Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **assimilated Regulation No 1107/2009**
as it applies in Great Britain

Inpyrfluxam

Volume 3 – B.6 (AS)

Toxicology & Metabolism Data

Great Britain

March 2026

Version History

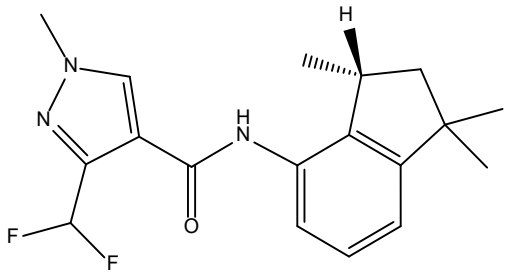
When	What
November 2025	Initial DAR
March 2026	Updates made after ECP

Contents

B.6. Toxicology and Metabolism Data

Inpyrfluxam (3- (Difluoromethyl) -1-methyl – N - [(3R) -1, 1, 3 – trimethyl - 2, 3 – dihydro - 1H-inden-4-yl]-1H-pyrazole-4-carboxamide), also known as S-2399, CAS 1352994-67-2) is a new fungicidal active substance, developed by Sumitomo Chemical Agro Europe S.A.S. It is intended to control various foliar diseases from *Puccinia* sp. on winter and spring cereals (wheat and barley).

The structure of inpyrfluxam is presented below:



Inpyrfluxam belongs to the succinate dehydrogenase inhibitor (SDHI) family of fungicides. Its mode of action is by inhibiting the activity of the enzyme succinate dehydrogenase (SDH), which is a key component of both the Krebs cycle (tricarboxylic acid cycle) and the mitochondrial electron transport chain (Complex II). Inhibition of SDH, specifically disrupts the Krebs cycle preventing normal cellular respiration and energy (ATP) production, and the mitochondrial electron transport by leading to energy deficiency and eventually cell death in the fungal pathogen.

The representative product for inpyrfluxam is S-2399 60 g/L EC which is an emulsifiable concentrate containing 60 g/L of inpyrfluxam.

S-2399 60 g/L EC is intended to be used as a single application on wheat (winter and spring) for the control of *Puccinia recondita* and *Puccinia striiformis* and on barley (winter and spring) for the control of *Puccinia hordei*.

This document uses the term ‘inpyrfluxam’ when referring to the active substance. However, the development code S-2399 has been used by the applicant within the individual study reports. The batches of inpyrfluxam used in the toxicology studies are considered representative of the technical specification (see Vol 4 for more details). All of the methods of analysis for the active substance in different matrices (diet, air, gavage solutions) used in the *in vivo* toxicological studies are either validated or fit for regulatory purposes (see document CA B5 and the individual studies within this B6 document for further details).

The classification of inpyrfluxam for Human Health effects has been addressed in an aligned MCL (Mandatory Classification and Labelling) dossier produced by HSE.

The data requirements of assimilated Regulation (EC) 1107/2009 and Regulation (EU) 283/2013 have been met and HSE concludes that there are no data gaps.

B.6.1. Absorption, Distribution, Metabolism and Excretion in mammals

The absorption, distribution, metabolism, and elimination (ADME) and plasma kinetics of inpyrfluxam were investigated by the oral route in two studies in rats, the first following single dosing and the second following repeated dosing for 14 days. Two in vitro metabolism studies are also available to assess the comparability of metabolism of inpyrfluxam across species.

[Pyrazolyl-4-^{14}C]- labelled inpyrfluxam and [Phenyl-^{14}C] - labelled inpyrfluxam were used in some, but not all the experiments. The radiolabel positions in the two moieties (denoted by *) are shown in figures 6.1-1 and 6.1-2 below.

Figure 6.1-1: Location of radiolabel in [pyrazolyl-4-^{14}C] labelled inpyrfluxam molecules.

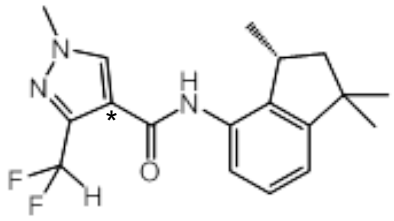
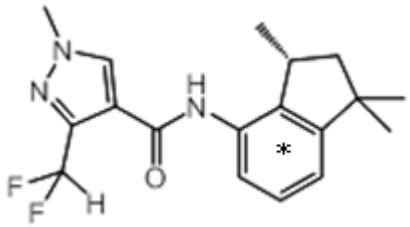


Figure 6.1-2: Location of radiolabel in [phenyl-^{14}C] labelled inpyrfluxam molecules.



B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

To meet the data requirements for ADME by the oral route, the applicant has submitted two in vivo studies in Wistar Hannover rats, which are GLP compliant and performed against the most recent 2010 OECD 417 test guideline.

1. Metabolism of inpyrfluxam in rats – single exposure

Reference:	KCA 5.1.1/01
Report Title:	Metabolism of S-2399 in rats

Author(s) & Year:	[redacted] (2016a)
Document No, Authority registration No	Study No. TPM-0026 [redacted]
Substance used:	Test Material radiolabelled: [pyrazolyl-4-^{14}C] S-2399 ([pyrazolyl-4-^{14}C] inpyrfluxam) Lot/Batch: CFQ41802 Purity: ≥98.4% Test Material radiolabelled: [phenyl-^{14}C] S-2399 ([phenyl-^{14}C] inpyrfluxam) Lot/Batch: CFQ41473 Purity: ≥99.3% Test Material unlabelled: S-2399 A.S (inpyrfluxam) Lot/Batch: YT3424G Purity: 99.9%; optical isomer ratio: 100.0%
Method of analysis:	Validated method of analysis not required
Guideline(s):	OECD TG 417, (2010)
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Method

The ADME of [pyrazolyl-4-^{14}C] or [phenyl-^{14}C] radiolabelled inpyrfluxam were investigated in Wistar Hannover rats.

Groups of male and female rats were dosed by oral gavage once with 1 or 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam in 0.5% methylcellulose or with 1 mg/kg bw of [phenyl-^{14}C] inpyrfluxam in 0.5% methylcellulose. The study design, including how many animals were used for each experiment and the investigations performed is described in the table below. In experiment D, bile-cannulated rats were used; these were given 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam. Rats were sacrificed 7-days post dosing. Total radioactivity was determined in plasma, urine, faeces and from the cage wash during the course of the study and in organ and tissue samples (including blood and plasma) at sacrifice.

Table 6.1.1-1: Experimental design for ADME investigation of [14C]-labelled inpyrfluxam

Group	Labelled position	Study type	Dose (mg/kg)	Number of animals	
				Male	Female
A	Pyrazolyl	^{14}C-Excretion ^{14}C-Tissue residue Metabolite profiling	1	4	4
B	Pyrazolyl	^{14}C-concentration in blood and plasma	1	4	4
C	Pyrazolyl	^{14}C-Distribution Metabolite profiling	1	12	12
D (bile duct-cannulated)	Pyrazolyl	^{14}C-Excretion Metabolite profiling	1	4	4
E	Pyrazolyl	^{14}C-Excretion ^{14}C-Tissue residue Metabolite profiling	150	4	4
F	Pyrazolyl	^{14}C-concentration in blood and plasma	150	4	4
G	Pyrazolyl	^{14}C-Distribution Metabolite profiling	150	12	12
H	Phenyl	^{14}C-Excretion Metabolite profiling	1	4	4

Animals were observed daily for mortality and clinical signs of toxicity until sacrifice. Groups A, C, D, E and G were dissected at termination, and any abnormality in organs and tissues were recorded.

Dose levels were selected based on the one-month oral toxicity study in rats ([redacted] T, 2015) where 500 ppm (31.7 mg/kg bw/day) resulted in no toxicity and the 90-day study in rats ([redacted] R, 2016) where toxicity was noted at 2000 ppm (123 mg/kg bw/day).

Results

A single female administered ([pyrazolyl-4-^{14}C] inpyrfluxam at 150 mg/kg bw (from group E) was found dead after 6h from dosing. This was considered incidental as no mortality, or severe clinical signs of toxicity were noted in any other group given 150 mg/kg bw inpyrfluxam.

No clinical signs of toxicity were noted in any of the groups.

Recovery and excretion

The total recovery of radioactivity was >90% at 2-3 days post dose for both radiolabels in males and females, with the majority of the dose (>67%) recovered within the first 24 hours. This is in accordance with the acceptability criteria of the test guideline (>90% recovery after 7 days).

For full details of the recovery of radioactivity, please refer to Table 6.1.1-2.

At the low dose, with either label position, complete excretion was noted within 7 days (for ([phenyl-^{14}C] inpyrfluxam and [pyrazolyl-4-^{14}C] inpyrfluxam- 97.2% of administered dose (AD) and 101.9% AD in males respectively, and 97.9% AD and 101.7% AD in females respectively). Excretion of radioactivity for the phenyl and pyrazolyl labels in urine were 49.2% AD and 59.7% AD respectively in males, and 58.5% AD and 61.0% AD respectively in females; excretion in faeces was 47.9% AD and 42.2% AD respectively in males, and 39.4 AD and 40.7% AD respectively in females. There was no excretion via expired air.

At the high dose of [pyrazolyl-4-^{14}C] inpyrfluxam, total excretion of radioactivity within 7 days of dosing was 98.9% AD (urine: 49.5% AD; faeces: 49.3% AD) in males, and 96.9% AD (urine: 53.3% AD; faeces: 43.6% AD) in females. No notable sex-, dose-, or radiolabel-related differences were noted in excretion.

