg/kg/day)	8000 ppm (526mg/kg/day)	Slight ↓ foetal weight; slight ↑ incidence of retarded ossification	
Rat developmental probe dietary XDE-729 Methyl 0-521-2083-4167-8333 ppm	Maternal: 2083 ppm (169 mg/kg/day)	4167 ppm (303 mg/kg/day)	↓ bodyweight gain; ↓ food consumption	[redacted] 2012 (111070)
	Developmental: 4167 ppm (303 mg/kg/day)	-	No adverse effects observed	
Rat developmental dietary XDE-729 Methyl 0-521-2083-4167-ppm	Maternal: 521 ppm (41 mg/kg/day)	2083 ppm (159 mg/kg/day)	↓ bodyweight gain; ↓ food consumption; ↑ liver weight, altered cytoplasmic homogeneity of hepatocytes	[redacted] 2012 (111071)
	Developmental: 2083 ppm (159 mg/kg/day)	4167 ppm (323 mg/kg/day	Slight ↓ foetal weight	
Rabbit developmental probe oral dietary XDE-729 Acid 0-5000-10000-15000-23000 ppm	Maternal: 23000 ppm (929 mg/kg/day)	-	No adverse effects observed	[redacted] 2011 (091142)
	Developmental: 23000 ppm (929 mg/kg/day)	-	No adverse effects observed	
Rabbit developmental oral dietary XDE-729 Acid 0-4000-10000-28292 ppm	Maternal: 10000 ppm (434 mg/kg/day)	28292 ppm (1094 mg/kg/day)	Slight ↓ bodyweight gain; slight ↓ food consumption	[redacted] 2011 (091143)
	Developmental: 28292 ppm (1094 mg/kg/day)	-	No adverse effects observed	
Rabbit develop. probe dietary XDE-729 Methyl 0-521-2083-4167-8333-10417-15625-24500ppm	Maternal: -	521 ppm (21.5 mg/kg/day)	↑ liver weight	[redacted] 2012 (111045)
	Developmental: 521 ppm (21.5 mg/kg/day)	2038 ppm (83.3 mg/kg/day)	↓ foetal weight	
Rabbit developmental dietary XDE-729 Methyl 0-122-391-1539 ppm	Maternal: 122 ppm (5.78 mg/kg/day)	391 ppm (18.5 mg/kg/day)	Liver: ↑ weight, slight hypertrophy and altered tinctorial properties of periportal hepatocytes	[redacted] 2012 (111137)
	Developmental: 391 ppm (18.5 mg/kg/day)	1539 ppm (71.6 mg/kg/day)	↓ foetal weight, delayed ossification of pubis	
Rat acute neurotoxicity, oral, gavage XDE-729 Acid 0-250-750-2000 mg/kg/day	General toxicity: M&F:250 mg/kg/day	M&F: 750 mg/kg/day	M&F: ↓ bodyweight	[redacted] 2010 (101016)
	Neurological: M&F: 250 mg/kg/day	M&F: 750 mg/kg/day	M&F: equivocal evidence of ↓ activity several hours after dosing	
Rat 90-day neurotoxicity, oral dietary	General toxicity: M: 260mg/kg/day F: 772 mg/kg/day	M: 260 mg/kg/day F: -	M: ↓ bodyweight gain, ↓ food consumption F: no adverse effects observed	[redacted] 2011 (101006)

Study	NOAEL	LOAEL	Effects at LOAEL	Reference (study ID)
XDE-729 Acid 0-50-250-750 mg/kg/day target	Neurological: M&F: ~772 mg/kg/day	M&F: -	M&F: no adverse effects observed	
Rat 28-day immunotoxicity, oral dietary XDE-729 Methyl 0-52-500 mg/kg/day target	Immunotox: F: 505 mg/kg/day	F: -	F: no adverse effects observed	[redacted] 2012 (121004)
	General toxicity: F: 52 mg/kg/day	F: 505 mg/kg/day	F: ↓ haemoglobin conc, ↓ haematocrit, increased reticulocyte count, ↑ liver weight, ↓ thymus weight	

Toxicokinetics

Absorption

XDE-729 Acid and XDE-729 Methyl are rapidly and extensively absorbed following oral administration, with plasma C_{max} occurring in the rat within 30 minutes post-dosing. Absolute oral bioavailability, calculated from the dose-corrected plasma AUC data for the low oral and IV dose groups is close to 100% for both female and male rats. In dogs, absorption is fairly rapid with t_{max} at 0.5 to 1 hour post-dosing for both sexes. Oral bioavailability in the dog is also high with about 80% of the administered dose eliminated in the urine.

Distribution

The presence of a high proportion of administered radioactivity in the blood plasma indicates widespread systemic circulation of both XDE-729 Na salt and XDE-729 Methyl metabolites. There is no evidence of preferential distribution to any particular tissue, or of accumulation. Foetal exposure to XDE-729 Acid in systemic circulation is demonstrated.

Metabolism

Metabolism of XDE-729 Acid is limited in rats, mice and dogs evidenced by the parent compound being the major radiolabelled component in urine and faeces, accounting for 82-98% of administered dose in rats. Minor metabolites of XDE-729 Acid are the acyl-glucuronide conjugate of XDE-729 (accounting for up to ~7% of radioactivity dose in rats) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (~6% of dose).

The major radiolabelled component present in urine and faeces following administration of XDE-729 Methyl is also XDE-729 Acid, demonstrating that XDE-729 Methyl is rapidly and extensively hydrolysed to XDE-729 Acid. The Acid accounts for about 80% of administered radioactivity, in males and females, respectively. As is the case for XDE-729 Acid, minor metabolites of XDE-729 Methyl are the acyl-glucuronide conjugate of XDE-729 (accounting for up to ~6% of radioactive dose) and O-demethyl-XDE-729 and the corresponding sulphate and glucuronide conjugates (up to ~11%). The primary analyte in blood samples is XDE-729 Acid, with no parent compound being detected, confirming the rapid hydrolysis of XDE-729 Methyl *in vivo*. Toxicokinetic investigations in the 90-day oral dietary study in the rat (IIA 5.3.2/02) identified an additional metabolite of XDE-729 Methyl, the glucuronide conjugate of demethyl XDE-729 Methyl, detected in blood, liver and urine. The amount of this metabolite excreted in urine in females accounts for up to 5.5% of the dose of XDE-729 Methyl (adjusted for equivalence) at a dose level of 3 mg/kg/day and 12.4% at 500 mg/kg/day. This indicates that not

all administered XDE-729 Methyl undergoes hydrolysis to XDE-729 Acid, even at dose levels as low as 3 mg/kg/day.

For the dermal route of exposure to XDE-729 Methyl, systemic exposure is shown also to be primarily to XDE-729 Acid following application of concentrations up to 7.5 g/L for 10 hours.

Excretion

Absorbed XDE-729 Acid is rapidly and extensively excreted mainly via urine (68-92% of the administered dose), with the proportion in urine being highest among females. The majority of the urinary elimination occurs within the first 24 hours post-dosing. A smaller percent of the oral dose is eliminated in faeces, the majority of which (78-93%) occurred within 24 h of dosing. The extent and rates of urinary and faecal elimination of absorbed XDE-729 Methyl is similar to the XDE-729 Acid salts.

Acute toxicity, irritation and sensitisation

The acute toxicity, irritancy and skin sensitisation potential of XDE-729 Acid and XDE-729 Methyl have been investigated in standard GLP and OECD guideline compliant studies. These studies showed that both XDE-729 Acid and XDE-729 Methyl are of low acute oral and dermal toxicity, with LD_{50} values above those required for classification, and that neither is irritating to the skin and eye or show skin sensitising potential. Acute inhalation studies were not conducted because it is not technically possible to generate respirable test atmospheres; furthermore, human inhalation exposure is unlikely as both XDE-729 Acid and XDE-729 Methyl are classed as very slightly volatile.

Short-term toxicity

The oral dietary studies demonstrate that the main target organ for XDE-729 Acid is the kidney in rats and dogs. The primary changes in the rat kidney are renal tubular degeneration together with hypertrophy and vacuolation of the collecting duct epithelium. In the dog, degenerative and regenerative changes in the tubules and slight degeneration of collecting ducts are induced at 28 and 90 days and, additionally, slight fibrosis or focal granuloma in cortex and slight glomerulosclerosis at 1 year. Other findings in the dog include decreased red blood cell (RBC) mass and subsequent extramedulary hematopoiesis in the spleen and liver. In mice the only noteworthy finding are a slight inflammation of the urinary bladder mucosa in the 90 day study and a reduction in red blood cell count in the 26 day study. The most sensitive NOAELs are 252 mg/kg/day in rats, 11 mg/kg/day in dogs and 269 mg/kg/day in mice. By the dermal route, XDE-729 Acid does not cause local effects or systemic toxicity in rats. Inhalation studies have not been conducted because XDE-729 Acid and XDE-729 Methyl are non-volatile substances and inhalation is not expected to be a significant route of exposure to humans.

In contrast, oral dietary studies identify the liver as the main target for XDE-729 Methyl in rats. The primary changes in the liver are hepatocyte hypertrophy, mitotic figures and vacuolisation consistent with fatty change, observed at exposure levels of 52 mg/kg/day and above. Other targets, affected only at higher exposure levels, are kidneys, thyroids, thymus and blood. The kidney changes are slight multifocal hypertrophy of collecting duct epithelial cells; for the thyroid, organ weight is increased, accompanied by a diffuse hypertrophy of follicular cells; in the thymus, lymphoid tissue atrophy are induces; in blood, haemoglobin concentration, haematocrit and red cell count are reduced. For XDE-729 Methyl, a short-term oral NOAEL of

10 mg/kg/day is identified for liver toxicity is identified in the repeated dose toxicity studies, based on the presence liver toxicity in rat at 52 mg/kg/day. However, in the XDE-729 Methyl rabbit developmental toxicity study a lower oral dietary NOAEL for maternal toxicity of 5.78 mg/kg/day was identified, based on the observation of increased liver weight and slight hypertrophy and altered tinctorial properties of periportal hepatocytes at 18.5 mg/kg/day.

Genotoxicity

The genotoxicity of XDE-729 Acid has been adequately investigated in the standard tests. This substance tests negative in *in vitro* assays for gene mutation and clastogenicity and in an *in vivo* micronucleus test. It is therefore concluded that XDE-729 Acid is not genotoxic. XDE-729 Methyl also tests negative in standard *in vitro* assays for gene mutation and clastogenicity, and it can therefore be concluded that XDE-729 Methyl is not genotoxic. A technical batch of XDE-729 Methyl (Lot 201101758-63B, TSN302167, 93.3% purity) is negative in an *in vitro* bacterial assay for gene mutation.

Long-term toxicity and carcinogenicity

A standard carcinogenicity study in the rat and one in the mouse demonstrate that XDE-729 Acid is not carcinogenic. This conclusion is also applicable to XDE-729 Methyl at oral exposure levels of up 10 mg/kg/day.

Regarding the long-term non-neoplastic toxicity, kidneys are identified at the principal target for XDE-729 Acid in the rat, as is the case for short-term exposure (see section B.6.3). Kidney changes occur at exposure levels of 400 mg/kg/day and above. The kidney effects seen at 12 months are chronic progressive glomerulonephropathy, hyperplasia of the pelvic epithelium, hypertrophy of collecting duct epithelium, increased number of mitotic figures in the collecting duct epithelium, chronic interstitial inflammation of the medulla, necrosis of collecting duct epithelium and vacuolization of collecting duct epithelium in the papilla, accompanied by increase kidney weight. Additional pathology changes in the kidney are observed at 24 months, namely tubular degeneration with regeneration and necrosis of individual tubular epithelial cells and, in occasional males, the presence of calculi in the pelvis. At 24 months there are also changes in the urinary bladder, characterised by hyperplasia of the transitional epithelium and inflammation in the submucosa beneath the hyperplastic epithelium and, in one animal, the presence of microscopic calculi in the lumen. Secondary to the kidney toxicity, slight hypertrophy and vacuolisation of the adrenal zona glomerulosa is seen at 24 months. The kidney toxicity is accompanied by reduced bodyweight gain from 400 mg/kg/day and increased urinary volume and, at the highest dose level, increased blood urea nitrogen and reduced survival. A NOAEL of 102 mg/kg/day (target 100 mg/kg/day) is identified for the long-term toxicity of XDE-729 Acid in the rat.

In mice the urinary bladder and kidneys are identified as the principal toxicity targets of XDE-729 Acid, affecting only males. At 250 mg/kg/day and above inflammation of the urinary bladder and microscopic calculi are present in the urinary bladder lumen of males. At 750 mg/kg/day the effects on the urinary bladder are additionally characterised by hyperplasia, increased numbers of mitotic figures, necrosis of individual cells and ulceration of the transitional epithelium; also, kidney tubular degeneration with regeneration and dilatation were present. The urinary bladder and kidney toxicity is associated with increased mortality among males at 750 mg/kg/day. Treatment-related hypertrophy of the intercalated cells of the kidney was present in males at 750 mg/kg/day and females at 250 and 1000 mg/kg/day; this is interpreted as being an adaptive

response, possibly related to maintaining acid-base homeostasis. NOAELs of 50 mg/kg/day in males and 1004 mg/kg/day (target 1000 mg/kg/day) in females are identified for the long-term toxicity of XDE-729 Acid in the mouse.