Table 6.1.1-2: Cumulative excretion of radioactivity in male and female rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Fraction	% of dosed ^{14}C						
	Time after administration						
	0-6 h	0-12 h	0-1 day	0-2 days	0-3 days	0-5 days	0-7 days
Male rats							
Urine	29.5 ± 8.39	43.3 ± 9.24	56.4 ± 5.64	59.2 ± 5.40	59.5 ± 5.41	59.6 ± 5.40	59.7 ± 5.39
Faeces	--	--	15.9 ± 9.01	38.1 ± 5.14	41.2 ± 4.70	42.1 ± 4.52	42.2 ± 4.51
Expired air*	--	--	<0.0	<0.0	<0.0	<0.0	<0.0
Total	**29.5 ± 8.39**	**43.3 ± 9.24**	**72.3 ± 7.31**	**97.3 ± 0.57**	**100.7 ± 1.08**	**101.7 ± 1.23**	**101.9 ± 1.23**
Female rats							
Urine	23.6 ± 15.16	41.3 ± 17.17	54.6 ± 17.31	59.7 ± 13.95	60.6 ± 12.81	60.9 ± 12.43	61.0 ± 12.32
Faeces	--	--	12.9 ± 11.38	36.0 ± 7.25	39.7 ± 9.49	40.5 ± 10.37	40.7 ± 10.68
Expired air*	--	--	<0.0	<0.0	<0.0	<0.0	<0.0
Total	**23.6 ± 15.16**	**41.3 ± 17.17**	**67.6 ± 17.50**	**95.6 ± 8.65**	**100.3 ± 4.61**	**101.4 ± 3.59**	**101.7 ± 3.33**

*: expired air was determined until 1 day after administration; --: not analysed

Table 6.1.1-3: Cumulative excretion of radioactivity in male and female rats after a single oral dose at 1 mg/kg bw of [phenyl-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Fraction	% of dosed ^{14}C						
	Time after administration						
	0-6 h	0-12 h	0-1 day	0-2 days	0-3 days	0-5 days	0-7 days
Male rats							
Urine	26.3 ± 12.11	38.3 ± 11.56	46.3 ± 9.73	48.7 ± 8.15	49.1 ± 8.03	49.2 ± 8.02	49.2 ± 8.03
Faeces	--	--	28.8 ± 2.00	44.9 ± 6.45	47.1 ± 7.77	47.8 ± 8.03	47.9 ± 8.07
Total	**26.3 ± 12.11**	**38.3 ± 11.56**	**75.1 ± 11.66**	**93.6 ± 1.74**	**96.2 ± 0.28**	**97.0 ± 0.07**	**97.2 ± 0.13**
Female rats							
Urine	33.7 ± 12.10	45.7 ± 11.80	55.1 ± 10.71	57.9 ± 8.77	58.4 ± 8.25	58.4 ± 8.17	58.5 ± 8.18
Faeces	--	--	24.1 ± 7.91	37.4 ± 6.98	39.0 ± 7.86	39.3 ± 8.14	39.4 ± 8.15
Total	**33.7 ± 12.10**	**45.7 ± 11.80**	**79.2 ± 9.65**	**95.3 ± 2.74**	**97.3 ± 1.15**	**97.8 ± 1.05**	**97.9 ± 0.99**

--: not analysed

Table 6.1.1-4: Cumulative excretion of radioactivity in male and female rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Fraction	% of dosed ^{14}C						
	Time after administration						
	0-6 h	0-12 h	0-1 day	0-2 days	0-3 days	0-5 days	0-7 days
Male rats							
Urine	4.6 ± 3.56	10.2 ± 6.23	30.9 ± 13.60	44.7 ± 12.36	47.8 ± 10.79	49.2 ± 9.99	49.5 ± 9.93
Faeces	--	--	8.0 ± 3.41	31.3 ± 5.01	43.2 ± 8.52	48.7 ± 10.48	49.3 ± 10.70
Total	**4.6 ± 3.56**	**10.2 ± 6.23**	**38.9 ± 13.77**	**76.0 ± 12.03**	**91.0 ± 8.58**	**97.9 ± 7.27**	**98.9 ± 7.17**
Female rats							
Urine	2.9 ± 0.98	6.8 ± 2.62	20.0 ± 7.78	44.5 ± 11.82	52.0 ± 8.31	53.1 ± 7.82	53.3 ± 7.80
Faeces	--	--	2.5 ± 3.36	17.1 ± 0.96	37.1 ± 8.97	43.2 ± 8.03	43.6 ± 8.30
Total	**2.9 ± 0.98**	**6.8 ± 2.62**	**22.5 ± 9.06**	**61.6 ± 12.28**	**89.1 ± 4.23**	**96.3 ± 0.76**	**96.9 ± 1.05**

--: not analysed

Absorption

In the bile-duct experiment (low dose of the pyrazolyl label), total excretion of radioactivity within 3 days was 98.3% AD in males (urine: 26.7% AD; bile: 68.9% AD; faeces: 2.7%AD)

and 98.1% AD in females (urine: 48.4% AD; bile: 46.9% AD; faeces: 2.8% AD). The radioactivity excreted in urine in these bile-duct animals was significantly lower than in the intact animals and the amount excreted in bile was much higher than that found in faeces in intact animals. This indicates that entero-hepatic re-circulation occurs with this substance. The percentage of oral absorption of inpyrfluxam at the low dose of 1 mg/kg bw (sum of radioactivity in urine, bile and carcass) was determined to be 96% AD in both sexes. Overall, an oral absorption value of 100% is considered appropriate at the low dose of 1 mg/kg bw.

Table 6.1.1-5: Cumulative excretion of radioactivity in bile duct-cannulated male and female rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-14C] inpyrfluxam (mean ± SD of 4 rats)

Sex	Fraction	% of dosed ^{14}C					
		Time after administration					
		0-6 h	0-12 h	0-1 day	0-2 days	0-3 days*	Total
Male	Urine	13.7 ± 8.73	23.5 ± 11.58	26.5 ± 10.85	26.7 ± 10.82	22.9 ± 9.41	26.7 ± 10.78
	Bile	33.7 ± 12.80	61.6 ± 13.34	68.6 ± 11.95	68.9 ± 11.93	73.6 ± 9.00	68.9 ± 11.96
	Faeces	--	--	1.6 ± 1.14	2.6 ± 0.43	2.6 ± 0.45	2.7 ± 0.40
	Carcass	--	--	--	--	--	0.2 ± 0.12
	GIT contents	--	--	--	--	--	0.0 ± 0.03
	Total	47.3 ± 11.27	85.1 ± 12.15	96.7 ± 2.21	98.2 ± 1.86	99.2 ± 1.05	**98.5 ± 1.77**
Female	Urine	29.5 ± 28.56	44.0 ± 24.98	47.8 ± 24.76	48.3 ± 24.64	48.4 ± 24.60	48.4 ± 24.60
	Bile	36.5 ± 26.55	45.6 ± 23.29	46.6 ± 23.31	46.8 ± 23.37	46.9 ± 23.45	46.9 ± 23.45
	Faeces	--	--	1.5 ± 0.91	2.6 ± 0.59	2.8 ± 0.64	2.8 ± 0.64
	Carcass	--	--	--	--	--	0.4 ± 0.28
	GIT contents	--	--	--	--	--	0.0 ± 0.01
	Total	66.0 ± 31.33	89.5 ± 6.43	95.9 ± 1.58	97.7 ± 2.04	98.1 ± 2.01	**98.5 ± 1.75**

*: Mean and SD of three male rats; GIT: gastrointestinal tract: --: not analysed

Radioactive levels in blood and plasma

At the low dose of the pyrazolyl label, maximal plasma concentrations (C_{max}) of radioactivity were recorded at 1 h after administration. There was a rapid decrease in the plasma levels of radioactivity after 1 h, with half-lives ($t_{1/2}$) and area under the curve (AUC) of 13 h and 1.77 µg equivalents/g·h respectively in males, and 12 h and 1.63 µg equivalents/g·h respectively in females.

At the high dose of the pyrazolyl label, maximal plasma concentrations (C_{max}) of radioactivity were recorded at 8 h after administration. There was rapid decrease in the plasma levels of

radioactivity after 8 h, with half-lives ($t_{1/2}$) and area under the curve (AUC) of 14 h and 270 µg equivalents/g·h respectively in males, and 17 h and 382 µg equivalents/g·h respectively in females.

Mean blood/plasma radioactivity ratios of males and females were between 0.5-2 at different time points at both dose levels. Due to the large variation, no clear conclusion can be drawn about greater affinity of radioactivity for the erythrocytes. There was no remarkable sex-related difference in the concentrations of radioactivity in blood or plasma, and the ratio of AUC at the high and low dose was proportional to the dose ratio.

Table 6.1.1-6: ^{14}C-Concentrations and pharmacokinetic parameters of ^{14}C in plasma after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-14C] inpyrfluxam (mean ± SD of 4 rats)

Time after administration [h]	^{14}C-Concentration [µg eq. of inpyrfluxam/g]					
	Male			Female		
	Blood	Plasma	Blood/Plasma ratio	Blood	Plasma	Blood/Plasma ratio
0.25	0.057 ± 0.0498	0.062 ± 0.0554	0.9	0.104 ± 0.0844	0.052 ± 0.0304	2.0
0.5	0.148 ± 0.1162	0.118 ± 0.1094	1.2	0.114 ± 0.0558	0.114 ± 0.0510	1.0
1	0.105 ± 0.0270	0.161 ± 0.1243	0.7	0.079 ± 0.0147	0.144 ± 0.0533	0.5
2	0.034 ± 0.0111	0.120 ± 0.0414	0.3	0.013 ± 0.0031	0.096 ± 0.0221	0.1
4	0.046 ± 0.0268	0.111 ± 0.0272	0.4	0.092 ± 0.0422	0.093 ± 0.0332	1.0
8	0.119 ± 0.0565	0.083 ± 0.0129	1.4	0.080 ± 0.0152	0.065 ± 0.0145	1.2
12	0.080 ± 0.0288	0.034 ± 0.0118	2.4	0.058 ± 0.0088	0.040 ± 0.0116	1.4
24	0.041 ± 0.0149	0.013 ± 0.0018	3.2	0.014 ± 0.0040	0.015 ± 0.0043	0.9
48	0.007 ± 0.0011	0.007 ± 0.0015	1.0	0.005 ± 0.0020	0.006 ± 0.0022	1.0
72	0.003 ± 0.0006	0.003 ± 0.0005	1.2	<0.002	0.002 ± 0.0001	-
120	<0.002	<0.002	-	<0.002	<0.001	-
168	<0.002	<0.001	-	<0.002	<0.001	-
T_{max} [h]	1			1		
C_{max} [µg eq. of S-2399/g]	0.161			0.144		
$t_{1/2}$ [h]	13			12		
AUC [µg eq. of S-2399.h/g]	1.77			1.63		

Pharmacokinetic parameters were calculated from the mean values of ^{14}C-concentration in plasma

Table 6.1.1-7: ^{14}C-Concentrations and pharmacokinetic parameters of ^{14}C in plasma after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-14C] inpyrfluxam (mean ± SD of 4 rats)

Time after administration [h]	^{14}C-Concentration [µg eq. of inpyrfluxam/g]					
	Male			Female		
	Blood	Plasma	Blood/Plasma ratio	Blood	Plasma	Blood/Plasma ratio
0.25	1.1 ± 0.47	1.2 ± 0.52	0.9	3.3 ± 1.60	4.4 ± 2.57	0.8
0.5	2.9 ± 1.25	3.2 ± 1.47	0.9	3.5 ± 1.29	4.6 ± 2.10	0.8
1	4.6 ± 1.49	4.9 ± 1.73	1.0	4.1 ± 1.48	4.8 ± 1.83	0.9
2	6.4 ± 2.21	6.2 ± 2.25	1.0	4.2 ± 1.51	4.9 ± 1.89	0.8
4	6.2 ± 1.97	6.2 ± 1.85	1.0	3.8 ± 1.50	4.7 ± 1.60	0.8
8	8.0 ± 3.95	8.0 ± 3.98	1.0	4.5 ± 1.10	5.4 ± 1.26	0.8
12	6.9 ± 0.77	6.8 ± 0.68	1.0	5.0 ± 1.23	5.8 ± 1.52	0.9
24	5.6 ± 2.06	6.0 ± 2.13	0.9	5.8 ± 2.21	7.2 ± 2.65	0.8
48	1.3 ± 0.49	1.2 ± 0.54	1.1	4.0 ± 2.55	4.8 ± 2.78	0.8
72	0.6 ± 0.09	0.4 ± 0.15	1.4	1.0 ± 0.51	1.0 ± 0.33	1.0
120	<0.3	<0.2	-	<0.3	<0.2	-
168	<0.3	<0.1	-	<0.3	<0.2	-
T_{max} [h]	8			24		
C_{max} [µg eq. of S-2399/g]	8.0			7.2		
$t_{1/2}$ [h]	14			17		
AUC [µg eq. of S-2399.h/g]	270			382		

Pharmacokinetic parameters were calculated from the mean values of ^{14}C-concentration in plasma

Distribution

At the low dose, there were rapid increases in radioactivity concentrations in all organs and tissues of both male and female rats, reaching the maximal concentrations (C_{max}) at 0.25 to 1 h after dosing, except for the gastrointestinal tract or its content. After reaching the $C_{max,,}$ concentrations declined with time. Relatively high levels of radioactivity were recorded in the liver, kidney, adrenal and heart. The mean elimination half-lives were 2 to 8 h after dosing, in examined tissues, except for the gastrointestinal tract or its content. At 168 h after administration, the total radioactive residue in the carcass was 0.2% AD in males and 0.1% AD in females.