Reproductive and developmental toxicity

The reproductive toxicity of XDE-279 Acid has been adequately investigated in a rat two generation study and in a rat developmental toxicity study and a rabbit developmental toxicity study. Also, the developmental toxicity of XDE-279 Methyl has been investigated in a rat and a rabbit study.

The two generation study demonstrates that XDE-729 Acid does not have an adverse effect on fertility or general reproductive performance and classification for this endpoint is not warranted. A NOAEL of 443 mg/kg/day, the highest dose level investigated, is identified for effects on fertility and general reproductive performance. This conclusion can be extrapolated to XDE-729 Methyl.

In the rat, there is evidence that both XDE-729 Acid and XDE-729 Methyl can cause marginal developmental effects, such as a slight ossification delay or slightly reduced foetal weight, but only in association with maternal toxicity. In the rabbit, XDE-729 Acid does not cause developmental toxicity; however, XDE-729 Methyl causes developmental toxicity, manifested as reduced foetal weight and a slight ossification delay, at an exposure level that also causes slight maternal liver toxicity. NOAELs for developmental toxicity of 159 mg/kg/day for XDE-729 Acid (based the rat study) and 18.5 mg/kg/day for XDE-729 Methyl (based on the rabbit study) are identified. As the only developmental changes observed for both XDE-729 Acid and XDE-729 Methyl occurred in association with maternal toxicity, and were of the type that can be assumed to secondary non-specific consequences of maternal toxicity, the criteria for classification for developmental toxicity are not met.

Neurotoxicity

No evidence of specific irreversible neurotoxicity has been observed in an acute and a 90 day neurotoxicity study on XDE-729 Acid or in the general toxicity studies XDE-729 Acid and XDE-729 Methyl.

Immunotoxicity

Dietary administration of XDE-729 Methyl for 28 days to the rat at dose levels of up to 500 mg/kg/day did not elicit immunotoxicity, based on the absence of an influence the primary immune response to sheep red blood cells.

Studies on metabolites

O-demethyl XDE-729 Acid (X11449757) is negative in *in vitro* assays for gene mutation and clastogenicity.

Bridging from XDE-729 Acid to XDE-729 Methyl

The core registration toxicological studies were conducted with XDE-729 Acid. Bridging studies have been conducted with XDE-729 Methyl with the aim of demonstrating toxicological

equivalence between XDE-729 Acid and XDE-729 Methyl. The bridging studies included a toxicokinetics study in rats, genotoxicity studies, acute toxicity studies, a 28-day and 90 day repeat exposure (oral dietary) studies, and a developmental toxicity study in both the rat and rabbit.

Toxicokinetic investigations in rats, mice and rabbits demonstrated that post-hepatic systemic exposure following oral administration of XDE-729 Methyl is mainly to XDE-729 Acid, and that the systemic levels are quantitatively similar following oral administration of equivalent doses of XDE-729 Methyl and XDE-729 Acid (i.e. XDE-729 Methyl and XDE-729 Acid have 'toxicokinetic equivalence'). XDE-729 Methyl and XDE-729 Acid have also been shown to be toxicologically equivalent for acute toxicity and genotoxicity endpoints, and both do not cause specific developmental toxicity in rats and rabbits. However, on 28 and 90 day repeated oral dietary administration in rats XDE-729 Methyl and XDE-729 Acid have different main toxicity target organs; for XDE-729 Methyl it is the liver (short-term NOAEL 10 mg/kg/day) and for XDE-729 Acid it is the kidney (short-term NOAEL 250 mg/kg/day in rat).

MoA studies with XDE-729 Methyl demonstrated that the liver effects are mediated through activation of the aryl hydrocarbon receptor (AhR). The Applicant conducted additional MoA studies to investigate the AhR-mediated MoA and hydrolysis of XDE-729 Methyl to XDE-729 Acid both *in vivo* and *in vitro* studies across multiple species.

Integrating the results of the MoA studies, the bridging studies and core registration studies, it can be concluded that:

1. Systemic (post-hepatic) exposure following dietary intake of XDE-729 Methyl is to XDE-729 Acid

Additional toxicokinetic investigations consistently confirm the presence of negligible levels of XDE-729 Methyl in blood or urine of rats, mice and rabbits in a wide range of studies at various oral dose levels of XDE-729 Methyl, ranging from 10 to 782 mg/kg/day. These data demonstrate that post-hepatic systemic exposure following the administration of XDE-729 Methyl is mainly to its hydrolysis product XDE-729 Acid. Evidence of systemic exposure to a direct metabolite of the parent, namely a glucuronide conjugate of O-demethyl XDE-729 Methyl, has been seen in some XDE-729 Methyl rat studies, but this conjugate was present in blood only at dose levels above the rat NOAEL of 10 mg/kg/day and no free O-demethyl XDE-729 Methyl has been detected in blood, liver or urine at any exposure level of XDE-729 Methyl, indicating low toxicological concern for this direct metabolite, certainly at the rat NOAEL for liver toxicity of 10 mg/kg/day. Overall, in relation to potential effects in the organs other than the gastrointestinal tract (local effects) and liver, the toxicity studies on XDE-729 Acid in rats, mice and rabbits can be regarded as predictive for XDE-729 Methyl for the oral route, especially at dose levels of 10 mg/kg/day and below.

PBPK modelling predicted comparable peak concentrations and 24-hr AUCs of XDE-729 Methyl in liver and blood between rats and humans at XDE-729 Methyl dietary exposure levels of 0.0001, 0.1 and 10 mg/kg/day, providing evidence that in humans systemic exposure is also mainly to the hydrolysis product. The PBPK modelling also predicted negligible (i.e. considerably below the LOQ) liver and post-hepatic systemic exposure to XDE-729 Methyl at a human exposure levels of 0.0001 and 0.1 mg/kg/day, exposure levels relevant to predicted human exposure and a proposed ADI, respectively.

2. Hepatic exposure to XDE-729 Methyl causes AhR-mediated liver toxicity and the 90-day dietary rat study defined the NOAEL for these effects as 10 mg/kg/day in the rat

In a XDE-729 Methyl 90 day dietary study conducted at dose levels 3, 10, 52, 261 and 500 mg/kg/day, a NOAEL of 10 mg/kg/day was identified. At dose levels of 52 mg/kg/day and above, the induction of hepatic *Cyp1a1* gene expression was notable and liver toxicity (increased organ weight, hepatocyte vacuolation) was observed. The severity of the hepatic changes correlated with the levels of XDE-729 Methyl detected in the liver. Thyroid changes were observed in the 90-day rat study, from 261 mg/kg/day, interpreted as being secondary to increased glucuronidation in the liver because markedly increased hepatic *Ugt1a6* gene expression was seen from 261 mg/kg/day. The induction of hepatic *Cyp1a1* and *Ugt1a6* gene expression, which correlated with the observed liver and thyroid changes, provides evidence of activation of the AhR signalling pathway. A 28-day dietary MoA study in rats showed that XDE-729 Methyl induced liver toxicity, hepatocellular proliferation, and hepatic *Cyp1a1* gene expression which are reversible after a short recovery period.

Comparison of the results of 7 day dietary toxicity in rats and mice indicate that mice are less sensitive than rats to XDE-729 Methyl induced liver toxicity and hepatic *Cyp1a1* gene expression in relation to a short exposure period.

3. XDE-729 Methyl is the AhR agonist and not an impurity, metabolite or XDE-729 acid

AhR transactivation assays demonstrate that XDE-729 Methyl is a weak AhR agonist in a mouse reporter cell line and essentially has no activity in a human reporter cell line. The agonist activity of the XDE-729 Methyl samples is not quantitatively influenced by the level of purity, indicating that it is unlikely to be an impurity that is responsible for the observed AhR agonist activity. XDE-729 Acid has no AhR agonist activity in either the mouse or human reporter cell lines. A cell-free *in vitro* photoaffinity ligand competition-binding assay, using AhR sourced from Sprague Dawley male rat liver cytosol, demonstrates that XDE-729 Methyl is an AhR ligand in this species.

4. Rats are significantly more sensitive to AhR activation by XDE-729 Methyl than humans

In vitro Cyp1a1 and Cyp1a2 gene expression studies in primary cultures of freshly isolated human, mouse and rat hepatocytes provide evidence of rats being more sensitive to AhR activation by XDE-729 Methyl than humans. For expression of Cyp1a1/CYP1A1 by XDE-729 Methyl, the rat was the most sensitive and human being the least. For Cyp1a2/CYP1A2 expression, the rat was also the most sensitive, and the mouse being the least. For both enzymes, all tested species were much more sensitive to induction by the positive control, 3-MC, than by XDE-729 Methyl.

As mentioned in point 3 above, AhR transactivation assays demonstrate that XDE-729 essentially has no AhR agonist activity in a human reporter cell line. This provides additional evidence that humans may have relatively low sensitivity to AhR activation by XDE-729 Methyl.

5. The NOAEL of 10 mg/kg/day established for AhR-mediated liver toxicity in the rat 90-day dietary study with XDE-729 Methyl is applicable to the human health risk assessment for longer-term exposures

The 90 day XDE-729 Methyl dietary study identified a NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity in the rat. A framework analysis shows that it is appropriate to make a conservative assumption that this MoA is relevant to humans. The 90 day NOAEL is considered to also be applicable for XDE-729 Methyl exposures of longer duration because there was no increase in severity of liver toxicity in the 90 day XDE-729 Methyl dietary study in rats as compared to the 28 day XDE-729 Methyl dietary study (both studies identified NOAELs and LOAELs of 10 and ~50 mg/kg/day, respectively). This NOAEL of 10 mg/kg/day for AhR-mediated liver toxicity in rats is probably conservative in relation to humans because humans are likely to be less sensitive than rats to AhR activation and to subsequent induction of CYP1A1/CYP1A2 by XDE-729 Methyl.

Human post-hepatic systemic exposure following XDE-729 Methyl intake by the oral route will primarily be to the Acid, for which there is a full set of regulatory toxicity studies. Bridging studies are available for XDE-729 Methyl which show that XDE-729 Methyl and XDE-729 Acid are toxicologically equivalent in rats at dose levels of up to 10 mg/kg/day based on the absence of AhR-mediated effects in the liver at this dose level and below in the XDE-729 Methyl studies, although XDE-729 Methyl and XDE-729 Acid have different target organs and NOAELs at higher dose levels. Although a NOAEL of 10 mg/kg/day has been identified for AhR-mediated liver toxicity in the rat, a lower NOAEL of 5.78 mg/kg/day was identified for XDE-729 Methyl mediated liver toxicity in the rabbit in a developmental toxicity study (Ellis-Hutchings et al 2012, IIA 5.6.11/04). This lower NOAEL will be used as the starting point for deriving a dietary reference dose for the human risk assessment.

Regarding the dog, the toxicokinetics of XDE-729 Methyl have not been investigated in this species. Therefore, it must be assumed that the toxicokinetics of XDE-729 Methyl in the dog will be similar to the rat (i.e. post hepatic systemic exposure in the dog following XDE-729 Methyl is mainly to its hydrolysis product XDE-729 Acid) for the XDE-729 Acid dog studies to be regarded as predictive of the post-hepatic systemic toxicity of XDE-729 Methyl; in the opinion of the RMS, this is a reasonable assumption especially for dose levels up to the rat NOAEL of 10 mg/kg/day. To consider the liver as the target organ of XDE-729 Methyl, it is likely that the dog will be less sensitive than the rat to XDE-729 Methyl AhR-mediated liver toxicity, based on published studies showing that dog AhR has relatively low binding affinity for an AhR ligand and the absence of liver toxicity in dogs following administration of a compound which is metabolised to a potent AhR agonist.

For the dermal route of exposure to XDE-729 Methyl, systemic exposure is shown also to be primarily to XDE-729 Acid following the single application of concentrations up to 7.5 g/L for 10 hours. Therefore, the results of the oral XDE-729 Acid studies can be extrapolated to the dermal route.

No information is available on the toxicokinetics for the inhalation route. However, human exposure is likely to be to material with particle size greater that 10 µm and so inhaled particles will cleared to the stomach rather than penetrating the deep lung. Thus, oral XDE-729 Acid studies are relevant for the inhalation route of exposure.

France (coRMS) comments on bridging from XDE-729 Acid to XDE-729 Methyl and the MoA investigations.

France agrees that it has been clearly demonstrated that XDE-729 Methyl causes liver toxicity, hepatocellular proliferation and hepatic CYP1A gene expression. However, France is of opinion

that the MoA of XDE-729 Methyl has not been not clearly demonstrated because the partial AhR agonism cannot alone explain the strong CYP 1A1 gene expression increase observed in rat after XDE-729 Methyl exposure. Nevertheless, a NOAEL can be established for the liver effects and the coRMS is agrees with the NOAEL of 5.78 mg/kg/day proposed by the UK RMS (based on liver toxicity observed in the rabbit developmental toxicity study after XDE-729 Methyl exposure). Also, France agrees that the bridging strategy based on the demonstration of the saturation of hydrolysis of XDE-729 Methyl may be acceptable.