At the high dose, radioactivity concentrations attained the C_{max} at 1 to 8 h after dosing, with relatively high levels in the liver, kidney and adrenal. The mean elimination half-lives in organs and tissues were 7 to 48 h after dosing, except for the gastrointestinal tract or content. At 168 h after administration, the total radioactivity residue in the carcass was 0.1% AD in males and 0.2% AD in females.

No remarkable sex-related difference was observed in the tissue C_{max} or mean elimination $t_{1/2}$ values at either dose, although the C_{max} and half-lives were longer at the high dose.

Table 6.1.1-8: Concentration of radioactivity in tissues in male rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Tissue	µg eq. of inpyrfluxam/g tissue [ppm] *% of dosed ^{14}C*			
	Time after administration			
	0.25 h	1 h	8 h	168 h
Adrenal	0.654 ± 0.2015 *0.0 ± 0.00*	0.769 ± 0.1338 *0.0 ± 0.01*	0.167 ± 0.0441 *0.0 ± 0.00*	<0.005 *<0.0001*
Blood	0.174 ± 0.0586 *0.8 ± 0.25*	0.226 ± 0.1094 *1.0 ± 0.47*	0.058 ± 0.0083 *0.3 ± 0.04*	<0.002 *<0.0101*
Blood cell	0.143 ± 0.0553	0.182 ± 0.1129	0.038 ± 0.0069	0.005 ± 0.0007
Plasma	0.190 ± 0.0662	0.260 ± 0.1070	0.072 ± 0.0099	<0.002
Bone	0.027 ± 0.0105	0.048 ± 0.0017	0.014 ± 0.0047	0.006 ± 0.0070
Bone marrow	0.131 ± 0.0480	0.179 ± 0.0407	0.043 ± 0.0078	<0.001
Brain	0.113 ± 0.0436 *0.1 ± 0.03*	0.109 ± 0.0107 *0.1 ± 0.01*	0.017 ± 0.0073 *0.0 ± 0.00*	<0.002 *<0.0013*
Caecum	0.096 ± 0.0400 *0.0 ± 0.02*	0.274 ± 0.1266 *0.1 ± 0.04*	2.109 ± 1.0326 *0.8 ± 0.46*	<0.009 *<0.0025*
Carcass	- *6.7 ± 2.1*	- *12.5 ± 2.47*	- *3.6 ± 0.76*	- *0.2 ± 0.05*
Eye	0.049 ± 0.0179 *0.0 ± 0.00*	0.072 ± 0.0134 *0.0 ± 0.00*	0.020 ± 0.0066 *0.0 ± 0.00*	<0.002 *<0.0001*
Fat	0.050 ± 0.0232	0.170 ± 0.0351	0.071 ± 0.0165	<0.003
Hair and skin	0.079 ± 0.0191	0.165 ± 0.0258	0.053 ± 0.0075	0.008 ± 0.0130
Heart	0.382 ± 0.1471 *0.1 ± 0.03*	0.358 ± 0.0280 *0.1 ± 0.01*	0.081 ± 0.0265 *0.0 ± 0.01*	<0.002 *<0.0005*
Kidney	0.873 ± 0.2642 *0.7 ± 0.20*	1.040 ± 0.1387 *0.8 ± 0.18*	0.423 ± 0.1879 *0.3 ± 0.14*	<0.002 *<0.0015*
Large intestine	0.170 ± 0.1144 *0.1 ± 0.06*	0.250 ± 0.1037 *0.1 ± 0.03*	0.937 ± 0.5298 *0.4 ± 0.26*	<0.005 *<0.0023*
Liver	1.737 ± 0.7990 *7.5 ± 3.52*	1.512 ± 0.1375 *6.2 ± 0.80*	0.461 ± 0.2155 *1.8 ± 0.94*	0.005 ± 0.0013 *0.0 ± 0.01*
Lung	0.266 ± 0.1078 *0.1 ± 0.04*	0.318 ± 0.0457 *0.1 ± 0.02*	0.090 ± 0.0304 *0.0 ± 0.01*	<0.002 *<0.0007*
Mandibular gland	0.235 ± 0.0740 *0.0 ± 0.02*	0.304 ± 0.0417 *0.0 ± 0.01*	0.062 ± 0.0176 *0.0 ± 0.00*	<0.001 *<0.0001*
Muscle	0.112 ± 0.0464	0.221 ± 0.0677	0.047 ± 0.0098	<0.002
Pancreas	0.353 ± 0.1441 *0.1 ± 0.04*	0.365 ± 0.0553 *0.1 ± 0.04*	0.108 ± 0.0745 *0.0 ± 0.02*	<0.002 *0.0007*
Pituitary gland	0.152 ± 0.0569 *0.0 ± 0.00*	0.295 ± 0.1627 *0.0 ± 0.00*	0.062 ± 0.0140 *0.0 ± 0.00*	<0.028 *<0.0001*
Sciatic nerve	0.024 ± 0.0050	0.089 ± 0.0200	0.023 ± 0.0079	<0.020
Small intestine	1.216 ± 0.7852 *1.7 ± 1.37*	3.378 ± 0.9297 *5.1 ± 1.40*	0.717 ± 0.5761 *1.2 ± 1.15*	<0.002 *<0.0021*
Spleen	0.218 ± 0.0928 *0.1 ± 0.02*	0.222 ± 0.0508 *0.0 ± 0.001*	0.056 ± 0.0220 *0.0 ± 0.01*	<0.002 *<0.0005*
Spinal cord	0.102 ± 0.0466	0.119 ± 0.0113	0.018 ± 0.0061	<0.002
Stomach	8.157 ± 0.5576 *4.2 ± 0.52*	3.184 ± 0.3399* *1.6 ± 0.51*	3.160 ± 5.5969 *1.6 ± 2.91*	<0.005 *<0.0022*

Testis	0.055 ± 0.0233 *0.0 ± 0.02*	0.124 ± 0.0203 *0.1 ± 0.01*	0.031 ± 0.0063 *0.0 ± 0.01*	<0.002 *<0.0021*
Thymus	0.118 ± 0.0477 *0.0 ± 0.01*	0.163 ± 0.0222 *0.0 ± 0.01*	0.041 ± 0.0123 *0.0 ± 0.00*	<0.002 *<0.0004*
Thyroid	0.308 ± 0.1113 *0.0 ± 0.00*	0.262 ± 0.0315 *0.0 ± 0.00*	0.139 ± 0.0912 *0.0 ± 0.00*	<0.018 *<0.0001*
Caecum contents	0.057 ± 0.0276 *0.1 ± 0.04*	0.308 ± 0.0862 *0.6 ± 0.31*	11.736 ± 1.7407 *26.4 ± 4.08*	0.002 ± 0.0008 *0.0 ± 0.00*
Large intestine contents	0.122 ± 0.1182 *0.1 ± 0.11*	0.178 ± 0.0377 *0.1 ± 0.06*	5.956 ± 2.4188 *4.0 ± 1.71*	0004 ± 0.0014 *0.0 ± 0.00*
Small intestine contents	2.115 ± 0.8246 *5.1 ± 2.17*	9.634 ± 4.2420 *21.4 ± 8.55*	11.185 ± 4.0342 *18.0 ± 8.21*	0.001 ± 0.0007 *0.0 ± 0.00*
Stomach contents	24.114 ± 5.4270 *53.5 ± 13.53*	16.472 ± 4.6310 *22.1 ± 2.63**	6.767 ± 4.1775 *1.8 ± 1.05*	<0.0005 *<0.0015*
Total %	*81.0 ± 9.63*	*73.3 ± 4.00**	*60.4 ± 10.76*	*0.2 ± 0.04*

*: mean and SD of three rats because stomach of animal No. 125 erroneously not weighed; - carcass value cannot be calculated