B.6.10.1 Acceptable Daily Intake (ADI)

The key repeated dose adverse health effect of XDE-729 Methyl is liver toxicity, observed in rats and rabbits. In rat, the liver toxicity has been shown to be AhR mediated, with a NOAEL of 10 mg/kg/day, identified in a 90 day dietary study in rats ([redacted] 2012, IIA 5.3.2/02). The level of toxicity seen after 90 days exposure is similar to that found at 28 days, indicating no progression with time and therefore this NOAEL is considered applicable to chronic exposures. However, in the rabbit a lower NOAEL of 5.78 mg/kg/day was identified in a developmental toxicity study [redacted] et al 2012, IIA 5.6.11/04). Humans are expected to be less sensitive to the AhR-mediated liver effects seen in the rat, but the mode of action for the liver effects in the rabbit is not clear, so the standard assessment factor of 100 is deemed appropriate. Therefore, dividing the NOAEL of 5.78 mg/kg/day by 100, an **ADI of 0.058 mg/kg/day** is derived.

B.6.10.2 Acute Reference Dose (ARfD)

The most appropriate starting point for deriving the ARfD is the NOAEL of 250 mg/kg identified in the acute oral neurotoxicity study on XDE-729 Acid ([redacted] 2010, IIA 5.7.1/01), based on the observation of slight reduced bodyweights and equivocal evidence of reduced activity several hours after dosing.

The other studies which potentially provide information on toxicity associated with acute exposure do not provide a suitable starting point for the ARfD derivation. In the general acute toxicity studies, the only dose levels investigated were much greater than the 250 mg/kg acute neurotoxicity LOAEL. Also, no adverse effects that could clearly be associated with the first few days exposure were observed in the short-term repeated dose toxicity and developmental toxicity studies.

The standard assessment factor of 100 is deemed appropriate. So, dividing the NOAEL of 250 mg/kg/day by 100, an **ARfD of 2.5 mg/kg/day** is derived.

B.6.10.3 Admissible Operator Exposure Level (AOEL)

A systemic AOEL based on the NOAEL of 5.78 mg/kg/day identified in the developmental toxicity study ([redacted] 2012, IIA 5.6.11/04) is proposed as this dose level is relevant to a short-term repeated exposure pattern experienced by operators. There is no reason to apply a safety factor other than the standard one of 100. Therefore, dividing the NOAEL of 5.78 mg/kg/day by 100, an **AOEL of 0.058 mg/kg/day** is derived.

B.6.10.4 Maximum Allowable Concentration (MAC: drinking water limit)

A health-based MAC of 0.174 mg/L can be calculated, on the basis that exposure via drinking water should not exceed 10% of the ADI and assuming water consumption of 2 L/day and a bodyweight of 60 kg. However, the levels of all active substances in drinking water are limited to 0.1 µg/L under EU legislation.

B.6.10.5 Proposal for classification and labelling

No classification with respect to human health is proposed.

B.6.11 Acute toxicity, irritancy and skin sensitisation of GF-2573 (IIIA 7.1)

GF-2573 is an emulsifiable concentrate (EC) formulation containing 7.6 g/L XDE-729 Methyl. In use spray dilutions will be 0.12g/L and 0.020g/L.

B.6.11.1 Acute oral toxicity (IIIA 7.1.1)

GF-2573	
Study	IIIA1 7.1.1/01 Acute oral toxicity up and down procedure in rats
Reference	[redacted] (2011a)
Date performed	March – April 2011
Test facility	[redacted]
Report reference	[redacted] study no. 31820, Dow study no. 110305
Guideline(s)	OECD 425
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573, Lot #E2837-57,TSN031424-0002, containing 0.86% w/w (7.8 g/L) XDE-729 Methyl
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 11 weeks old, were used. Dosing was by gavage. The animals were fasted prior to dosing. The test substance was administered undiluted. The stability of the formulation was not determined as part of this study.
One group of three females received a single dose of 5000 mg/kg bodyweight, administered by gavage. As all three animals survived at this dose level, no further testing was necessary.

Animals were examined for mortality and clinical signs during the first several hours post-dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

RESULTS

All animals survived test substance administration and gained body weight during the study. Following administration, one animal was hypoactive and exhibited ano-genital staining, clear oral discharge and reduced faecal volume. This animal recovered from these symptoms by day 2 and along with the other two animals appeared active and healthy for the remainder of

the 14-day observation period. No gross abnormalities were noted for any of the animals at necropsy on day 14.

CONCLUSION

The acute oral LD_{50} of GF-2573 in female rats is >5000 mg/kg. Accordingly, of GF-2573 is not classified for acute oral toxicity.

[redacted] (2011a)

B.6.11.2 Acute dermal toxicity (IIIA 7.1.2)

GF-2573	
Study	IIIA1 7.1.2/01 Acute dermal toxicity study in rats
Reference	[redacted])
Date performed	March – April 2011
Test facility	[redacted]
Report reference	[redacted] study no. 31821, Dow study no. 110306
Guideline(s)	OECD 402
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573, Lot #E2837-57,TSN031424-0002, containing 0.86% w/w (7.8 g/L) XDE-729 Methyl
Study acceptable	Yes

METHODS

Female Fischer 344 rats, 11 weeks old, were used. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk. The day of dosing was designated study day 0. The test material was applied, undiluted, to an area of clipped skin, measuring 5 x 7.5 cm (approximately 10% of the body surface) on each animal and covered with a 5 x 7.5 cm, 4-ply, gauze pad. The gauze pad and entire trunk of each animal were then wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. After an exposure period of 24 hours, the dressings were removed and dosing site was rinsed with a 3% soap solution followed by tap water.

A group of 5 males and 5 females received a single dose of 5000 mg/kg bodyweight.

Animals were examined for mortality and clinical signs during the first several hours post-dosing and at least once daily thereafter. Bodyweights were recorded on the day of application and on day 7 and 14. The study was terminated after a 14 day observation period. A gross necropsy was conducted on all animals, in which the external surfaces of the animal and the organs of the thoracic and abdominal cavities were examined.

RESULTS

All animals survived exposure to the test substance. Ocular discharge was observed in two females from days 3 through 5. Dermal irritation at the dose site was observed for all animals from day 1. In most animals, the signs of irritation persisted until termination on day 14. There were no other clinical findings recorded for any animal over the course of the observation period.

Although all rats lost body weight by day 7, all showed body weight gain between days 7 and 14. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

CONCLUSION

The acute dermal LD_{50} of GF-2573 in rats is >5000 mg/kg. Accordingly, GF-2573 is not classified for acute dermal toxicity.

[redacted] (2011b)

B.6.11.3 Acute inhalation toxicity (IIIA 7.1.3)

No inhalation studies have been conducted on GF-2573 because this product does not fall into any of the categories which require inhalation testing according to Directive 91/414/EEC (Annex III, 7.1.3 Inhalation).

The inhalation test must be carried out only where the product:

- a gas or liquified gas,
- is a smoke-generating formulation or fumigant,
- is a vapour releasing preparation,
- is used with fogging equipment,
- is an aerosol,
- contains an active substance with a vapour pressure >1 x 10^{-2} Pa and is to be used in enclosed spaces such as warehouses or glasshouses,
- is a powder containing a significant proportion of particles of diameter <50 μm (>1% on a weight basis),
- is to be applied from aircraft in cases where inhalation exposure is relevant,
- is to be applied in a manner which generates a significant proportion of particles or droplets of diameter <50 μm (>1 % on a weight basis).

B.6.11.4 Skin irritancy (IIIA 7.1.4)

GF-2573	
Study	IIIA1 7.1.4/01 Primary skin irritation study in rabbits
Reference	[redacted] (2009a)
Date performed	October 2009
Test facility	[redacted]
Report reference	[redacted] study no. 28348, Dow study no. 090537
Guideline(s)	OECD 404
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573, Lot #E2837-57,TSN031424-0002, containing 0.86% w/w (7.8 g/L) XDE-729 Methyl
Study acceptable	Yes

METHODS

GF-2573 was applied by semi-occlusive application of 0.5 g (undiluted) to a clipped area of intact skin on the dorsal area of the trunk of each of three young adult female New Zealand white

rabbits. The duration of treatment was 4 hours, after which the dressings were removed and dosing site was rinsed with a 3% soap solution followed by tap water.

The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study. Any skin reactions were assessed according to the numerical scoring system of OECD test guideline 404, approximately 1, 24, 48 and 72 hours and at 7, 10 and 14 days after the removal of the dressings. Additionally, for the first of the three animals tested, skin reactions were also assessed after 3 minutes and 1 hour exposure periods. Study was terminated at 14 days.

RESULTS

No deaths occurred. No signs of systemic toxicity were observed. The body weights of the rabbits appeared to be within a normal range of variability.

Well-defined erythema and slight oedema were observed in all three animals within 24 hours of patch removal. The overall incidence and severity of irritation decreased gradually with time. A brown area, new skin, hyperkeratosis and/or desquamation at the dose site were evident in the animals between 24 hours and day 14. Very slight erythema and desquamation persisted at the dose sites of all three animals through Day 14 (study termination).

In the first animal tested, there was no dermal irritation noted at the 3-minute application site. Very slight erythema was observed at the 1-hour site between 24 hours and day 7. Also desquamation was evident at the 1-hour site from 72 hours and persisted through Day 14 (study termination)

The mean irritation scores for the 24, 48 and 72 hours observations are shown in Table B.6.11.4-1. As a mean score of 2 for erythema or oedema has been observed in at least two of the three animals tested, the criteria for classification as R38; Irritating to skin (Directive 67/548/EEC) are met. However, the classification criteria for skin irritation under Regulation (EC) 1272/2008 are not met as the mean scores are not ≥ 2.3.

Table B.6.11.4-1: Mean skin irritation scores at 24, 48 and 72 h

Number tested	Score for each animal: mean of 24, 48 & 72 h observations		Reversibility (Yes/No)	Result
	Erythema	Oedema		
3 NZW rabbits	2, 2, 2	1.3, 2, 2	No	Irritating. R38

CONCLUSION

GF-2573 is a skin irritant, meeting the criteria for classification as R38; Irritating to skin under Directive 67/548/EEC. However, GF-2573 does not meet the criteria for classification for skin irritancy under Regulation (EC) 1272/2008).

([redacted] 2009a)

B.6.11.5 Eye irritancy (IIIA 7.1.5)

GF-2573	
Study	IIIA1 7.1.5/01 Primary eye irritation study in rabbits
Reference	[redacted] 2009b)
Date performed	October 2009
Test facility	[redacted]
Report reference	[redacted] study no. 28347, Dow study no. 090538
Guideline(s)	OECD 405
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573, Lot #E2837-57,TSN031424-0002, containing 0.86% w/w (7.8 g/L) XDE-729 Methyl
Study acceptable	Yes

METHODS

A 0.1 ml aliquot of GF-2573 was installed into one eye of each of three young adult female New Zealand white rabbits. The other eye, which was untreated, served as a control. Scoring of eye reactions, according to the numerical system of OECD test guideline 405, was conducted at 1, 24, 48 and 72 hours and at 4 days. The animals were checked daily for signs of systemic toxicity and mortality. Bodyweights were recorded at the start and finish of the study

RESULTS

No deaths occurred. No systemic signs of toxicity were reported.

One hour after test substance instillation, two treated eyes exhibited iritis and all three treated eyes exhibited conjunctivitis. The overall incidence and severity of irritation decreased with time. All animals were free of ocular irritation by Day 4. There was no corneal opacity observed in any treated eye during this study.

The mean irritation scores for the 24, 48 and 72 hours observations are shown in Table B.6.11.11-1. The mild reactions observed did not meet the criteria for classification as an eye irritant.

Table B.6.11.5–1: Mean irritation scores at 24, 48 and 72 h

Number tested	Score for each animal: mean of 24, 48 & 72 h observations				Reversibility Yes/no	Result
	Cornea	Iris	Conjunctiva			
			Redness	Chemosis		
3 NZW rabbits	0, 0, 0	0.3, 0.3, 0	1, 1.7, 1.7	0.3, 0.7, 0.7	Yes	Not irritating

CONCLUSION

GF-2573 did not induce significant or irreversible damage to the eye and therefore is not classified for eye irritancy.

[redacted] (2009b)

B.6.11.6 Skin sensitisation (IIIA 7.1.6)

	GF-2573
Study	IIIA1 6.11.6/01 Local lymph node assay in CBA/J mice
Reference	[redacted] (2010)
Date performed	January 2010
Test facility	[redacted]
Report reference	Laboratory study ID 101001
Guideline(s)	OECD 429 (2002)
Deviations from the guideline	The positive control group did not achieve the required ≥3 SI. However, the study is considered acceptable because a positive sensitisation result for GF-2537 is claimed by the Applicant.
GLP	Yes
Test material	GF-2573, Lot #E2837-57,TSN031424-0002, containing 0.86% w/w (7.8 g/L) XDE-729 Methyl
Study acceptable	Yes

METHODS

Female mice of the CBA/J strain, aged 9-12 weeks, were used.

In a screening study, 3 daily topical applications (25 µl to dorsal surface of each ear) of 1%, 5%, 25%, 50%, 75%, or 100% GF-2573 were given to one animal at each dose level. Erythema was absent in the mice exposed to up to 25% and present with concentration-related severity had the higher concentrations. Bodyweights were unaffected in all dose groups. Results from this study were used to select concentrations for main LLNA.

In the LLNA, six female mice/group received 3 daily topical applications (25 µl to dorsal surface of each ear) of 5%, 20%, or 80% of GF-2573. A vehicle and positive control groups were similarly treated with 1% Pluronic L92 surfactant or 30% α-hexylcinnamaldehyde (HCA), respectively. Three days after the third application, all the animals were injected, via the tail vein, with approximately 250µl of phosphate buffered saline (PBS) containing 20µCi of a 2.0Ci/mmol specific activity ^{3}H-methyl thymidine. Approximately 5 hours later, the animals were killed and the draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in the group, were placed in a container of PBS. A single cell suspension of the auricular lymph nodes from each mouse was prepared by gentle mechanical disaggregation using a tissue homogenizer. The cells were washed two times and were suspended in 3 ml of 5% trichloroacetic acid (TCA) for approximately 18 hours. The suspended precipitates were centrifuged (200 x g for 10 minutes) and the supernatant removed. The pellet from each mouse was reconstituted in 1 ml of 5% TCA and subsequently transferred to a scintillation vial containing 10 ml of Aquasol-2 scintillation cocktail. Two additional 2 ml aliquots of water were used to rinse the tubes and the rinses were added to the scintillation vials containing the 1 ml of pellet in TCA and cocktail. The radioactivity in each precipitate was measured using a β-scintillation counter and reported as disintegrations per minute (dpm) per mouse. The mean activity of each test group is then divided by the mean activity of the vehicle control group to give a test:control ratio known as the stimulation index (SI), for each concentration. The criterion for a positive response is that the SI for one or more concentrations is ≥3.

During the study any clinical signs were recorded, the ears were examined for erythema, and bodyweights were recorded.

RESULTS

There were no clinical signs of toxicity or adverse effects on bodyweights. Erythema of the ear was absent in the mice treated with 5% and 20%, while at 80% slight erythema was seen day 3, which resolved by day 6.

As shown in Table B.6.11.6-1, the SI value for the GF-2573 80% group was ≥3, indicating a positive response. An EC_3 of 32.4% was calculated. The criteria for classification as R43; May cause sensitisation by skin contact (Directive 67/548/EEC) and Skin Sens. 1B. H317 (Regulation (EC) 1272/2008) are met.

For the positive control group, the SI was 2.9; as the response was <3, the satisfactory conduct of the study could not be confirmed. However, because the Applicant is claiming a positive result, this assay is considered to be acceptable. Reference to recent laboratory historical control data (see Table B.6.11.6-2) suggests that the low SI for the positive control group in the current study was the result of an unusually high disintegrations/min count for the vehicle control group.

The UK RMS notes that GF-2573 contains a co-formulant that is a known skin sensitiser.

Table B.6.11.6–1: Group mean disintegrations/min and stimulation indices

Dose group	Mean disintegrations/min	SI
0 (L92 vehicle control)	1954	1.0
GF-2573 5%	2080	1.1
GF-2573 20%	4654*	2.4
GF-2573 80%	10360*	5.3
HCA 30% (positive control)	5680*	2.9

*significantly different from control, $p<0.05$

Table B.6.11.6–1: Laboratory historical control data: vehicle and positive control groups

Study ID	Report Date	Vehicle	Vehicle control: mean disintegrations/min	30% HCA: mean disintegrations/min	SI
091025	April 14, 2009	1% L92	1083	6838	6.3
091071	September 8, 2009	1% L92	433	6729	15.5
091046	June 22, 2009	1% L92	770	2847	3.7
091052	July 8, 2009	1% L92	555	4183	7.5
091092	September 24, 2009	1% L92	1001	5370	5.4

CONCLUSION

GF-2573, with an SI value of ≥3 (and EC_3 of 32%) demonstrates skin sensitising potential in the LLNA. The criteria for classification as R43; May cause sensitisation by skin contact (Directive 67/548/EEC) and Skin Sens. 1B. H317 (Regulation (EC) 1272/2008) are met.

([redacted] 2010)

B.6.11.7 Summary of acute toxicity, irritation and skin sensitisation studies

Test	Species	Result	Classification		Reference (study ID)
			67/548/EEC	EC1272/2008	
Acute oral	Rat	LD_{50} >5000 mg/kg	-	-	[redacted] 2011a (110305)
Acute dermal	Rabbit	LD_{50} >5000 mg/kg	-	-	[redacted] 2011b (110306)
Acute inhalation	Study not conducted because product does not fall into any of the categories which require inhalation testing				
Skin irritation	Rabbit	Positive- irritating	R38	-	[redacted] 2009a (090537)
Eye irritation	Rabbit	Negative	-	-	[redacted] 2009b (090538)
Skin sensitisation	Mouse (LLNA)	Positive: EC_3 = 32%	R43	Skin Sens. 1B	[redacted] 2010 (101001)

B.6.11.8 Toxicological data on non active substances (IIIA 7.4)

The information provided on the non-active substances present in GF-2573 indicate no specific toxicological properties of concern.

B.6.11.9 Proposal for classification and labelling

Directive 67/548/EEC

R38; Irritating to skin

R43; May cause sensitisation by skin contact

Regulation (EC) 1272/2008

Skin Sens. 1B. (H317)

B.6.11.10 Potential for interaction between ASs in the product

B.6.12 Dermal absorption studies (IIIA 7.3)

B.6.12.1 Dermal absorption *in vitro*

	GF-2573
Study	IIIA1 7.6.2/01 *In vitro* percutaneous absorption of [14C]-XR-729 methyl, formulated as GF-2573 through human and rat skin membranes
Reference	[redacted] (2011)
Date performed	May – July 2010
Test facility	[redacted]
Report reference	[redacted] study code V8995
Guideline(s)	OECD 428
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573 containing 7.5 g acid equivalents/L XDE-729 Methy with 10% of a.i. radiolabelled GF-2573 containing 7.5 g acid equivalents/L XDE-729 Methyl, with 100% of a.i. radiolabelled [14C]-XDE-729 Methyl, labeled at UL-phenyl position, batch XS9-100040-28, purity 98.5%, specific activity 45.3 mCi.mmol^{-1} Non-radiolabelled XDE-729 Methyl, batch XW7-38246-49, purity 98.9%
Study acceptable	Yes

METHODS

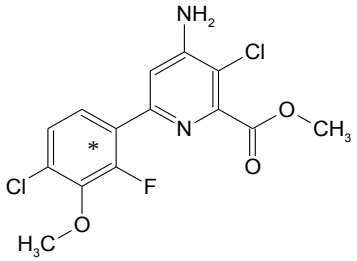
The test substance was tested at three target concentrations, representing the maximal concentration possible when handling the undiluted formulation and concentrations recommended for use in the field, in human and rat skin according to the following design:

Table B.6.12.1-1 *In vitro* percutaneous absorption from GF-2573: study design

Test group	No. of replicates	Species	Concentration of XDE-729 Methyl	Mean dose of XDE-729 Methyl	Exposure duration	Sampling of receptor fluid
A	6	Human	7.5 g a.e./L	71.2 µg a.e./cm^2	10 h	0-24 h
B	6	Human	0.12 a.e./L	1.23 µg a.e /cm^2	10 h	0-24 h
C	6	Human	0.020 a.e./L	0.21 µg a.e /cm^2	10 h	0-24 h
D	6	Rat	7.5 a.e./L	71.2 µg a.e /cm^2	10 h	0-24 h
E	6	Rat	0.12 a.e./L	1.23 µg a.e /cm^2	10 h	0-24 h
F	6	Rat	0.020 a.e./L	0.21 µg a.e /cm^2	10 h	0-24 h

a.e. = acid equivalents

The test substance was labelled on the phenyl ring, as indicated by an asterisk below.



The study was performed in flow-through diffusion cells (PermeGear Inc., Riegelsville, PA, USA). The contact time was 10 hours and the post exposure time was a further 14 hours. The amount of radioactivity in the receptor fluid (determined at 1-2 h intervals), skin washes after 10 and 24 h, in the skin (including up to 20 skin strippings), and receptor and donor diffusion cell compartment washings was measured by liquid scintillation counting.

Human skin membranes were prepared from three separate donors. Rat skin membranes were prepared from male rat. After thawing, the skin was dermatomed using a Dermatome 25 mm (Nouvag GmbH, Germany) to a recorded thickness of *ca* 400 µm. The thickness of the skin preparations were measured with a digimatic micrometer (Mitutoyo Corporation, Japan).

The integrity of the skin membranes was assessed using tritiated water. For human skin, skin membranes with a permeability coefficient (K_p) of less than 2.5 x 10^{-3} cm/h[1] were used, while for rat skin membranes with a K_p of less than 3.5 x 10^{-3} cm/h were used.

The solubility of XR-729 Methyl in the receptor fluid (saline containing 6 % polyoxyethylene 20-oleyl ether (w/v)) is considered to meet the requirements of the OECD guideline. The solubility was determined to be *ca* 37 µg/mL. The highest dose of XR-729 Methyl (*ca* 44.8 µg a.i./skin membrane) over 24 h, assuming 10 % absorption, would mean 4.48 µg XR-729 methyl in 38.4 mL of receptor fluid, i.e. 0.12 µg/mL. The solubility of the test substance in this receptor fluid composition is therefore considered sufficient, especially considering the fact that the maximal amount entering the receptor fluid was only 2.85 µg/cm^2 over 24 h, or 3.98 % of the highest dose (rat skin).

RESULTS

The key results are summarised in Tables B6.12.1-2 and B6.12.1-3.

Dermal absorption was calculated as the sum of radioactivity in the receptor fluid, the receptor compartment washings, the skin tape strips excluding the first two strips and skin, and was normalised to 100% recovery for test groups with total radioactivity recoveries of <95%. For the concentrate (7.5 mg/L) absorption was 0.9% and 5.2% of administered dose for human and rat skin, respectively. For the 0.12 mg/L dilution absorption was 16.1% and 44.1% of administered dose and for the 0.02 mg/L dilution absorption was 26.5% and 42.7% of administered dose for human and rat skin, respectively.

In the groups with radioactivity recovery of <95%, there was no evidence that the loss of radioactivity had occurred preferentially from either the absorbed or unabsorbed factions, and therefore normalisation of the absorbed dose to 100% recovery was considered appropriate.

Based on maximum flux values, dermal absorption of XDE-729 Methyl through human skin was about 9-fold lower from the concentrate, 4-fold lower from the 0.12 mg/L dilution and about 50% lower, compared with rat skin.

Table B.6.12.1-2. Dermal absorption of XDE-729 Methyl from GF-2573 through human skin

XDE-729 Methyl conc. [g/L]	**7.5**		**0.12**		**0.020**	
Administered dose [µg/cm^2]	71.2		1.23		0.21	
Penetration into the receptor fluid after 24 h	% of dose	µg/cm^2	% of dose	µg/cm^2	% of dose	µg/cm^2
	0.51	0.36	9.69	0.12	15.8	0.033
Maximal flux [µg/cm^2/h]	0.024		0.006		0.002	
Lag time [h]	6.9		2.8		1.4	
Absorbed dose [as % of dose]*	0.8 ± 0.2		14.9 ± 2.1		26.5 ± 5.4	
Absorbed dose [as % of dose]**, adjusted for significant variance	1.0		NA		NA	
Absorbed dose [% of dose], normalised for total recovery	1.1		16.1		26.5	
Recovery of radioactivity (% of dose, mean ± SD)						
Receptor fluid	0.51 ± 0.17		9.69 ± 1.33		15.8 ± 3.7	
Receptor compartment wash	0.03 ± 0.01		0.36 ± 0.06		0.66 ± 0.16	
Skin tape strips (3 – last)	0.09 ± 0.04		1.36 ± 0.39		2.91 ± 0.94	
Skin	0.18 ± 0.07		3.54 ± 0.60		7.17 ± 2.33	
Donor compartment	0.16 ± 0.13		0.78 ± 1.23		0.45 ± 0.26	
Skin wash t = 10h	92.3 ± 2.6		70.0 ± 6.1		61.2 ± 5.7	
Skin wash t = 24h	0.51 ± 0.06		6.27 ± 1.78		10.6 ± 2.5	
Total skin wash + tape strips 1 & 2	92.8 ± 2.6		76.8 ± 4.8		73.2 ± 6.2	
Total recovery	93.8 ± 2.6		92.5 ± 3.4		100.2 ± 4.0	

*The absorbed dose is defined as the sum of radioactivity found in the receptor fluid, the receptor compartment wash, the stratum corneum (first two skin tape strips excluded) and skin

** According to EFSA Guidance on Dermal Absorption, when variance is significant (i.e. SD is ≥25% of mean) the standard deviation should be added to the mean

Table B.6.12.1-3. Dermal absorption of XDE-729 Methyl from GF-2573 through rat skin