Table 6.1.1-9: Concentration of radioactivity in tissues in female rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Tissue	µg eq. of S-2399/g tissue [ppm] *% of dosed ^{14}C*			
	Time after administration			
	0.25 h	1 h	8 h	168 h
Adrenal	0.572 ± 0.1866 *0.0 ± 0.01*	1.059 ± 0.0963 *0.0 ± 0.00*	0.165 ± 0.0266 *0.0 ± 0.00*	<0.005 *<0.0001*
Blood	0.129 ± 0.0279 *0.6 ± 0.13*	0.163 ± 0.0168 *0.7 ± 0.08*	0.045 ± 0.0046 *0.2 ± 0.02*	<0.002 *<0.0092*
Blood cell	0.098 ± 0.0204	0.117 ± 0.0155	0.035 ± 0.0051	0.003 ± 0.0003
Plasma	0.152 ± 0.0377	0.205 ± 0.0231	0.053 ± 0.0054	<0.002
Bone	0.025 ± 0.0087	0.080 ± 0.0452	0.026 ± 0.0160	0.014 ± 0.0061
Bone marrow	0.118 ± 0.0214	0.237 ± 0.0090	0.062 ± 0.0129	<0.001
Brain	0.149 ± 0.0579 *0.1 ± 0.05*	0.279 ± 0.0206 *0.2 ± 0.02*	0.021 ± 0.0087 *0.0 ± 0.01*	<0.002 *<0.0015*
Caecum	<0.072 *<0.0220*	0.229 ± 0.0681 *0.1 ± 0.03*	3.052 ± 2.0155 *1.1 ±0.71*	<0.010 *<0.0034*
Carcass	- *6.0 ± 1.56*	- *16.8 ± 0.81*	- *4.1 ± 0.41*	- *0.1 ± 0.04*
Eye	0.046 ± 0.0120 *0.0 ± 0.00*	0.099 ± 0.0090 *0.0 ± 0.00*	0.026 ± 0.0092 *0.0 ± 0.00*	<0.002 *<0.0002*
Fat	0.048 ± 0.0180	0.305 ± 0.0153	0.165 ± 0.0185	<0.003
Hair and skin	0.067 ± 0.0197	0.234 ± 0.0275	0.065 ± 0.0274	0.008 ± 0.0133
Heart	0.441 ± 0.1359 *0.1 ± 0.04*	0.678 ± 0.0411 *0.2 ± 0.02*	0.089 ± 0.0187 *0.0 ± 0.01*	<0.002 *<0.0006*
Kidney	0.643 ± 0.1265 *0.5 ± 0.07*	1.119 ± 0.3183 *0.8 ± 0.21*	0.300 ± 0.0791 *0.2 ± 0.06*	<0.002 *<0.0014*
Large intestine	0.114 ± 0.0433 *0.1 ± 0.02*	0.320 ± 0.0555 *0.1 ± 0.02*	1.015 ± 0.5507 *0.4 ± 0.21*	<0.007 *<0.0028*
Liver	2.016 ± 0.6785 *7.6 ± 2.64*	1.692 ± 0.1497 *6.9 ± 0.85*	0.382 ± 0.0282 *1.4 ± 0.15*	0.003 ± 0.0006 *0.0 ± 0.00*

Lung	0.276 ± 0.0671 *0.1 ± 0.03*	0.457 ± 0.0401 *0.2 ± 0.03*	0.089 ± 0.0162 *0.0 ± 0.01*	<0.002 *<0.0009*
Mandibular gland	0.248 ± 0.0740 *0.0 ± 0.02*	0.506 ± 0.0312 *0.1 ± 0.01*	0.084 ± 0.0173 *0.0 ± 0.00*	<0.001 *<0.0002*
Muscle	0.095 ± 0.0211	0.339 ± 0.0160	0.062 ± 0.0210	<0.002
Ovary	0.215 ± 0.0511 *0.0 ± 0.00*	0.417 ± 0.0402 *0.0 ± 0.00*	0.071 ± 0.0104 *0.0 ± 0.00*	<0.002 *<0.0001*
Pancreas	0.320 ± 0.0660 *0.1 ± 0.02*	0.578 ± 0.0712 *0.2 ± 0.05*	0.091 ± 0.0120 *0.0 ± 0.01*	<0.002 *<0.0009*
Pituitary gland	0.180 ± 0.0558 *0.0 ± 0.00*	0.350 ± 0.0413 *0.0 ± 0.00*	0.072 ± 0.0138 *0.0 ± 0.00*	<0.024 *<0.0001*
Sciatic nerve	0.040 ± 0.0332	0.181 ± 0.0412	0.027 ± 0.0087	<0.012
Small intestine	0.839 ± 0.5164 *1.2 ± 0.76*	1.925 ± 1.1586 *2.2 ± 1.81*	1.767 ± 1.4764 *1.7 ± 1.20*	<0.003 *<0.0032*
Spleen	0.203 ± 0.0395 *0.0 ± 0.01*	0.306 ± 0.0252 *0.1 ± 0.00*	0.052 ± 0.0075 *0.0 ± 0.00*	<0.002 *<0.0004*
Spinal cord	0.103 ± 0.0491	0.281 ± 0.0250	0.023 ±0.0107	<0.003
Stomach	14.323 ± 9.1286 *9.3 ± 8.05*	5.367 ± 4.9602 *2.5 ± 2.36*	0.174 ± 0.0415 *0.1 ± 0.01*	<0.006 *<0.0034*
Thymus	0.114 ± 0.0267 *0.0 ± 0.01*	0.241 ± 0.0211 *0.1 ± 0.01*	0.041 ± 0.0080 *0.0 ± 0.00*	<0.002 *<0.0004*
Thyroid	0.751 ± 0.6312 *0.0 ± 0.01*	0.531 ± 0.1214 *0.0 ± 0.00*	0.077 ± 0.0027 *0.0 ± 0.00*	<0.015 *<0.0001*
Uterus	0.130 ± 0.0198 *0.0 ± 0.01*	0.263 ± 0.0404 *0.0 ± 0.00*	0.063 ± 0.0121 *0.0 ± 0.00*	<0.002 *<0.0004*
Caecum contents	0.037 ± 0.0070 *0.1 ± 0.01*	0.212 ± 0.0480 *0.4 ± 0.16*	14.278 ± 4.8174 *31.5 ± 7.06*	0.007 ± 0.0129 *0.0 ± 0.03*
Large intestine contents	0.049 ± 0.0158 *0.0 ± 0.02*	0.163 ± 0.0210 *0.2 ± 0.03*	4.916 ± 1.5218 *4.4 ± 1.20*	0.007 ± 0.0123 *0.0 ± 0.00*
Small intestine contents	1.188 ± 0.5538 *2.5 ± 1.20*	8.089 ± 1.3499 *22.7 ± 2.95*	6.826 ± 2.9239 *13.8 ± 7.44*	0.005 ± 0.0084 *0.0 ± 0.02*
Stomach contents	73.985 ± 38.9226 *61.5 ± 19.86*	25.509 ± 23.0070 *21.4 ± 2.31*	0.925 ± 0.7823 *0.3 ± 0.16*	<0.0005 *<0.0011*
Total %	*89.9 ± 9.55*	*75.9 ± 4.50*	*59.5 ± 10.69*	*0.1 ± 0.02*

- carcass value cannot be calculated

Table 6.1.1-10: Concentration of radioactivity in tissues in male rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Tissue	µg eq. of S-2399/g tissue [ppm] *% of dosed ^{14}C*			
	Time after administration			
	1 h	8 h	24 h	168 h
Adrenal	32.6 ± 8.53 *0.0 ± 0.00*	37.0 ± 6.08 *0.0 ± 0.00*	12.6 ± 2.01 *0.0 ± 0.00*	<0.8 *<0.0001*
Blood	9.4 ± 2.51 *0.3 ± 0.08*	9.8 ± 2.10 *0.3 ± 0.07*	4.1 ± 0.84 *0.1 ± 0.03*	<0.3 *<0.0117*
Blood cell	8.6 ± 3.00	8.4 ± 2.23	3.8 ± 0.91	0.5 ± 0.13
Plasma	10.4 ± 2.59	10.6 ± 2.34	4.3 ± 0.57	<0.3
Bone	1.8 ± 0.33	2.5 ± 0.97	1.1 ± 0.32	<0.2

Bone marrow	8.1 ± 2.25	8.7 ± 1.60	3.3 ± 0.63	<0.2
Brain	6.3 ± 1.64 *0.0 ± 0.01*	5.7 ± 0.74 *0.0 ± 0.01*	1.1 ± 0.29 *0.0 ± 0.00*	<0.3 *<0.0014*
Caecum	8.4 ± 2.52 *0.0 ± 0.01*	73.3 ± 12.81 *0.2 ± 0.06*	214.6 ± 175.38 *0.7 ± 0.53*	<1.0 *<0.0026*
Carcass	- *4.8 ± 1.35*	- *5.0 ± 1.22*	- *3.5 ± 0.47*	- *0.1 ± 0.02*
Eye	3.6 ± 0.41 *0.0 ± 0.00*	3.9 ± 0.78 *0.0 ± 0.00*	1.6 ± 0.25 *0.0 ± 0.00*	<0.3 *<0.0002*
Fat	8.2 ± 1.33	18.8 ± 2.35	11.4 ± 2.77	<0.5
Hair and skin	9.3 ± 3.35	9.4 ± 1.95	4.3 ± 0.53	0.3 ± 0.56
Heart	14.4 ± 2.97 *0.0 ± 0.00*	14.0 ± 1.68 *0.0 ± 0.00*	4.9 ± 1.13 *0.0 ± 0.00*	<0.3 *<0.0006*
Kidney	41.9 ± 15.61 *0.2 ± 0.08*	48.5 ± 17.10 *0.3 ± 0.09*	17.1 ± 5.90 *0.1 ± 0.03*	<0.3 *<0.0018*
Large intestine	10.0 ± 1.66 *0.0 ± 0.00*	36.8 ± 10.17 *0.1 ± 0.004*	136.2 ± 83.81 *0.5 ± 0.24*	<0.8 *<0.0027*
Liver	43.8 ± 10.97 *1.3 ± 0.31*	54.1 ± 6.39 *1.5 ± 0.20*	25.6 ± 0.82 *0.8 ± 0.05*	0.7 ± 0.06 *0.0 ± 0.00*
Lung	16.0 ± 3.41 *0.0 ± 0.01*	15.0 ± 1.96 *0.0 ± 0.00*	5.6 ± 1.22 *0.0 ± 0.00*	<0.3 *<0.0008*
Mandibular gland	13.5 ± 3.23 *0.0 ± 0.00*	13.1 ± 1.59 *0.0 ± 0.00*	4.3 ± 0.84 *0.0 ± 0.00*	<0.1 *<0.0002*
Muscle	8.8 ± 1.72	8.5 ± 0.73	3.2 ± 0.68	<0.3
Pancreas	19.2 ± 4.61 *0.0 ± 0.01*	18.1 ± 2.60 *0.0 ± 0.01*	7.3 ± 0.49 *0.0 ± 0.00*	<0.3 *<0.0008*
Pituitary gland	11.7 ± 3.69 *0.0 ± 0.00*	12.7 ± 1.43 *0.0 ± 0.00*	<5.0 *<0.0001*	<5.9 *<0.0001*
Sciatic nerve	4.1 ± 1.16	4.7 ± 0.99	<1.3	<1.2
Small intestine	49.7 ± 8.19 *0.4 ± 0.05*	158.4 ± 119.99 *1.4 ± 0.83*	118.1 ± 86.52 *1.2 ± 0.88*	<0.4 *<0.0030*
Spleen	11.5 ± 2.21 *0.0 ± 0.01*	9.8 ± 1.06 *0.0 ± 0.00*	3.7 ± 0.52 *0.0 ± 0.00*	<0.3 *<0.0004*
Spinal cord	8.1 ± 2.61	6.0 ± 0.57	1.6 ± 0.33	<0.3
Stomach	173.3 ± 96.16 *0.6 ± 0.35*	139.5 ± 30.29 *0.4 ± 0.10*	43.1 ± 29.09 *0.1 ± 0.10*	<0.7 *<0.0025*
Testis	6.3 ± 1.38 *0.0 ± 0.01*	7.2 ± 2.05 *0.0 ± 0.01*	2.6 ± 0.58 *0.0 ± 0.00*	<0.3 *<0.0021*
Thymus	8.3 ± 1.95 *0.0 ± 0.01*	7.7 ± 1.45 *0.0 ± 0.00*	2.5 ± 0.68 *0.0 ± 0.00*	<0.3 *<0.0005*
Thyroid	26.8 ± 17.05 *0.0 ± 0.00*	13.4 ± 1.18 *0.0 ± 0.00*	<4.5 *0.0 ± 0.00*	<5.7 *<0.0001*
Caecum contents	12.2 ± 4.53 *0.2 ± 0.09*	361.4 ± 102.85 *5.1 ± 1.14*	722.2 ± 370.31 *16.6 ± 11.32*	1.0 ± 0.70 *0.0 ± 0.02**
Large intestine contents	9.0 ± 3.38 *0.0 ± 0.03*	153.8 ± 88.89 *0.8 ± 0.44*	789.9 ± 220.00 *6.9 ± 1.00*	1.3 ± 0.72 *0.0 ± 0.00*
Small intestine contents	256.3 ± 110.75 *4.0 ± 1.47*	881.2 ± 516.4 *12.0 ± 8.323*	710.9 ± 341.06 *14.6 ± 7.45*	1.1 ± 1.00 *0.0 ± 0.02*
Stomach contents	4391.9 ± 1528.65 *75.8 ± 8.62*	4589.1 ± 633.61 *57.8 ± 3.65*	363.5 ± 468.09 *7.8 ± 8.60*	<0.1 *<0.0026*
Total %	*88.0 ± 10.21*	*85.1 ± 6.63*	*52.9 ± 12.79*	*0.2 ± 0.06**