XDE-729 Methyl conc. [g/L]	**7.5**		**0.12**		**0.020**	
Administered dose [µg/cm^2]	71.5		1.23		0.21	
Penetration into the receptor fluid after 24 h	% of dose	µg/cm^2	% of dose	µg/cm^2	% of dose	µg/cm^2
	3.98	2.85	26.6	0.33	23.8	0.050
Maximal flux [µg/cm^2/h]	0.212		0.022		0.003	
Lag time [h]	2.5		2.5		2.1	
Absorbed dose [as % of dose]*	4.9 ± 0.6		41.1 ± 2.9		42.7 ± 3.2	
Absorbed dose [% of dose], normalised for total recovery	5.2		44.1		42.7	
Recovery of radioactivity (% of dose, mean ± SD)						
Receptor fluid	3.98 ± 0.49		26.6 ± 2.0		23.8 ± 5.9	
Receptor compartment	0.05 ± 0.02		0.58 ± 0.19		0.69 ± 0.13	
Tape strips (3 – last)	0.33 ± 0.10		7.03 ± 1.49		10.7 ± 4.2	
Skin	0.51 ± 0.13		6.85 ± 1.60		7.42 ± 1.82	
Donor compartment	0.14 ± 0.15		0.13 ± 0.03		0.19 ± 0.08	
Skin wash t = 10h	87.2 ± 1.9		44.7 ± 3.5		42.3 ± 5.2	
Skin wash t = 24h	0.66 ± 0.18		5.95 ± 1.49		12.0 ± 2.1	
Total skin wash + tape strips 1 & 2	88.0 ± 1.8		52.0 ± 2.7		57.6 ± 3.7	
Total recovery	93.0 ± 2.1		93.2 ± 2.1		100.5 ± 0.8	

*The absorbed dose is defined as the sum of radioactivity in the receptor fluid, the receptor compartment wash, the stratum corneum (first two skin tape strips excluded) and skin

CONCLUSION

In vitro dermal absorption of XDE-729 Methyl from GF-2573 over a 24 hour period was:

Table B.6.12.1-4. Dermal absorption of XDE-729 Methyl from GF-2573 through rat skin: conclusion

Species/parameter	**Concentrate 7.5 g/L**	**Field dilution 0.12 g/L**	**Field dilution 0.02 g/L**
Rat % absorption	5.2	44.1	42.7
Human % absorption	1.1	16.1	26.5
Ratio rat/human (% absorption)	4.7	2.7	1.6
Ratio rat/human (maximal flux)	8.3	3.7	1.5

[redacted] (2011)

B.6.12.2 Dermal absorption *in vivo*

	GF-2573
Study	IIIA1 7.6.1/01 *In vivo* percutaneous absorption of [14C]-XR-729 methyl, formulated as GF-2573, in rats
Reference	[redacted] (2011)
Date performed	August 2010 – July 2011
Test facility	[redacted]
Report reference	[redacted] study code V9029
Guideline(s)	OECD 427
Deviations from the guideline	None
GLP	Yes
Test material	GF-2573 containing 7.5 g acid equivalents/L XDE-729 Methyl with 10% of a.i. radiolabelled, batch E3026-87A, radiochemical purity 99.1% GF-2573 containing 7.5 g acid equivalents/L XDE-729 Methyl, with 100% of a.i. radiolabelled. [14C]-XDE-729 Methyl, labelled at UL-phenyl position, batch XS9-100040-28, purity 98.5%, specific activity 45.3 mCi.mmol^{-1} Non-radiolabelled XDE-729 Methyl, batch XW7-38246-49, purity 98.9%
Study acceptable	Yes

METHODS

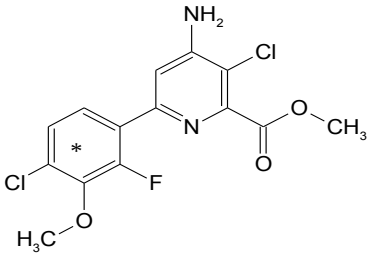
The radiolabelled test substance was tested at three target concentrations, representing the maximal concentration possible when handling the undiluted formulation and concentrations recommended for use in the field, in Wistar male rat skin according to the following design:

Table B.6.12.2-1 *In vivo* percutaneous absorption from GF-2573: study design

Test group	No. of rats/ sacrifice time	Nominal concentration of XDE-729 Methyl	Nominal dose of XDE-729 Methyl	Exposure duration	Sacrifice times
Concentrate	4	7.5 g a.e./L	75 µg a.e./cm^2	10 h	24, 48, 72 & 144 h
Field dilution 1	4	0.15 g a.e. g/L	1.5 µg a.e./cm^2	10 h	24, 48, 96 & 192 h
Field dilution 2	4	0.025 a.e. g/L	0.25 µg a.e./cm^2	10 h	24, 48, 96 & 192 h

a.e. = acid equivalents

The test substance was labelled on the phenyl ring, as indicated by an asterisk below.



Following administration (100 µL of test preparation to 10 cm^2 area of skin), the shaved application site was protected by an 'O'-ring enclosing the treated area and covered with a protective dressing. The animals were placed in metabolism cages designed specifically for the quantitative collection of urine and faeces.

After an exposure period of 10 h, the protective dressings were removed retained for analysis. The unabsorbed test substance was removed from the application site by washing with a mild lukewarm soap solution using cotton swabs. Urine and faeces were quantitatively collected at various intervals until termination. Blood samples were taken from the tail vein 4 h after application and at the end of the 10 h application period, and from the abdominal aorta at termination. At termination the application site was washed and skin stripped. The stripped skin was removed and retained for analysis. As control, a skin sample of a non-treated shaved area was also collected. The gastro-intestinal tract and residual carcass were retained for analysis.

The radioactivity in urine, faeces, cage washes, skin wash, skin tape strips, application site skin, non-treated skin, GI tract, residual carcass, blood collected at necropsy, dressings plus 'O' ring were measured by liquid scintillation counting and expressed % of total administered radioactivity.

Blood plasma samples were also analysed for XDE-729 Methyl and XDE-729 Acid, by HPLC and by LC-MS/MS

RESULTS

There were no treatment related deaths or clinical signs of toxicity.

The distribution of radioactivity is presented in Table B.6.12.2-1.

Mean total recoveries were high in all groups, ranging from 97.06% to 99.73%. In all groups, the majority of the administered radioactivity was recovered in the skin surface washing swabs.

Most of the systemic absorption of radioactivity occurred within the first 24 h hours of the study, and therefore it can be assumed that all of the radioactivity in the 20 skin tape strips will represent material that will not become bioavailable due to desquamation. Thus, the total amount absorbed can be calculated by summing the amounts of radioactivity in urine, faeces, cage wash, GI tract, carcass and stripped skin. Allowing for natural variation the total absorbed appeared to be essentially complete by 48 h and so an absorption value can be calculated as the mean of the 48 h, 72/96 h and 144/192 h values, which are 9.0%, 13.8% and 22.4% for the concentrate, field dilution 1 and field dilution 2, respectively.

Table B.6.12.2-1 Mean distribution of radioactivity expressed as % of administered dose

Sample	GF-2573 concentrate (7.5 g a.e.L^{-1})				GF-2573 field dilution 1 (0.15 g a.e.L^{-1})				GF-2573 field dilution 2 (0.025 g a.e.L^{-1})			
	Termination time				Termination time				Termination time			
	24 h	48 h	72 h	144 h	24 h	48 h	96 h	192 h	24 h	48 h	96 h	192 h
Absorbed radioactivity												
Urine (total)	5.30	5.72	5.80	7.88	7.31	8.01	11.38	10.46	12.58	19.16	13.77	17.17
Faeces (total)	1.45	1.68	1.65	2.81	2.66	2.91	4.06	3.19	3.43	5.72	3.91	5.06
Cage wash	0.38	0.08	0.04	0.13	0.40	0.13	0.16	0.03	0.32	0.23	0.14	0.09
Blood	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01	<0.02	<0.01
Control skin	<0.01	<0.01	<0.01	0.08	<0.01	0.01	0.05	<0.01	<0.01	<0.01	0.02	<0.03
GI-tract	0.41	0.08	0.02	0.02	0.15	0.06	0.02	0.02	0.33	<0.07	<0.09	<0.09
Carcass	0.22	0.12	0.15	0.42	0.14	0.24	0.27	0.07	<0.40	<0.38	<0.46	<0.43
Stripped skin	0.37	0.85	0.18	0.15	0.21	0.19	0.15	0.06	0.24	0.20	0.12	0.05
Total absorbed	8.15	8.55	7.86	11.49	10.89	11.56	16.10	13.85	17.33	25.78	18.53	22.93
Total absorbed: mean of 48, 72/96 & 144/192 h	**9.0%**				**13.8%**				**22.4%**			
Non-absorbed radioactivity												
Skin wash	85.21	84.68	87.59	84.12	80.69	79.39	72.24	79.05	74.55	69.46	76.16	67.62
Skin strips 1&2	0.45	0.35	0.59	0.88	1.87	2.96	6.45	3.52	3.69	1.13	2.25	6.45
Skin strips 3-20	1.26	0.98	0.82	0.23	2.88	2.66	1.68	1.04	2.46	2.44	1.48	1.54
'O' ring, dressings etc.	2.64	2.95	1.97	2.79	0.85	0.86	0.61	0.82	0.44	0.39	0.65	1.20
Total not absorbed	89.56	88.96	90.97	88.02	86.29	85.87	80.98	84.43	81.14	73.42	80.54	76.81
Recovery												
Total recovery	97.69	97.51	98.81	97.46	97.19	97.43	97.06	98.25	98.47	99.22	99.09	99.73

Analysis of individual plasma samples for XDE-729 Methyl and XDE-729 Acid by HPLC were unsuccessful due to the analysis system being insufficiently sensitive. Further analysis by LC-MS/MS of pooled 4 h and 10 h samples from the 7.5 g/L group found that XDE-729 Methyl was not present (limit of detection 1 ng/g) and that XDE-729 Acid was present at 47 and 22 ng/g, respectively.

CONCLUSION

The following *in vivo* dermal absorption values in the rat for XDE-729 Methyl from GF-2573 are determined:

- 9.0% for the concentrate (7.5 g/L)
- 13.8% for field dilution 1 (0.15 g/L)
- 22.4% for field dilution 2 (0.025 g/L)

XDE-729 Methyl was not present in the plasma at 4 and 10 h of animals receiving the highest dose level.

[redacted] (2011)

B.6.12.3 Summary of dermal absorption and calculation of dermal absorption values

Estimated human *in vivo* values can be calculated from the *in vitro* and *in vivo* dermal absorption studies as follows:

Table B.6.12.3-1. Dermal absorption values for XDE-729 Methyl from GF-2573

Parameter	Concentrate (7.5 g a.e./L)	Field dilution 2 (0.12-0.15 g a.e./L)	Field dilution 1 (0.02- 0.025 g a.e./L)
Rat *in vivo* % absorption	9.0	13.8	22.4
Ratio *in vitro* rat/human maximal flux	8.3	3.7	1.5
Estimated human *in vivo* % absorption value	**1%**	**4%**	**15%**

It is noted that the co-RMS (France) would prefer to use dermal absorption values that are based on the ratios of the *in vitro* rat/human % absorption ratios (these ratios are presented in Table B.6.12.1-4). Using the % absorption ratios, dermal absorption values for the concentrate, field dilution 2 and field dilution 1, respectively, of **2%**, **5%** and **14%** are calculated. However, the RMS (UK) considers that using dermal absorption values based on the % absorptions ratios, instead of maximum flux ratios, does not influence the outcome of the risk assessments for operators, workers and bystanders.

The safener cloquintocet-mexyl is a co-formulant in GF-2573. This is an existing safener and has previously been consider in the EU as part of the EU review of clodinafop (see Draft Assessment Report on clodinafop-propargyl prepared by the Netherlands, dated October 2003 and the EFSA Conclusion, finalised 10 August 2005). Appropriate dermal absorption values will need to be considered at Member State level when evaluating product applications.

B.6.13 Toxicological data on non active substances (IIIA 7.4)

The information provided on the non-active substances present in GF-2573 indicates no specific toxicological properties of concern.

B.6.14 Exposure data (IIIA 7.2)

B.6.14.1 Operator exposure (IIIA 7.2.1)

XDE-729 methyl is a new active substance developed by Dow AgroSciences. GF-2573, containing XDE-729 methyl is the representative formulation for the EU registration of XDE-729 methyl. GF-2573, containing 7.82 g/L of the active substance XDE-729 methyl is intended to be used on cereals as a post emergence herbicide.

Estimates of non-dietary exposure and associated summary calculations are presented below with full calculation sheets detailed in Appendix 2.

Details of 'GF - 2573'pertinent to operator, bystander and worker exposure are summarised in Table B.6.14.1-1 below.

Table B.6.14.1 -1 'GF-2573' - Critical uses and end points pertaining to operator, bystander and worker exposure

	'GF-2573'
Formulation type	Emulsifiable Concentrate (EC) containing 7.82 g/l XDE-729 methyl
Use	Herbicide for use on cereals targeting broadleaf weed species (outdoor)
Application method	Field crop boom sprayer
Max individual dose	1L / product/ ha Equivalent to 7.82 g / ai / ha XDE-729 methyl
Application volume	Field crop boom sprayer - 100 litres of spray solution/ha
Max spray concentration	Field crop boom sprayer - 0.0782 g/L XDE-729 methyl
Max total dose	2 applications (autumn & spring) equivalent to 15.64 g / ai / ha XDE-729 methyl
Latest time of application	BBCH 45
Packaging	PET & F-HDPE container – 0.2 L, 0.5 L, 1 L, 2 L, 3 L, 5 L, 10 L and 20 L
Classification	Xi 'Irritant' R38 'Irritating to skin' R43 'May cause sensitisation by skin contact'
Active substance	XDE-729 methyl
AOEL	0.058 mg/kg bw/day
Dermal absorption	1% for concentrate *15% in spray dilution

* Dermal absorption value derived from spray dilution of 0.02-0.25 g a.e/L as detailed in Table B.6.12.3-1.