*: mean and SD of three rats because caecum of animal No. 154 erroneously not weighed; - carcass value cannot be calculated

Table 6.1.1-11: Concentration of radioactivity in tissues in female rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Tissue	µg eq. of S-2399/g tissue [ppm] *% of dosed ^{14}C*			
	Time after administration			
	8 h	24 h	48 h	168 h
Adrenal	30.0 ± 6.73 *0.0 ± 0.00*	25.1 ± 2.88 *0.0 ± 0.00*	10.7 ± 7.63 *0.0 ± 0.00*	<0.4 *<0.0001*
Blood	5.1 ± 0.97 *0.2 ± 0.03*	5.3 ± 0.84 *0.2 ± 0.03*	2.7 ± 1.60 *0.1 ± 0.05*	<0.3 *<0.0094*
Blood cell	3.4 ± 0.99	4.4 ± 0.79	2.2 ± 1.30	<0.3
Plasma	6.3 ± 1.12	6.4 ± 0.79	3.4 ± 1.97	<0.3
Bone	1.7 ± 0.34	1.8 ± 0.43	0.9 ± 0.43	<0.2
Bone marrow	7.4 ± 0.79	7.0 ± 1.02	3.1 ± 1.86	<0.4
Brain	6.8 ± 0.60 *0.0 ± 0.00*	6.3 ± 1.26 *0.0 ± 0.01*	1.5 ± 1.49 *0.0 ± 0.01*	<0.2 *<0.0016*
Caecum	38.0 ± 15.21 *0.1 ± 0.06*	146.1 ± 103.10 *0.4 ± 0.31*	272.2 ± 223.53 *0.6 ± 0.50*	<1.2 *<0.0031*
Carcass	- *3.7 ± 0.21*	- *4.4 ± 0.68*	- *3.0 ± 1.49*	- *0.1 ± 0.04*
Eye	2.8 ± 0.04 *0.0 ± 0.00*	3.1 ± 0.54 *0.0 ± 0.00*	1.5 ± 0.56 *0.0 ± 0.00*	<0.3 *<0.0002*
Fat	19.7 ± 1.93	27.4 ± 6.36	15.8 ± 11.06	<0.5
Hair and skin	8.3 ± 1.40	7.8 ± 1.62	5.5 ± 1.73	<0.2
Heart	12.3 ± 0.66 *0.0 ± 0.00*	12.1 ± 1.38 *0.0 ± 0.01*	4.5 ± 3.18 *0.0 ± 0.01*	<0.3 *<0.0006*
Kidney	30.1 ± 13.00 *0.2 ± 0.06*	21.1 ± 1.60 *0.1 ± 0.02*	13.0 ± 7.42 *0.1 ± 0.04*	<0.3 *<0.0014*
Large intestine	20.6 ± 4.86 *0.1 ± 0.02*	78.4 ± 21.03 *0.3 ± 0.10*	131.0 ± 76.80 *0.4 ± 0.24*	<0.9 *<0.0033*
Liver	41.6 ± 5.48 *1.1 ± 0.19*	44.6 ± 6.87 *1.2 ± 0.15*	22.4 ± 14.43 *0.6 ± 0.33*	0.5 ± 0.05 *0.0 ± 0.00*
Lung	12.7 ± 1.11 *0.0 ± 0.00*	13.8 ± 1.89 *0.0 ± 0.01*	5.1 ± 3.48 *0.0 ± 0.01*	<0.3 *<0.0007*
Mandibular gland	11.3 ± 0.68 *0.0 ± 0.00*	10.8 ± 1.00 *0.0 ± 0.01*	3.9 ± 3.02 *0.0 ± 0.00*	<0.1 *<0.0002*
Muscle	7.1 ± 0.59	6.2 ± 0.43	3.0 ± 2.16	<0.2
Ovary	12.5 ± 0.23 *0.0 ± 0.00*	11.4 ± 0.41 *0.0 ± 0.00*	4.0 ± 3.03 *0.0 ± 0.00*	<0.3 *<0.0001*
Pancreas	16.1 ± 2.24 *0.1 ± 0.02*	15.0 ± 2.31 *0.1 ± 0.03*	6.9 ± 5.30 *0.0 ± 0.01*	<0.2 *<0.0007*
Pituitary gland	10.6 ± 0.57 *0.0 ± 0.00*	10.2 ± 1.47 *0.0 ± 0.00*	<3.9 *<0.0001*	<4.5 *<0.0001*
Sciatic nerve	5.4 ± 0.84	5.5 ± 1.25	2.1 ± 1.86	<2.1
Small intestine	65.7 ± 39.85 *0.7 ± 0.43*	182.0 ± 93.74 *1.6 ± 0.93*	142.0 ± 127.22 *1.1 ± 0.84*	<0.4 *<0.0034*

Spleen	8.0 ± 0.83 *0.0 ± 0.00*	7.3 ± 0.72 *0.0 ± 0.00*	3.2 ± 2.50 *0.0 ± 0.00*	<0.2 *<0.0004*
Spinal cord	7.3 ± 0.44	7.8 ± 1.67	2.0 ± 2.25	<0.2
Stomach	152.3 ± 28.95 *0.5 ± 0.09*	160.1 ± 73.74 *0.6 ± 0.30*	43.2 ± 38.36 *0.1 ± 0.13*	<0.8 *<0.0035*
Thymus	6.3 ± 0.43 *0.0 ± 0.00*	6.0 ± 0.80 *0.0 ± 0.00*	2.2 ± 1.71 *0.0 ± 0.00*	<0.2 *<0.0004*
Thyroid	20.9 ± 7.94 *0.0 ± 0.00*	10.4 ± 1.41 *0.0 ± 0.00*	<3.8 *0.0 ± 0.00*	<2.6 *<0.0001*
Uterus	7.1 ± 0.83 *0.0 ± 0.00*	6.3 ± 0.69 *0.0 ± 0.00*	3.2 ± 2.55 *0.0 ± 0.00*	<0.3 *<0.0005*
Caecum contents	236.5 ± 62.87 *3.4 ± 1.06*	654.7 ± 376.48 *9.0 ± 3.51*	822.6 ± 629.13 *10.5 ± 7.28*	0.9 ± 0.83 *0.0 ± 0.01*
Large intestine contents	91.4 ± 66.59 *0.5 ± 0.48*	615.2 ± 177.70 *4.0 ± 1.43*	848.8 ± 409.24 *4.4 ± 2.42*	0.6 ± 0.87 *0.0 ± 0.00*
Small intestine contents	398.7 ± 131.31 *3.5 ± 0.78*	707.5 ± 471.02 *10.9 ± 6.13*	464.9 ± 408.29 *6.5 ± 5.10*	0.5 ± 0.53 *0.0 ± 0.01*
Stomach contents	5867.9 ± 1581.92 *59.5 ± 21.71*	3965.2 ± 802.46 *34.8 ± 7.79*	418.3 ± 484.41 *1.6 ± 1.89*	<0.1 *<0.0024*
Total %	*73.6 ± 22.81*	*67.7 ± 7.17*	*29.1 ± 14.85*	*0.1 ± 0.04*

- carcass value cannot be calculated

Table 6.1.1-12: Biological half-lives of ^{14}C in tissues in male and female rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam

Tissue	Biological half-life [h]			
	1 mg/kg		150 mg/kg	
	Male	Female	Male	Female
Adrenal	3	3	10	19
Blood	4	4	13	25
Blood cell	3	4	14	24
Plasma	4	4	12	26
Bone	4	4	14	24
Bone marrow	3	4	12	20
Brain	3	2	7	12
Caecum	NA	NA	NA	NA
Eye	4	4	12	22
Fat	6	8	22	30
Hair and skin	4	4	14	48
Heart	3	2	11	17
Kidney	5	4	11	34
Large intestine	NA	NA	NA	NA
Liver	4	3	15	24
Lung	4	3	11	17
Mandibular gland	3	3	10	16
Muscle	3	3	11	23
Ovary	-	3	-	16
Pancreas	4	3	12	22
Pituitary gland	3	3	NA	NA
Sciatic nerve	4	3	NA	17
Small intestine	3	57	38	67

Spleen	4	3	11	20
Spinal cord	3	2	8	12
Stomach	660	1	9	13
Testis	3	-	11	-
Thymus	3	3	10	16
Thyroid	8	3	NA	NA
Uterus	-	3	-	25
Calculation interval	1-8 h	1-8 h	8-24 h	24-48 h

NA: not analysed because ^{14}C concentrations were below the limit of quantitation or not decreased in the interval

Metabolism

The total amounts of metabolites after administration of 1 mg/kg bw or 150 mg/kg bw [phenyl-^{14}C] or [pyrazolyl-4-^{14}C] labelled inpyrfluxam were found to be similar between males and females. No radiolabel specific metabolite was recorded at either dose level. Inpyrfluxam was extensively metabolized, with 44, 44 and 43 metabolites detected in urine, bile and faeces respectively. In addition to the parent, a total of 12 metabolites, including two conjugates, were identified and quantified. Although differences in the amount of some metabolites between males and females were noted, these metabolites were qualitatively similar in both males and females at the two tested doses.