The personal protective equipment (PPE) requirements arising from the classification of 'GF-2573' as 'R38 – Irritating to skin' and 'R43 – May cause sensitisation by skin contact' and H317 'May cause allergic skin reaction' are as follows.

- Operators must wear suitable protective clothing (coveralls) and suitable protective gloves when handling the concentrate**.**

Estimates of operator exposure have been conducted in line with the critical GAP(s) identified above in Table B.6.14.1 -1 and using the following models:

- UK POEM[3]
- German Model[4]

Operator exposure estimates for 'GF-2573' are summarised below and presented in full at the end of this section (Appendix 2)

Model: UK POEM

Scenario: Outdoor use on cereals
Application method: Field crop boom sprayer
Formulation type: Organic solvent based
Container: 10 L
Dose: 1 L/ha
Water volume: 100 L/ha
Work rate: 50 ha/day
Duration of spraying: 6 hours
Operator body weight: 60kg

Table B.6.14.1-2 Operator exposure to XDE-279 methyl resulting from the use of 'GF-2573' on cereals (UK POEM for field crop boom sprayers)

	Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure	
Active Substance	Mix/loading	Application	Mix/loading	Application	mg/kg bw/day	% of AOEL
	No PPE					
XDE-729 methyl	1.96	3.25	Negligible	0.0047	0.0085	15

A pack size of 10L has been assumed throughout as a realistic choice for treatment of 50 ha from the proposed range of packaging types.

The predicted operator exposure is calculated to be equivalent to 15% of the AOEL for XDE-729 methyl for an operator wearing no PPE.

The UK POEM estimates summarised above (Table B.6.14.1-2) indicate that the use of 'GF-2573' on cereals through tractor-mounted field crop boom sprayers will result in an acceptable level of systemic exposure to XDE-279 methyl for an operator wearing no PPE.

It is noted that the predicted levels of operator exposure will be further reduced due to the PPE requirements arising from the classification (R38 'Irritating to skin' & R43 'May cause sensitisation due to skin contact') of GF-2573. The classification of 'GF-2573' as R43 & R38 requires the use of coveralls and gloves when handling the concentrate.

[3] Estimation of Exposure and Absorption of Pesticides by Spray Operators, Scientific subcommittee on Pesticides and British Agrochemical association Joint Medical Panel Report (UK MAFF), 1986 and the Predictive Operator Exposure Model (POEM) V 1.0, (UK MAFF), 1992, 2007 version. ("UK POEM").

[4] Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection), Mitteilungen aus der Biologischen Bundesanstalt für Land-und Forstwirtschaft, Berlin-Dahlem, Heft 277, 1992. ('German Model').

Model: German Model

Scenario: Outdoor use on cereals
Application method: Field crop boom sprayer
Formulation type: Liquid
Dose: 1 L/ha
Work rate: 20 ha/day
Operator body weight: 70kg

Table B.6.14.1-3 Operator exposure to XDE-279 methyl resulting from the use of 'GF-2573' on cereals (German Model for field crop boom sprayers)

	Dermal exposure mg/person/day		Inhalation exposure mg/person/day		Total systemic exposure	
Active Substance	Mix/loading	Application	Mix/loading	Application	mg/kg bw/day	% of AOEL
	No PPE					
XDE-729 methyl	0.38	0.32	0.000094	0.00016	0.0519	1

The predicted operator exposure is calculated to be equivalent to 1% of the AOEL for XDE-729 methyl for an operator wearing no PPE.

The German model estimates summarised above (Table B.6.14.1-3) indicate that the use of 'GF-2573' on cereals through tractor-mounted field crop boom sprayers will result in an acceptable level of systemic exposure to XDE-279 methyl for an operator wearing no PPE.

There are no PPE requirements arising from the UK POEM or German model operator exposure risk assessments.

The personal protective equipment (PPE) requirements arising from the classification of 'GF-2573' (Xi Irritant, R38 'Irritating to skin', R43 'May cause sensitisation by skin contact' and H317 'May cause allergic skin reaction') are as follows:

- Operators must wear suitable protective clothing (coveralls), suitable protective gloves when handling the concentrate**.**

Final PPE Phrases

The operator protection phrases must read:

- **Operators must wear suitable protective clothing (coveralls) and suitable protective gloves when handling the concentrate.**

B.6.14.2 Bystander exposure (IIIA 7.2.2)

Bystander exposure to vapour

Table B.6.14.2-1 – Vapour pressure at 25°C (Pa) of Chlorpyrifos and XDE-729 methyl

Active substance	Vapour pressure at 25°C (Pa)
Chlorpyrifos	3.35×10^{-3}
XDE-729 methyl	8×10^{-5}

Bystander exposure to XDE-279 methyl resulting from the proposed use of 'GF-2573' is likely to result primarily from spray drift. However, the level of bystander exposure to XDE-279 methyl vapour following the use of 'GF-2573' can be estimated using a surrogate value for residues in air adjacent to treated crops, derived from Californian Environmental Protection Agency studies [5]. In these studies, a 24 ha orange orchard was treated with chlorpyrifos using broadcast air-assisted sprayers. During application, wind speeds ranged from 2 to 20 km/h and the maximum temperature was 42 °C. Chlorpyrifos residues in air adjacent to the orchard were monitored over 72 hours. The highest 24 hour time-weighted average residue in air was15 µg/m^3.

Based on these measurements and assuming:

- a body weight of 60 kg for an adult (based on the 50th percentile value for females aged 16 to 24 years in 1995-7 Health Surveys for England);
- a body weight of 15 kg for a small child (based on the average value for male and female children aged 2 and 3 years in 1995-7 Health Surveys for England);
- a respired volume of 15.2 m^3/day (based on mean values for the long term inhalation rate for adult males aged 19 to >65 years published in the United States Environmental Protection Agency (US EPA) Exposure Factors Handbook); and
- a respired volume of 8.3 m^3/day (based on mean values for the long term inhalation rate for children aged 3 to 5 years published in the US EPA Exposure Factors Handbook);

Potential exposure to vapour is estimated to be 0.0038 mg/kg bw/day for an adult and 0.0083 mg/kg bw/day for a child.

Table B.6.14.2-2 Bystander exposure to XDE-279 methyl vapour resultant from the use of 'GF-2573' on cereals.

Parameter/active	XDE-729 methyl
Adult systemic exposure	0.003800
% AOEL adult	6.55
Child systemic exposure	0.0083
% AOEL child	14.3

[5] California Environmental Protection Agency, Air Resources Board (1998). Report for the application and ambient air monitoring for chlorpyrifos (and the oxon analogue) in Tulare County during spring/summer 1996.

The predicted bystander exposure to vapour is calculated to be 7% of the AOEL for an adult and 14% of the AOEL for a child for XDE-729 methyl. The calculated bystander exposure to vapour values are within acceptable limits and no further assessment is required.

Bystander exposure to spray drift

Application method: Field crop boom sprayer

Bystander exposure through dermal and inhalation exposure to spray drift can be estimated on the basis of direct measurements of simulated bystander exposure for field crop sprayers in a UK study[6].

In this study, a single pass of the sprayer resulted in a mean potential dermal exposure (PDE) of 0.1 ml of spray solution on a bystander positioned 8 m downwind from the edge of the treatment area. Mean potential inhalation exposure (PIE) was 0.006 ml of spray solution.

Systemic exposure = (PDE x SC x DA) + (PIE x SC x 100%)/BW

Where:

PDE = potential dermal exposure (ml spray)
PIE = potential inhalation exposure (ml spray)
SC = concentration of active substance in spray
DA = percentage dermal absorption (%)
BW = bodyweight (60 kg)

Table B.6.14.2-3 Bystander exposure to XDE-279 methyl resultant from spray drift following the use of 'GF-2573' on cereals.

Parameter/active	XDE-729 methyl
MID (g a.s./ha)	7.82
Water volume (L/ha)	100
Dermal absorption (%)	15.0%
AOEL (mg/kg bw/d)	0.058
SC (mg/ml)	0.0782
PDE (ml/spray)	0.1
PIE (ml spray)	0.006
Body weight (kg)	60
Dermal route (mg/day)	0.001173
Inhalation route (mg/day)	0.0004692
Tot systemic mg/kg bw/day	2.74E-05
% AOEL	0.047

[6] Lloyd G.A. and Bell G.J. (1983). Hydraulic nozzles: comparative spray drift study (MAFF/ADAS).

The predicted bystander exposure to spray drift is calculated to be equivalent to <1% of the AOEL for XDE-729 methyl. The calculated bystander exposure to spray drift value is within acceptable limits and no further assessment is required.

Although these estimates are based on a situation in which acute exposure to the spray solution occurs, as the levels of systemic exposure are compared with AOELs considered appropriate for assessing the risks associated with repeated exposures for operators, this exposure assessment is also relevant to situations of repeated bystander exposure to spray drift (for example, the exposure of residents in property adjoining sprayed crops).

Bystander exposure to fallout (children's model).

It is also possible that spray drift fallout may be deposited in gardens adjacent to the treated area and that users of these gardens may become exposed through contact with deposits.

The following calculations predict the amount of XDE-729 methyl and cloquintocet-mexyl likely to be deposited in gardens next to the treated crop (due to fallout from spray drift) and the level of exposure likely to result when children playing in the garden are exposed through dermal, hand-to-mouth and object-to-mouth routes.

Estimates of fallout from spray drift are based on the following published data.

- Rautmann, D., Streloke, M. and Winkler, R. (2001). New basic drift values in the authorisation procedure for plant protection products. In Forster, R. and Streloke, M. Workshop on risk assessment and risk mitigation measures in the context of the authorisation of plant protection products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Heft 381.

Exposure estimates for children playing on contaminated turf are based on the following published data.

- USA EPA (1998). Occupational and residential exposure test guidelines: Group B, Post-application exposure monitoring test guidelines. Series 875 v 5.4.
- USA EPA (2001). Recommended revisions to the standard operating procedures (SOPs) for residential exposure assessment. Science Advisory Council for Exposure Policy, 12.
- USA EPA (1999). Overview of issues related to the standard operating procedures for residential exposure assessment. Presentation to the FIFRA Scientific Appraisal Panel.

<u>Spray drift fallout for field crop sprayers</u>

Allowing for an untreated headland of 1 m, the level of fallout from spray drift at the boundary with a neighbouring area is predicted to be equivalent to 2.77% of the applied dose. This level of fallout is predicted to decline to 0.57% at a distance of 5 m from the boundary. By integration, the average level of fallout over the whole area from the boundary to a point 3 m outside is estimated to be about 1%.

It is probable that children may play directly on areas of treated amenity turf and therefore the child bystander exposure assessment has been conducted using a drift fallout value of 100%. The selection of this value represents the risk envelope for neighbouring residential properties.

Children's dermal exposure

A child's systemic exposure resulting from dermal contact with a lawn contaminated by spray drift during the application of XDE-729 methyl calculated as follows.

SE(d) = (AR X DF X TTR x TC x H x DA) / BW

Where:
SE(d) = Systemic exposure via the dermal route
AR = total application rate of a.s. in µg/cm^2 (= 10x rate in kg a.s./ha)
DF = drift fallout value of 100% of the applied dose for boom sprayers
TTR = turf transferable residue value of 5% (EPA default value)
TC = transfer coefficient of 5200 cm^2/h (standard EPA value for this situation)
H = duration of exposure of 2 hours per day (standard EPA 75th percentile value)
DA = dermal absorption of the a.s. in the spray solution
BW = body weight of 15 kg

Children's hand-to-mouth exposure

Additional systemic exposure to XDE-729 methyl resulting from ingestion of turf residues transferred from contaminated hands to the mouth is calculated as follows.

SE(h) = (AR x DF x TTR x (SE/100) x SA x Freq x H) / BW

Where:
SE(h) = Systemic exposure via the hand-to-mouth route
AR = total application rate of a.s. in µg/cm^2 (= 10x rate in kg a.s./ha)
DF = drift fallout value of 100% of the applied dose for boom sprayers
TTR = turf transferable residue value of 5% (EPA default value for wet hands)
SE = saliva extraction factor of 50% (EPA default value)
SA = surface area of the hands in contact with the mouth (the value of 20 cm^2/event represents the palmar surface of three fingers)
Freq = frequency of hand to mouth events/hour (the value of 20 events/hour is the 90th percentile of observations ranging from 0 to 70 events/hour)
H = duration of exposure of 2 hours per day (standard EPA 75th percentile value)
BW = body weight of 15 kg

Children's object-to-mouth exposure

Additional systemic exposure to XDE-729 methyl resulting from direct ingestion of turf residues is calculated as follows.

SE(o) = (AR x DF x TTR x IgR)/BW

Where:
SE(o) = Systemic exposure via mouthing activity
AR = total application rate of a.s. in µg/cm^2 (= 10x rate in kg a.s./ha)
DF = drift fallout value of 100% of the applied dose for boom sprayers
TTR = turf transferable residue value of 20% (EPA default value for object-to-mouth assessments)
IgR = ingestion rate for mouthing of 25 cm^2 grass/day (EPA default value)
BW = body weight of 15 kg

Table B.6.14.2-4 Bystander exposure to XDE-279 methyl fallout (children's model) following the use of 'GF-2573' on cereals.

Parameter/Active	XDE-729 methyl
MTD (kg a.s./ha)	0.01564
Dabs (%)	15.0%
AOEL (mg/kg bw/d)	0.058
AR µg/cm^2	0.1564
DF %	1.0%
TTR % (d, HtM)	5%
TTR % (OtM)	20%
TC cm^2/hour	5200
SE%	50%
SA cm^2/event	20
Frequency events/hour	20
Hours	2
BW (kg)	15
IgR cm^2/day	25
SE(d) ug/kg bw/d	0.008
SE(h) ug/kg bw/d	0.002
SE(o) ug/kg bw/d	0.001
Tot mg/kg bw/day	1.07E-05
% AOEL	0.02

The predicted child bystander exposure to spray drift is calculated to be equivalent to <1% of the AOEL for XDE-729 methyl. This value is within acceptable limits and as such no further assessment is required for this route of exposure.

B.6.14.3 Worker exposure (IIIA 7.2.3)

Worker exposure estimates using the EUROPOEM II worker re-entry model[7] are presented below.

The potential dermal exposure (D) for an unprotected worker is estimated to be:

D = DFR x AR x TC x H

Where

DFR = Dislodgeable Foliar Residue (µg/cm^2)
AR = Application Rate (maximum total dose in kg a.s./ha)
TC = Transfer Coefficient (cm^2/person/h)
H = Duration of task (hours)

The proposed use is as an herbicide on wheat, barley, triticale, spelt and rye (outdoor) and as such the worker exposure scenario is restricted to re-entry crop inspection activities. The predicted worker exposure has been calculated using task duration of 2 hours and a transfer coefficient (TC) of 5000 cm^2/person/h for cereals post growth stage BBCH 31.

Table B.6.14.3-1Worker exposure to XDE-279 methyl following the use of 'GF-2573' on cereals.

Parameter / Active	XDE-729 methyl
MTD (kg a.s./ha)	0.016
Dermal absorption (%)	15.00%
AOEL (mg/kg bw/day)	0.058
DFR (µg/cm^2/kg a.s. applied)	3
TC (cm^2/person/h)	5000
T (hours)	2
Body weight (kg)	60
Dermal exposure (ug a.s./person/day)	469.2
Systemic exposure (mg/kg bw/day)	0.001173
% AOEL	2

The predicted exposure for a worker without personal protective equipment is calculated to be equivalent to 2% of the AOEL for XD-729 methyl. This exposure value is within acceptable limits and no further assessment is required.

[7] van Hemmen et al (2002). Post-application exposure of workers to pesticides in agriculture. Report of the re-entry working group, EUROPOEM II project: FAIR3-CT96-1406

B.6.14.4 Conclusions

Operator exposure assessments undertaken using the German model and UK POEM indicate that the proposed application of 'GF 2573' will result in an acceptable risk to operators (as detailed below in Table B.6.14.4-1)

Table B.6.14.4-1 Operator exposure to XDE-279 methyl resulting from the proposed use of 'GF-2573': summary of estimates indicating an acceptable risk

Proposed use	Application method	Model/data	Operator protection	% of AOEL
Cereals	Tractor-mounted field crop boom sprayer	German model	NO PPE	1%
		UK POEM	NO PPE	15%

On the basis of the above estimates and considering the hazard classification of the formulation, the risk to operators resulting from the proposed use of 'GF-2573' is considered to be acceptable subject to the following operator protection requirements.

- Operators must wear suitable protective gloves and coveralls when handling the concentrate.

Bystander and worker exposure assessments also indicate an acceptable level of risk, as surmised below in tables B.6.14.4-2 and B.6.14.4-3.

Table B.6.14.4-2 Bystander exposure to XDE-279 methyl resulting from the proposed use of 'GF 2573': summary of estimates indicating an acceptable risk for unprotected bystanders

Proposed use	Application method	Model/data	% of AOEL
Cereals	Tractor-mounted field crop boom sprayer	Vapour, Californian EPA surrogate study	7% adults 14% children
Cereals	Tractor-mounted field crop boom sprayer	Simulated bystander exposure measurements (Lloyd and Bell)	<1 %
Cereals	Tractor-mounted field crop boom sprayer	US EPA values for residential exposure	<1 %

Table B.6. 14.4-3 Worker exposure to XDE-279 methyl resulting from the proposed use of 'GF-2573': summary of estimates indicating an acceptable risk for unprotected workers undertaking crop inspection activities

Proposed use	Application method	Model/data	% of AOEL
Cereals	Tractor-mounted field crop boom sprayer	EUROPOEM II worker re-entry model	2%

Label amendments:

The personal protective equipment (PPE) requirements arising from the classification of 'GF-2573' (Xi Irritant, R38 'Irritating to skin', R43 'May cause sensitisation by skin contact') are as follows.

- Operators must wear suitable protective clothing (coveralls), suitable protective gloves when handling the concentrate**.**

B.6.15 References relied on

Active substance-By Annex Point

Annex Point/ Reference Number	**Author(s)**	**Year**	**Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not**	**Data Protection claimed (Y/N)**	**Owner**
KIIA 5.1.1/01	[redacted]	2009	XR-729: Probe Study to Determine Absorption, Distribution, Metabolism, and Elimination in F344/DUCRL Rats and CRL:CS1(ICR) Mice [redacted] DAS Report No.: 081108 (Accession Number) 2009646 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.1.1/02	[redacted]	2012	XDE-729: An Investigation of [14C]-Labeled XDE-729 Metabolism and Excretion Balance in Beagle Dogs Following A Single Oral (Gavage) Administration [redacted] DAS Report No.: 101055, [redacted]410030 (Accession Number) 2012844 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.1.2 /01	[redacted]	2010	XDE-729 and XDE-729 methyl: Pharmacokinetics and metabolism in F344/DuCrl Rats [redacted] DAS Report No.: 091098 (Accession Number) 2006542 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.1/01	[redacted]	2010a	XDE-729 Acid technical grade active ingredient: acute oral toxicity up and down procedure in rats [redacted] DAS Report No.: 090506, 28268 (Accession Number) 2004339 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.1/02	[redacted]	2011a	XDE-729 methyl: Acute Oral toxicity Study in F344/DUCRL Rats (Up and down Procedure) [redacted] DAS Report No.: 110543, 32454 (Accession Number) 2009534 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.2.2 /01	[redacted]	2009a	XDE-729 acid technical grade active ingredient: acute dermal toxicity study in rats [redacted] DAS Report No.: 090507, 28269 (Accession Number) 2003690 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.2 /02	[redacted]	2011b	XDE-729 methyl: Acute Dermal Toxicity Study in Rats - Limit Test [redacted] DAS Report No.: 102069, 31306 (Accession Number) 2007964 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.3/01	[redacted]	2011	XDE-729 and XDE-729 methyl: Acute Dust Aerosol Inhalation Toxicity Studies in F344/DUCRL Rats [redacted] DAS Report No.: 091096 (Accession Number) 2008264 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.4 /01	[redacted]	2009b	XDE-729 acid technical grade active ingredient: primary skin irritation study in rabbits [redacted] DAS Report No.: 090508, 28271 (Accession Number) 2003691 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.4 /02	[redacted]	2011c	XDE-729 methyl: Primary skin irritation in rabbits [redacted] DAS Report No.: 102070, 31308 (Accession Number) 2007965 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.2.5/01	[redacted]	2010b	XDE-729 Acid Technical Grade Active Ingredient: Primary Eye Irritation Study in Rabbits [redacted] DAS Report No.: 090509, 28270 (Accession Number) 2003977 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.5/02	[redacted]	2011d	XDE-729 Methyl Technical Grade Active Ingredient: Primary Eye Irritation Study in Rabbits [redacted] DAS Report No.: 102071, 31307 (Accession Number) 2008821 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.6/01	[redacted]	2011a	XDE-729: Local Lymph Node Assay in CBA/J Mice [redacted] DAS Report No.: 091092 (Accession Number) 2002106 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.2.6/02	[redacted]	2011b	XDE-729 methyl: Local Lymph Node Assay in CBA/J Mice [redacted] DAS Report No.: 101177 (Accession Number) 2008283 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.1/01	[redacted]	2009	XDE-729 methyl: 28-day Dietary Toxicity Study in F344 DUCRL Rats [redacted] DAS Report No.: 081115 (Accession Number) 2001102 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.3.1/02	[redacted]	2011	XR-729: 28-day Dietary Toxicity Study in F344 DUCRL Rats [redacted] DAS Report No.: 111005 (Accession Number) 2009533 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.1/03	[redacted]	2009	XR-729: 28-Day Dietary Toxicity Study in Crl:CD1(ICR) Mice [redacted] DAS Report No.: 081116 (Accession Number) 2001561 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.1/04	[redacted]	2010	XR-729: Palatability Probe and 28-Day Dietary Toxicity Study in Beagle Dogs [redacted]. DAS Report No.: 081127 (Accession Number) 2005396 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.2/01	[redacted]	2010	XDE-729: 90-Day Dietary Toxicity Study in F344/DUCRL Rats [redacted] DAS Report No.: 091016 (Accession Number) 2005530 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.2/02	[redacted]	2012	XDE-729 Methyl: 90-Day Dietary Toxicity Study in F344/DUCRL Rats [redacted] DAS Report No.: 111082 (Accession Number) 2012633 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.3.2/03	[redacted]	2010	XDE-729: 90-Day Dietary Toxicity Study in Crl:CD1(ICR) Mice [redacted] DAS Report No.: 091056 (Accession Number) 2005426 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.3/01	[redacted]	2011	XDE-729: 90-Day Dietary Toxicity Study in Beagle Dogs [redacted] DAS Report No.: 091070, 133-122 (Accession Number) 2008549 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.4/01	[redacted]	2012	XDE-729: A One-Year (Dietary) Toxicity Study in Beagle Dogs [redacted] DAS Report No.: 101163, 1797-006 (Accession Number) 2013693 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.3.7/01	[redacted]	2010	XDE-729: 28-Day Dermal Toxicity Study in F344/DuCrl Rats [redacted] DAS Report No.: 101031 (Accession Number) 2006365 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.1/01	Dakoulas, E. M. and VanDyke, M. R.	2010	XDE-729: Salmonella – Escherichia coli, Mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with XDE-729 BioReliance DAS Report No.: 091134, AD00LY.503001.BTL (Accession Number) 2004957 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.4.1/02	Dakoulas, E. M. and VanDyke, M. R.	2011	XDE-729 methyl: Salmonella – Escherichia coli, Mammalian-microsome reverse mutation assay preincubation method with a confirmatory assay with XDE-729 methyl BioReliance DAS Report No.: 111006 (Accession Number) 2008603 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.1/03	[redacted]	2012a	Bacterial Reverse Mutation Test of XDE-729 Methyl TGAI using *Salmonella typhimurium* [redacted] DAS Report No.: 120589, 481-1-06-4562 (Accession Number) 2013614 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.2/01	[redacted]	2010a	Evaluation of XDE-729 (4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-2-pyridinecarboxylic acid) in an in vitro chromosomal aberration assay utilizing rat lymphocytes [redacted] DAS Report No.: 091116 (Accession Number) 2006095 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.2/02	[redacted]	2012	Evaluation of XDE-729 Methyl in an in vitro Chromosomal Aberration Assay Utilizing Rat Lymphocytes [redacted] DAS Report No.: 101203 (Accession Number) 2011699 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

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KIIA 5.4.2/03	[redacted]	2012b	In vitro Mammalian Chromosome Aberration Test of XDE-729 Methyl TGAI in Human Peripheral Blood Lymphocytes [redacted] DAS Report No.: 120590, 488-1-06-4564 (Accession Number) 2013864 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.3/01	[redacted]	2010	Evaluation of XDE-729 in the Chinese Hamster Ovary Cell-Hypoxanthine-Guanine-phosphoribosyl transferase (CHO-HGPRT) Forward Mutation Assay [redacted] DAS Report No.: 091118 (Accession Number) 2006097 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.3/02	[redacted]	2011	Evaluation of XDE-729 Methyl in the Chinese Hamster Ovary Cell-Hypoxanthine-Guanine-phosphoribosyl transferase (CHO-HGPRT) Forward Mutation Assay [redacted] DAS Report No.: 101204 (Accession Number) 2009895 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.4.3/03	[redacted]	2012c	In vitro Mammalian Cell Gene Forward Mutation Test at the hgprt Locus of the Chinese Hamster Ovary (CHO)-K1 Cell Line using XDE-729 Methyl TGAI [redacted] DAS Report No.: 120591, 482-1-06-4563 (Accession Number) 2013865 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.4.4/01	[redacted]	2010b	Evaluation of XDE-729 (4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methoxyphenyl)-2-pyridinecarboxylic acid) in the mouse peripheral blood micronucleus assay. [redacted] DAS Report No.: 091117 (Accession Number) 2006159 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.1/01	[redacted]	2012	XDE-729: Two-year Chronic Toxicity/Oncogenicity Study in F344/DuCrl Rats [redacted], DAS Report No.: 091121 (Accession Number) 2013713 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.2/01 (See KIIA 5.5.1/01)	[redacted]	2012	XDE-729: Two-year Chronic Toxicity/Oncogenicity Study in F344/DuCrl Rats [redacted], DAS Report No.: 091121 (Accession Number) 2013713 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.5.3/01	[redacted]	2012	XDE-729: 18-month Dietary Oncogenicity Study in Crl:CD1(icr) Mice [redacted], DAS Report No.: 101021 (Accession Number) 2012991 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/01	[redacted]	2012	Hepatic Gene Expression and Biomarker Analyses in Male F344/DuCrl Rats Administered XDE-729 or XDE-729 Methyl for Seven Days [redacted] DAS Report No.: 110088 (Accession Number) 2012743 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/02	[redacted]	2012	XDE-729 Methyl: 7 Day Dietary Toxicity Probe Study in CRL:CD1 (ICD) Mice [redacted], DAS Report No.: 110177 (Accession Number) 2012634 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/03	[redacted]	2012	XDE-729 Methyl: Evaluation of Molecular and Cellular Changes in the livers of Male F344/DuCrl Rats After a Four Week Dietary Exposure and a Four Day or 28 Day Recovery Period [redacted] DAS Report No.: 120037 (Accession Number) 2013730 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