Urine and faeces

At either dose level, 1′-COOH-S-2840 and N-desMe-1′-COOH-S-2840 were the major metabolites in urine (approx. > 10% AD) in both sexes regardless of the label position. The parent compound was only detected in low amounts in faeces.

Table 6.1.1-13: Metabolites levels in urine and faeces in male rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose					
	0-6 h	6-12 h	12 h-1 d*	1-2 d	2-3 d	Total
Urine						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	4.7	2.6	2.7	0.5	--	10.5
1',1'-bis(CH_2OH)-S-2840	6.1	2.5	2.2	0.2	--	11.0
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	0.3	0.2	0.2	0.1	--	0.7
glucuronide of 1'-CH_2OH-S-2840	0.4	0.1	0.2	0.1	--	0.9
N-des-Me-1'-CH_2OH-3'-OH-S-2840	1.2	0.7	0.9	0.3	--	3.0
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	1.1	0.4	0.4	0.0	--	1.9
***N*-des-Me-1'-COOH-S-2840**	4.1	2.6	2.9	0.6	--	**10.2**
1'-COOH-S-2840	8.8	3.1	2.6	0.5	--	**15.0**
Others**	2.8	1.7	1.0	0.5	--	5.9
Total urine	29.5	13.9	13.1	2.8	--	**59.2**

Faeces						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	2.5	3.8	0.5	6.8
1',1'-bis(CH_2OH)-S-2840	--	--	1.1	0.9	ND	2.0
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	0.7	1.2	0.3	2.2
glucuronide of 1'-CH_2OH-S-2840	--	--	0.4	1.1	0.4	1.8
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	1.2	1.4	0.2	2.8
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	-- --	1.2	1.4	0.1	2.6
N-des-Me-1'-COOH-S-2840	--	--	1.3	2.1	ND	3.5
1'-CH_2OH-S-2840	--	--	0.6	1.2	0.3	2.1
1'-COOH-S-2840	--	--	1.0	0.7	ND	1.7
7'-OH-S-2399	--	--	0.3	0.6	0.2	1.1
N-des-Me-S-2840	--	--	0.3	0.4	ND	0.7
S-2399	--	--	0.2	0.4	0.1	0.8
Others***	--	--	1.8	2.3	0.6	4.6
Subtotal	--	--	12.7	17.5	2.6	32.7
acidic methanol extracts	--	--	0.7	1.0	0.1	1.8
basic methanol extracts	--	--	1.4	1.8	0.2	3.4
Unextractable	--	--	1.2	1.9	0.2	3.3
Total faeces	--	--	15.9	22.2	3.0	**41.2**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 22 unidentified metabolites which are below 1.2% of the dose; ***: sum of the 12 unidentified metabolites which are below 1.3% of the dose

Table 6.1.1-14: Metabolites levels in urine and faeces in female rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose					
	0-6 h	6-12 h	12 h-1 d*	1-2 d	2-3 d	Total
Urine						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	2.2	2.8	1.8	0.5	--	7.2
1',1'-bis(CH_2OH)-S-2840	2.2	2.2	1.5	0.4	--	6.3
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	2.1	1.1	1.0	0.6	--	4.8
glucuronide of 1'-CH_2OH-S-2840	0.9	0.4	0.3	0.1	--	1.8
N-des-Me-1'-CH_2OH-3'-OH-S-2840	1.0	0.9	1.0	0.5	--	3.4
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.9	0.4	0.5	0.1	--	1.8
***N*-des-Me-1'-COOH-S-2840**	7.8	6.0	5.0	2.2	--	**21.1**
1'-COOH-S-2840	5.4	2.9	1.7	0.5	--	**10.5**
Others**	1.2	1.0	0.6	0.1	--	2.8
Total urine	23.6	17.6	13.4	5.0	--	**59.7**
Faeces						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	1.7	2.7	0.4	4.7
1',1'-bis(CH_2OH)-S-2840	--	--	0.5	ND	ND	0.5
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	1.1	2.9	0.6	4.6

glucuronide of 1'-CH_2OH-S-2840	--	--	1.5	2.6	0.5	4.6
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	1.2	1.8	ND	3.0
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	-- --	2.4	3.1	0.8	6.3
N-des-Me-1'-COOH-S-2840	--	--	1.2	2.6	0.8	4.5
1'-CH_2OH-S-2840	--	--	0.6	0.2	ND	0.8
1'-COOH-S-2840	--	--	0.2	0.4	ND	0.7
7'-OH-S-2399	--	--	ND	0.3	ND	0.3
N-des-Me-S-2840	--	--	ND	0.2	ND	0.2
S-2399	--	--	0.5	0.7	ND	1.2
Others***	--	--	0.5	2.3	0.4	3.2
Subtotal	--	--	11.5	19.7	3.4	34.6
acidic methanol extracts	--	--	0.4	0.9	0.1	1.5
basic methanol extracts	--	--	0.6	1.1	0.1	1.8
Unextractable	--	--	0.5	1.2	0.1	1.9
Total faeces	--	--	12.9	23.0	3.7	**39.7**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 21 unidentified metabolites which are below 1.0% of the dose; ***: sum of the 12 unidentified metabolites which are below 0.8% of the dose

Table 6.1.1-15: Metabolites levels in urine and faeces in male rats after a single oral dose at 1 mg/kg bw of [phenyl-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose					
	0-6 h	6-12 h	12 h-1 d*	1-2 d	2-3 d	Total
Urine						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	3.6	1.7	1.0	0.9	--	7.2
1',1'-bis(CH_2OH)-S-2840	5.0	1.9	1.0	ND	--	8.0
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	0.5	0.2	0.2	ND	--	0.9
glucuronide of 1'-CH_2OH-S-2840	0.7	0.2	0.4	ND	--	1.3
N-des-Me-1'-CH_2OH-3'-OH-S-2840	0.9	0.9	0.7	ND	--	2.5
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	1.1	0.5	0.4	ND	--	2.0
***N*-des-Me-1'-COOH-S-2840**	4.0	2.5	1.9	ND	--	**8.4**
1'-COOH-S-2840	7.8	2.8	1.9	1.5	**--**	**14.0**
Others**	2.8	1.1	0.5	ND	--	4.4
Total urine	26.3	12.0	8.1	2.4	--	**48.7**
Faeces						
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	3.2	1.7	ND	4.9
1',1'-bis(CH_2OH)-S-2840	--	--	1.4	0.9	ND	2.3
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	1.2	1.0	ND	2.1
glucuronide of 1'-CH_2OH-S-2840	--	--	1.2	0.7	ND	1.9
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	2.3	2.0	ND	4.3
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	-- --	2.2	0.7	ND	2.9
N-des-Me-1'-COOH-S-2840	--	--	2.3	2.9	ND	5.2
1'-CH_2OH-S-2840	--	--	1.9	1.0	ND	2.9

1'-COOH-S-2840	--	--	1.4	0.7	ND	2.1
7'-OH-S-2399	--	--	0.5	0.5	ND	1.0
N-des-Me-S-2840	--	--	0.7	ND	ND	0.7
S-2399	--	--	0.5	ND	ND	0.5
Others***	--	--	2.9	0.9	ND	3.7
Subtotal	--	--	21.6	12.9	1.8****	36.3
acidic methanol extracts	--	--	1.3	0.8	0.1	2.2
basic methanol extracts	--	--	1.9	0.8	0.1	2.9
Unextractable	--	--	3.9	1.6	0.2	5.7
Total faeces	--	--	28.8	16.1	2.2	**47.1**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 11 unidentified metabolites which are below 1.0% of the dose; ***: sum of the 7 unidentified metabolites which are below 0.8% of the dose; ****: sum of the minor metabolites which are below the detection limit

Table 6.1.1-16: Metabolites levels in urine and faeces in female rats after a single oral dose at 1 mg/kg bw of [phenyl-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose				
	0-6 h	6-12 h	12 h-1 d*	1-2 d	Total
Urine					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	3.6	1.4	1.2	0.5	6.8
1',1'-bis(CH_2OH)-S-2840	3.6	1.0	1.1	0.4	6.1
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	1.0	0.5	0.7	ND	2.1
glucuronide of 1'-CH_2OH-S-2840	0.5	0.2	ND	ND	0.7
N-des-Me-1'-CH_2OH-3'-OH-S-2840	1.3	0.4	ND	ND	1.7
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	2.4	1.2	0.5	ND	4.1
***N*-des-Me-1'-COOH-S-2840**	10.0	5.0	4.4	1.8	**21.2**
1'-COOH-S-2840	9.2	1.5	1.6	ND	**12.3**
Others**	2.1	0.8	ND	ND	2.9
Total urine	33.7	11.9	9.4	2.8	**57.9**
Faeces					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	2.3	2.3	4.6
1',1'-bis(CH_2OH)-S-2840	--	--	0.8	ND	0.8
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	0.3	ND	0.3
glucuronide of 1'-CH_2OH-S-2840	--	--	1.4	ND	1.4
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	0.7	ND	0.7
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	-- --	5.5	3.1	8.6
N-des-Me-1'-COOH-S-2840	--	--	3.7	5.0	8.7
1'-CH_2OH-S-2840	--	--	0.7	ND	0.7
1'-COOH-S-2840	--	--	2.4	ND	2.4
S-2399	--	--	0.7	0.9	1.7
Others***	--	--	1.3	ND	1.3
Subtotal	--	--	19.8	11.3	31.1
acidic methanol extracts	--	--	1.0	0.6	1.5
basic methanol extracts	--	--	1.0	0.4	1.4

Unextractable	--	--	2.4	1.0	3.3
Total faeces	--	--	24.1	13.3	**37.4**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 7 unidentified metabolites which are below 0.8% of the dose; ***: sum of the 2 unidentified metabolites which are below 1.1% of the dose

Table 6.1.1-17: Metabolites levels in urine and faeces in male rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose						
	0-6 h	6-12 h	12 h-1 d*	1-2 d	2-3 d	3-5 d	Total
Urine							
N-des-Me-1',1'-bis(CH_2OH)-S-2840	0.2	0.3	0.5	0.4	0.2	--	1.6
1',1'-bis(CH_2OH)-S-2840	0.3	0.4	1.4	0.7	0.2	--	3.0
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	0.1	0.2	0.6	0.6	ND	--	1.4
glucuronide of 1'-CH_2OH-S-2840	0.1	0.1	0.4	0.4	ND	--	1.1
N-des-Me-1'-CH_2OH-3'-OH-S-2840	0.2	0.4	1.5	0.7	0.3	--	3.1
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.3	0.4	1.6	0.9	0.2	--	3.4
***N*-des-Me-1'-COOH-S-2840**	1.1	1.2	4.9	4.7	1.6	--	**13.4**
1'-COOH-S-2840	1.5	1.5	6.1	3.1	0.7	--	**13.0**
Others**	0.8	1.1	3.7	2.3	ND	--	7.9
Total urine	4.6	5.6	20.7	13.8	3.1	--	**47.8**
Faeces							
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	0.2	0.7	0.2	ND	1.1
1',1'-bis(CH_2OH)-S-2840	--	--	0.3	1.0	0.4	ND	1.7
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	0.1	0.5	0.3	ND	0.8
glucuronide of 1'-CH_2OH-S-2840	--	--	0.4	0.9	0.4	ND	1.7
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	0.8	1.5	0.7	ND	3.0
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	--	1.2	4.2	2.5	ND	7.8
N-des-Me-1'-COOH-S-2840	--	--	0.8	2.7	2.3	4.8	10.6
1'-CH_2OH-S-2840	--	--	0.9	2.4	0.8	ND	4.1
1'-COOH-S-2840	--	--	0.5	1.4	0.5	ND	2.4
7'-OH-S-2399	--	--	0.1	0.6	0.1	ND	0.8
N-des-Me-S-2840	--	--	0.1	0.6	0.3	ND	1.0
S-2399	--	--	0.3	0.5	0.1	ND	0.9
Others***	--	--	1.2	2.8	1.9	ND	5.9
Subtotal	--	--	7.0	19.7	10.4	4.8	41.9
acidic methanol extracts	--	--	0.3	1.0	0.5	0.2	1.9
basic methanol extracts	--	--	0.4	1.3	0.5	0.2	2.3
Unextractable	--	--	0.4	1.4	0.6	0.5	2.5
Total faeces	--	--	8.0	23.3	11.9	5.4	**48.7**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 20 unidentified metabolites which are below 1.8% of the dose; ***: sum of the 18 unidentified metabolites which are below 0.8% of the dose. 3'-OH-S-2840 was detected in trace amount by LC-MS using 2-day faeces extracts.

Table 6.1.1-18: Metabolites levels in urine and faeces in female rats after a single oral dose at 150 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose						
	0-6 h	6-12 h	12 h-1 d*	1-2 d	2-3 d	3-5 d	Total
Urine							
N-des-Me-1',1'-bis(CH_2OH)-S-2840	0.1	0.4	1.2	1.2	0.2	--	3.1
1',1'-bis(CH_2OH)-S-2840	0.2	0.3	0.8	1.3	0.4	--	2.9
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	0.3	0.4	1.6	3.1	0.9	--	6.3
glucuronide of 1'-CH_2OH-S-2840	0.2	0.2	0.8	1.2	0.2	--	2.6
N-des-Me-1'-CH_2OH-3'-OH-S-2840	0.2	0.3	1.0	1.9	0.4	--	3.8
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.2	0.2	0.6	1.0	ND	--	2.1
***N*-des-Me-1'-COOH-S-2840**	0.7	1.3	4.2	8.5	2.9	--	**17.5**
1'-COOH-S-2840	0.7	0.8	2.5	4.1	0.7	--	**8.8**
Others**	0.4	0.1	0.6	2.1	1.8	--	5.0
Total urine	2.9	3.9	13.2	24.5	7.5	--	**52.0**
Faeces							
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	0.2	0.9	1.3	ND	2.3
1',1'-bis(CH_2OH)-S-2840	--	--	0.5	2.1	2.8	1.8	7.3
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	--	--	0.2	0.8	1.1	ND	2.2
glucuronide of 1'-CH_2OH-S-2840	--	--	0.4	1.9	2.0	0.6	4.9
N-des-Me-1'-CH_2OH-3'-OH-S-2840	--	--	0.2	0.8	1.1	ND	2.1
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	--	-- --	0.2	1.5	2.8	0.7	5.3
N-des-Me-1'-COOH-S-2840	--	--	0.3	1.1	1.9	1.8	5.2
1'-CH_2OH-S-2840	--	--	ND	0.5	0.5	ND	1.0
1'-COOH-S-2840	--	--	ND	0.5	0.5	ND	1.0
7'-OH-S-2399	--	--	ND	0.3	0.2	ND	0.5
N-des-Me-S-2840	--	--	0.1	0.7	0.9	0.4	2.2
S-2399	--	--	0.1	0.2	0.3	ND	0.7
Others***	--	--	0.0	1.3	2.1	ND	3.4
Subtotal	--	--	2.2	12.7	17.6	5.4	37.9
acidic methanol extracts	--	--	0.1	0.6	0.7	0.3	1.6
basic methanol extracts	--	--	0.1	0.7	0.9	0.2	1.9
Unextractable	--	--	0.1	0.7	0.8	0.3	1.8
Total faeces	--	--	2.5	14.6	20.0	6.2	**43.2**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 11 unidentified metabolites which are below 1.7% of the dose; ***: sum of the 11 unidentified metabolites which are below 1.1% of the dose

Bile

In bile (experiment with the low dose of the pyrazolyl label), the glucuronide of 1′-CH_2OH-S-2840 was a major metabolite in both males and females.

Table 6.1.1-19: Metabolites levels in urine, bile and faeces in bile duct cannulated male rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose				
	0-6 h	6-12 h	12 h-1 d*	1-2 d	Total
Urine					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	1.3	1.2	1.2	--	3.7
1',1'-bis(CH_2OH)-S-2840	2.1	1.6	1.8	--	5.6
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	1.0	0.4	ND	--	1.4
glucuronide of 1'-CH_2OH-S-2840	0.9	0.5	ND	--	1.3
N-des-Me-1'-CH_2OH-3'-OH-S-2840	2.5	1.0	ND	--	3.6
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.3	0.3	ND	--	0.6
N-des-Me-1'-COOH-S-2840	1.1	1.1	ND	--	2.2
1'-COOH-S-2840	3.4	2.3	ND	--	5.7
Others**	1.1	1.3	ND	--	2.4
Total urine	13.7	9.8	3.0	--	**26.5**
Bile					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	1.6	1.9	0.7	--	4.2
1',1'-bis(CH_2OH)-S-2840	ND	0.5	ND	--	0.5
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	10.7	7.7	1.8	--	20.2
glucuronide of 1'-CH_2OH-3'-OH-S-2840					
glucuronide of 1'-CH_2OH-S-2840	15.0	11.6	2.3	--	**29.0**
N-des-Me-1'-CH_2OH-3'-OH-S-2840	0.4	ND	ND	--	0.4
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.2	1.3	0.4	--	1.9
N-des-Me-1'-COOH-S-2840	1.6	0.8	0.2	--	2.5
1'-CH_2OH-S-2840	0.4	0.5	0.2	--	1.1
1'-COOH-S-2840	1.7	1.2	0.4	--	3.2
Others***	2.1	2.6	0.9	--	5.5
Total bile	33.7	28.0	6.9	--	**68.6**
Faeces					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	0.7	ND	0.7
1',1'-bis(CH_2OH)-S-2840	--	--	0.7	ND	0.7
Subtotal	--	--	1.4	0.8****	2.3
acidic methanol extracts			0.1	0.1	0.1
basic methanol extracts			0.0	0.0	0.1
Unextractable	--	--	0.1	0.1	0.2
Total faeces	--	--	1.6	1.0	**2.6**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 18 unidentified metabolites which are below 0.5% of the dose; ***: sum of the 26 unidentified metabolites which are below 1.5% of the dose; ****: sum of the minor metabolites which are below the detection limit

Table 6.1.1-20: Metabolites levels in urine, bile and faeces in bile duct cannulated female rats after a single oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (data from pooled sample of 4 rats)

Metabolite	% of the dose				
	0-6 h	6-12 h	12 h-1 d*	1-2 d	Total
Urine					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	2.5	2.5	0.9	--	6.0
1',1'-bis(CH_2OH)-S-2840	4.1	1.7	0.6	--	6.4
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	5.6	1.5	0.2	--	7.3
glucuronide of 1'-CH_2OH-S-2840	2.5	0.6	ND	--	3.1
N-des-Me-1'-CH_2OH-3'-OH-S-2840	4.3	0.9	0.2	--	5.5
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.4	0.3	ND	--	0.7
N-des-Me-1'-COOH-S-2840	3.0	3.6	0.9	--	7.5
1'-COOH-S-2840	4.5	3.2	1.0	--	8.7
Others**	2.6	ND	ND	--	2.6
Total urine	29.5	14.4	3.8	--	**47.8**
Bile					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	1.4	0.5	ND	--	2.0
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	16.9	3.6	0.5	--	21.1
glucuronide of 1'-CH_2OH-3'-OH-S-2840					
glucuronide of 1'-CH_2OH-S-2840	12.3	1.7	0.5	--	**14.5**
N-des-Me-1'-CH_2OH-3'-OH-S-2840	ND	0.1	ND	--	0.1
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	0.3	0.4	ND	--	0.8
N-des-Me-1'-COOH-S-2840	1.2	0.7	ND	--	1.9
1'-CH_2OH-S-2840	0.2	0.3	ND	--	0.5
1'-COOH-S-2840	0.4	0.2	ND	--	0.6
Others***	3.7	1.5	ND	--	5.2
Total bile	36.5	9.1	1.0	--	**46.6**
Faeces					
N-des-Me-1',1'-bis(CH_2OH)-S-2840	--	--	0.9	1.0	1.9
1',1'-bis(CH_2OH)-S-2840	--	--	0.4	ND	0.4
Subtotal	--	--	1.4	1.0	2.3
acidic methanol extracts	--	--	0.1	0.1	0.1
basic methanol extracts	--	--	0.0	0.0	0.0
Unextractable	--	--	0.1	0.1	0.1
Total faeces	--	--	1.5	1.1	**2.6**

ND: not detected; --: not analysed; *: 0-1 day for faeces; **: sum of the 11 unidentified metabolites which are below 0.7% of the dose; ***: sum of the 21 unidentified metabolites which are below 2.5% of the dose

Plasma, liver and kidney

The parent compound, 1′-COOH-S-2840, 1′-CH_2OH-S-2840, and N-desMe-S-2840 were the most abundant metabolites detected in plasma, liver and kidney. Although concentrations

of inpyrfluxam and its major metabolites in liver and kidney were higher than in plasma, the profiles of metabolites were similar in tissues and plasma.