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KIIA 5.5.4/04	[redacted]	2011 (Amended 2012)	XDE-729 Methyl: Evaluation Of AhR Activation Potential Of XDE-729 Methyl Via Luciferase Reporter And Ligand Binding Assays [redacted], DAS Report No.: NS000063 (Accession Number) 2011767 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/05	[redacted].	2012	In Vitro Assessment of AhR (ARYL Hydrocarbon Receptor) Nuclear Receptor Activation and CYP 1A and Cyp 1a Induction Potential of XDE-729 Methyl in Primary Hepatocyte Cultures [redacted], DAS Report No.: SP0014001 (Accession Number) 2014153 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/06	[redacted]	2012 (revised 2013)	XDE-729 methyl: Determination of in vitro Hydrolysis Rates in Liver S9, Blood and Synthetic Gastric Fluid of Mouse, Rat, and Human and Physiologically-based Pharmacokinetic Simulations of Systemic Exposure in Rats and Humans [redacted] DAS Report No.: 110199 (Accession Number) 2013732 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.5.4/07	[redacted]	2012	XDE-729 Methyl: Mode of Action and Human Relevance Framework Analysis for XDE-729 Methyl-Induced Rodent Liver Effects [redacted], DAS Report No.: 120247 (Accession Number) 2014152 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.6.1/01	[redacted]	2010	XDE-729: Dietary Reproduction/Developmental Toxicity Probe Study in Crl:CD(SD) Rats [redacted] DAS Report No.: 091061 (Accession Number) 2006264 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.1/02	[redacted]	2011	XDE-729: Two Generation Dietary Reproductive Toxicity Study in CRL:CD (SD) Rats [redacted] DAS Report No.: 091148 (Accession Number) 2009408 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.10/01	[redacted]	2010	XDE-729: Dietary Developmental Toxicity Study in Crl:CD (SD) Rats [redacted] DAS Report No.: 091138 (Accession Number) 2006346 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.10/02	[redacted]	2012a	XDE-729 Methyl: Dietary Developmental Toxicity Probe Study in Crl:CD(SD) Rats [redacted] DAS Report No.: 111070 (Accession Number) 2011356 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.10/03	[redacted]	2012b	XDE-729 Methyl: Dietary Development Toxicity Study in Crl: CD(SD) Rats [redacted] DAS Report No.: 111071 (Accession Number) 2012173 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

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KIIA 5.6.11/01	[redacted]	2011a	XDE-729: Dietary Developmental Toxicity Probe Study in New Zealand White Rabbits [redacted] DAS Report No.: 091142 (Accession Number) 2007764 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.11/02	[redacted]	2011b	XDE-729: Dietary Developmental Toxicity Study in New Zealand White Rabbits [redacted] DAS Report No.: 091143 (Accession Number) 2007401 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.11/03	[redacted]	2012c	XDE-729 Methyl: Developmental Toxicity Probe Study in New Zealand White Rabbits [redacted] DAS Report No.: 111045 (Accession Number) 2012257 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.6.11/04	[redacted]	2012d	XDE-729 Methyl: Developmental Toxicity Study in New Zealand White Rabbits [redacted] DAS Report No.: 111137 (Accession Number) 2012628 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

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KIIA 5.7.1/01	[redacted]	2010	XDE-729: Acute Neurotoxicity in F344/DuCrl Rats [redacted] DAS Report No.: 101016 (Accession Number) 2006915 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.7.4/01	[redacted]	2011	XDE-729: 90-Day Dietary Neurotoxicity Study in F344/DuCrl Rats [redacted] DAS Report No.: 101006 (Accession Number) 2007765 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.8/01	[redacted]	2012d	Bacterial Reverse Mutation Test of X11449757 using Salmonella typhimurium [redacted] DAS Report No.: 120592, 481-1-06-4565 (Accession Number) 2013616 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.8/02	[redacted]	2012e	In vitro Mammalian Cell Gene Forward Mutation Test at the hgprt Locus of the Chinese Hamster Ovary (CHO)-K1 Cell Line using X11449757 [redacted] DAS Report No.: 120594, 482-1-06-4566 (Accession Number) 2013867 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIA 5.8/03	[redacted]	2012f	In vitro Mammalian Chromosome Aberration Test of X11449757 in Human Peripheral Blood Lymphocytes [redacted] DAS Report No.: 120593, 488-1-06-4567 (Accession Number) 2013866 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIA 5.10.1/01	[redacted]	2012	XDE-729 Methyl: Assessment of Immunotoxic Potential Using the Sheep Red Blood Cell Assay after 28-day Dietary exposure to Female f344/DuCrl Rats [redacted], DAS Report No.: 121004 (Accession Number) 2012621 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Plant Protection Product 'GF-2573'-By Annex Point

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 7.1.1/01	[redacted]	2011a	Acute Oral Toxicity Up and Down Procedure in Rats [redacted] DAS Report No.: 110305, 31820 (Accession Number) 2009078 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 7.1.2/01	[redacted]	2011b	Acute Dermal Toxicity Study in Rats [redacted] DAS Report No.: 110306, 31821 (Accession Number) 2009373 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 7.1.4/01	[redacted]	2011a	GF-2573: Primary Skin Irritation Study in Rabbits [redacted] DAS Report No.: 090537, 28348 (Accession Number) 2003902 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 7.1.5/01	[redacted]	2011b	GF-2573: Primary Eye Irritation Study in Rabbits [redacted] DAS Report No.: 090538, 28347 (Accession Number) 2003962 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 7.1.6/01	[redacted]	2011	GF-2573: Local Lymph Node Assay in CBA/J Mice [redacted] DAS Report No.: 101001, 101001 (Accession Number) 2005476 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Annex Point/ Reference Number	Author(s)	Year	Title Source (where different from the Company), Company, Report Number, GLP or GEP status (where relevant), Published or not	Data Protection claimed (Y/N)	Owner
KIIIA 7.6.1/01	[redacted]	2011	In vivo Percutaneous Absorption of (^{14}C) XR-729 Methyl, Formulated as GF-2573 in Rats [redacted] DAS Report No.: DR-0430-1422-020, V9029 (Accession Number) 2009680 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS
KIIIA 7.6.2/01	[redacted]	2011	In-vitro Percutaneous Absorption of [14C]-XR-729 Methyl, Formulated as GF-2573 Through Human and Rat Skin Membranes [redacted] DAS Report No.: GHE-T-1271, V8995 (Accession Number) 2009680 GLP/GEP (Y/N): Y Published (Y/N): N	Y	DAS

Appendix 1: Critical Uses – GAP table

Crop and/or situation (a)	Member State or Country	Product Name	F or G (b)	Pests or Group of pests controlled (c)	Formulation		Application			Application rate per treatment			PHI days (k)	Remarks (l)
					Type (d-f)	Conc. of a.s. (i) g/L	Method Kind (f-h)	Growth stage (j)	Number min max	kg as/hl min max	Water (l/ha) min max	kg as./ha min max		
Winter cereals (soft wheat, durum wheat, barley, spelt, rye, triticale)	EU	GF-2573	F	Broadleaf weeds	EC	XDE-729 methyl 7.817	Overall, Broadcast foliar spray	BBCH 10 - 29 and BBCH 13 - 45	1-2	0.00196-0.00782 followed by 0.00156 – 0.00625	100-400	0.00782 followed by 0.00625		The applications are made in autumn only (7.82 g/ha), or in spring only (6.25 g/ha) or in both autumn and spring (7.82 + 6.25 g/ha). Autumn applications are from BBCH 10 to 29. Spring applications are from BBCH 13 to 45.
Spring cereals (wheat, barley, durum wheat, rye)	EU	GF-2573	F	Broadleaf weeds	EC	XDE-729 methyl 7.817	Overall, Broadcast foliar spray	BBCH 13 to 45	1	0.00156 – 0.00625	100-400	0.00625		The application is made in spring only, from BBCH 13 to 45.

Remarks:
(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds
(d) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(h) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/l
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(l) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions

Appendix 2: Detailed exposure assessment sheets

THE UK PREDICTIVE OPERATOR EXPOSURE MODEL (POEM)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	**GF-2573**		Active substance	**XDE-729 methyl**	
Formulation type	organic solvent-based		a.s. concentration	**7.82**	mg/ml
Dermal absorption from product	**1**	%	Dermal absorption from spray	**15**	%
Container	10 litres 63 mm closure				
PPE during mix/loading	None		PPE during application	None	
Dose	**1**	l/ha	Work rate/day	**50**	ha
Application volume	**100**	l/ha	Duration of spraying	**6**	h

EXPOSURE DURING MIXING AND LOADING

Container size	10	litres
Hand contamination/operation	0.05	ml
Application dose	1	litres product/ha
Work rate	50	ha/day
Number of operations	5	/day
Hand contamination	0.25	ml/day
Protective clothing	None	
Transmission to skin	100	%
Dermal exposure to formulation	0.25	ml/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique	Tractor-mounted/trailed boom sprayer: hydraulic nozzles			
Application volume	100	spray/ha		
Volume of surface contamination	10	ml/h		
Distribution	Hands	Trunk	Legs	
	65%	10%	25%	
Clothing	None	Permeable	Permeable	
Penetration	100%	5%	15%	
Dermal exposure	6.5	0.05	0.375	ml/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	ml/day		

ABSORBED DERMAL DOSE

	Mix/load		Application	
Dermal exposure	0.25	ml/day	41.55	ml/day
Concen. of a.s. product or spray	7.82	mg/ml	0.0782	mg/ml
Dermal exposure to a.s.	1.955	mg/day	3.24921	mg/day
Percent absorbed	1	%	15	%
Absorbed dose	0.01955	mg/day	0.4873815	mg/day

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure	0.01	ml/h
Duration of exposure	6	h
Concentration of a.s. in spray	0.0782	mg/ml
Inhalation exposure to a.s.	0.004692	mg/day
Percent absorbed	100	%
Absorbed dose	0.004692	mg/day

PREDICTED EXPOSURE

Total absorbed dose	0.5116235	mg/day
Operator body weight	60	kg
Operator exposure	0.008527058	mg/kg bw/day
AOEL	0.058	mg/kg bw/day
% AOEL	14.70182471	

THE GERMAN MODEL (GEOMETRIC MEAN VALUES)

Application method	Tractor-mounted/trailed boom sprayer: hydraulic nozzles				
Product	GF-2573'		Active substance	XDE-729 methyl	
Formulation type	Liquid		a.s. concentration	7.82	g/l
Dermal absorption from product	1	%	Dermal absorption from spray	15	%
RPE during mix/loading	None		RPE during application	None	
PPE during mix/loading	None				
PPE during application: Head	None	Hands	None	Body	None
Dose	1	l product/ha	Work rate/day	20	ha

DERMAL EXPOSURE DURING MIXING AND LOADING

Hand contamination/kg a.s.	2.4	mg/kg a.s.
Hand contamination/day	0.37536	mg/day
Protective clothing	none	
Transmission to skin	100	%
Dermal exposure to a.s.	0.37536	mg/day

INHALATION EXPOSURE DURING MIXING AND LOADING

Inhalation exposure/kg a.s.	0.0006	mg/kg a.s.
Inhalation exposure/day	0.00009384	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.00009384	mg/day

DERMAL EXPOSURE DURING SPRAY APPLICATION

Application technique: Tractor-mounted/trailed boom sprayer: hydraulic nozzles

	Head	Hands	Rest of body	
Dermal contamination/kg a.s.	0.06	0.38	1.6	
Dermal contamination/day	0.009384	0.059432	0.25024	
Protective clothing	none	none	none	
Transmission to skin	100	100	100	%
Total dermal exposure to a.s.	0.319056	mg/day		

INHALATION EXPOSURE DURING SPRAYING

Inhalation exposure/kg a.s.	0.001	mg/kg a.s.
Inhalation exposure/day	0.0001564	mg/day
RPE	none	
Transmission through RPE	100	%
Inhalation exposure to a.s.	0.0001564	mg/day

ABSORBED DOSE

	Mix/load		Application	
Dermal exposure to a.s.	0.37536	mg/day	0.319056	mg/day
Percent absorbed	1	%	15	%
Absorbed dose (dermal route)	0.0037536	mg/day	0.0478584	mg/day
Inhalation exposure to a.s.	0.00009384	mg/day	0.0001564	mg/day
Total systemic exposure	0.00384744	mg/day	0.0480148	mg/day

PREDICTED EXPOSURE

Total systemic exposure	0.05186224	mg/day
Operator body weight	70	kg
Operator exposure	0.000740889	mg/kg bw/day
AOEL	0.058	mg/kg bw/day
% AOEL	1.277395074	