Conclusion

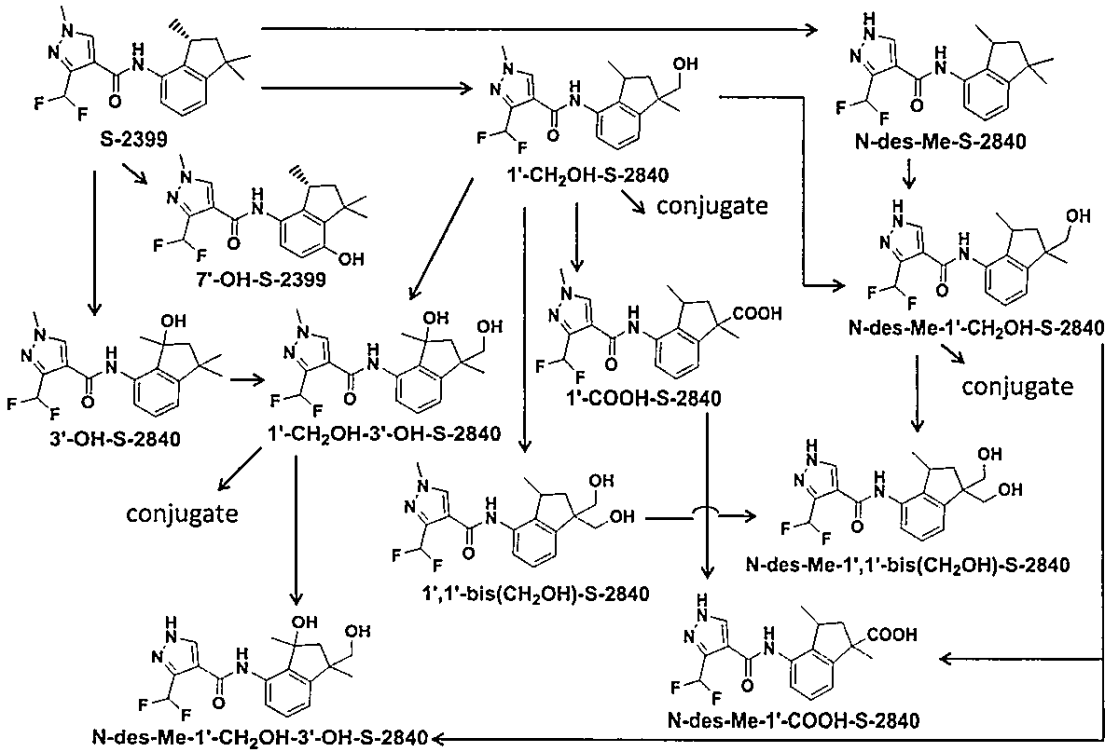
According to this GLP and guideline compliant ADME study, inpyrfluxam was almost completely absorbed (96%) by the oral route at the low dose of 1 mg/kg bw. Overall, an oral absorption value of 100% is considered appropriate at the low dose of 1 mg/kg bw. Inpyrfluxam and/or its metabolites were widely distributed, with higher levels found in blood, plasma, liver, kidney, adrenal and heart. No saturation in absorption and no bioaccumulation were noted at the high dose of 150 mg/kg bw.

Elimination was rapid, with >67% of the dose excreted within 24 hours of dosing and was essentially complete (>90% of the dose) within 2-3 days. Elimination was predominantly via urine (49.2% – 61% of the dose) and bile (46.9 – 68.9% of the dose). Faecal excretion of radioactivity was between 39.4% and 47.9% of the dose, at 168 hours, of which a significant proportion was excreted via bile. There was evidence of entero-hepatic re-circulation.

Inpyrfluxam was extensively metabolized, with 44, 44 and 43 metabolites detected in urine, bile and faeces respectively. In addition to the parent, a total of 12 metabolites, including two conjugates, were identified and quantified.

A metabolic pathway was proposed by the applicant with N-demethylation, oxidation of the 1',1'-dimethylgroup of the indane ring followed by further oxidation to carboxylic acid and glucuronide conjugation along with minor metabolic reactions such as 3'and 7'-hydroxylation of the indane ring.

Figure B.6.1.1-1: Proposed metabolic pathway of inpyrfluxam in rats.



([redacted] 2016a)

2. Metabolism of inpyrfluxam in rats – repeated exposure

Reference:	KCA 5.1.1/02
Report Title:	Metabolism of S-2399 in Rats (Repeated Oral Administration)
Author(s) & Year:	[redacted] (2016b)
Document No, Authority registration No	Study No. TPM-0027 [redacted]
Substance used:	Test Material radiolabelled: [pyrazolyl-4-^{14}C] S-2399 ([pyrazolyl-4-^{14}C] inpyrfluxam) Lot/Batch: CFQ41802 Purity: ≥98.4% Test Material unlabelled: S-2399 A.S (inpyrfluxam) Lot/Batch: YT3424G Purity: 99.9%
Method of analysis:	Validated method of analysis not required

Guideline(s):	OECD TG 417, (2010)
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Method

The ADME of [pyrazolyl-4-^{14}C] radiolabelled inpyrfluxam were investigated in Wistar Hannover rats following repeated exposure.

Groups of male and female rats were dosed by oral gavage once daily for 14 consecutive days with 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam in 0.5% methylcellulose. The study design, including how many animals were used for each experiment and the investigations performed is described in the table below. Rats were sacrificed 7-days post dosing. Total radioactivity was determined in plasma, blood, tissues, urine, faeces and from a cage wash during the course of the study and in organ and tissue samples (including blood and plasma) at sacrifice.

Table 6.1.2-1: Experimental design for ADME investigation of [pyrazolyl-4-^{14}C] - labelled inpyrfluxam

Group	Labeled position	Study type	Dose (mg/kg)	Number of animals	
				Male	Female
A	Pyrazolyl	^{14}C-Excretion ^{14}C-Tissue residue Metabolite profiling	1	Dosed: 6 Used for analysis: 4	Dosed: 5 Used for analysis: 4
B	Pyrazolyl	^{14}C-concentration in blood and plasma	1	Dosed: 7 Used for analysis: 4	Dosed: 6 Used for analysis: 4

Animals were observed daily for mortality and clinical signs of toxicity until sacrifice.

Dose levels were selected based on the previous metabolism study where single oral administration of inpyrfluxam at 1 mg/kg bw resulted in no toxicity, but it was high enough to identify metabolites in excreta ([redacted] T, 2016a).

Results

No mortality or clinical signs of toxicity were noted in any of the groups.

Recovery and excretion

The total recovery of radioactivity was >90% within 24 h post dose in males and females. This is in accordance with the acceptability criteria of the test guideline (>90% recovery after 7 days).

For full details of the recovery of radioactivity, please refer to Table 6.1.1.2-2.

Total excretion of radioactivity after 7 days from the end of dosing was 94.7% AD (urine: 33% AD; faeces: 61.5% AD) in males, and 96.4% AD (urine: 51.6% AD; faeces: 44.8% AD) in females. No notable sex related differences were noted in total excretion except that slightly higher amounts of radioactivity were recorded in urine in females.

Table 6.1.2-2: Cumulative excretion of radioactivity (% of total 14 doses) during and after repeated oral administration of 1 mg/kg of [pyrazolyl-4-^{14}C] inpyrfluxam to male and female rats (mean ± SD of 4 rats)

Time period	Cumulative excretion (% of total dose)					
	Male			Female		
	Urine	Faeces	Total	Urine	Faeces	Total
0-1 days	1.8 ± 0.51	1.7 ± 0.47	3.4 ± 0.90	3.2 ± 0.90	1.4 ± 0.62	4.6 ± 1.44
0-2 days	3.7 ± 1.00	5.1 ± 0.17	8.8 ± 1.13	6.6 ± 1.62	4.5 ± 1.43	11.1 ± 1.06
0-3 days	5.7 ± 1.44	9.5 ± 0.93	15.2 ± 0.95	9.9 ± 2.36	8.0 ± 2.15	17.9 ± 0.83
0-4 days	7.3 ± 1.94	13.8 ± 1.55	21.1 ± 0.70	13.4 ± 3.00	10.0 ± 2.26	23.4 ± 0.98
0-5 days	8.9 ± 2.24	17.4 ± 1.72	26.3 ± 0.89	16.4 ± 3.20	13.3 ± 2.46	29.7 ± 1.01
0-6 days	10.6 ± 2.63	21.2 ± 2.07	31.8 ± 1.07	19.8 ± 3.67	16.3 ± 3.81	36.2 ± 0.78
0-7 days	12.6 ± 3.10	26.5 ± 2.67	39.1 ± 0.73	23.5 ± 4.59	20.2 ± 4.92	43.6 ± 0.74
0-8 days	14.8 ± 4.19	29.9 ± 2.85	44.7 ± 2.53	27.1 ± 5.66	22.2 ± 5.72	49.2 ± 0.94
0-9 days	17.5 ± 5.00	34.5 ± 3.48	52.0 ± 3.60	31.3 ± 6.59	26.1 ± 5.84	57.4 ± 1.45
0-10 days	20.4 ± 5.52	39.4 ± 4.03	59.8 ± 3.01	35.4 ± 6.96	29.5 ± 6.73	64.8 ± 1.96
0-11 days	23.3 ± 6.05	44.5 ± 4.84	67.8 ± 2.51	39.2 ± 7.18	32.9 ± 7.46	72.1 ± 1.55
0-12 days	26.3 ± 6.70	49.1 ± 5.49	75.4 ± 1.79	43.3 ± 7.62	35.7 ± 6.90	79.1 ± 0.99
0-13 days	29.3 ± 7.28	53.9 ± 7.61	83.2 ± 1.55	47.2 ± 8.33	39.4 ± 7.44	86.6 ± 1.61
0-14 days	32.3 ± 7.88	57.3 ± 9.19	89.7 ± 2.32	51.2 ± 8.96	43.0 ± 8.70	94.2 ± 1.71
0-15 days	32.9 ± 8.14	60.1 ± 8.55	93.0 ± 1.53	51.5 ± 8.87	44.3 ± 8.98	95.8 ± 0.81
0-16 days	33.0 ± 8.11	61.1 ± 8.28	94.1 ± 1.37	51.5 ± 8.85	44.5 ± 9.02	96.0 ± 0.63
0-18 days	33.0 ± 8.16	61.4 ± 8.35	94.5 ± 1.42	51.6 ± 8.84	44.7 ± 9.14	96.3 ± 0.49
0-20 days	33.0 ± 8.16	61.5 ± 8.38	94.5 ± 1.43	51.6 ± 8.84	44.8 ± 9.16	96.3 ± 0.48
Carcass			0.1 ± 0.02			0.1 ± 0.01
Total			**94.7 ± 1.44**			**96.4 ± 0.48**

Radioactive levels in blood and plasma

Maximal plasma concentrations (C_{max}) of radioactivity were recorded at 1 h and 2 h after the end of dosing for 14 days in males and females respectively. There was a rapid decrease in the plasma levels of radioactivity after 1 h in males and 2 h in females, with half-lives ($t_{1/2}$)

and area under the curve (AUC) of 12 h and 2.04 µg equivalents/g·h respectively in males, and 9 h and 2.10 µg equivalents/g·h respectively in females.

Mean blood/plasma radioactivity ratios of males and females were between 0.8-1.7 at different time points. Due to the large variation, no clear conclusion can be drawn about greater affinity of radioactivity for the erythrocytes. There was no remarkable sex-related difference in the concentrations of radioactivity in blood or plasma.

Table 6.1.2-3: ^{14}C-Concentrations and pharmacokinetic parameters of ^{14}C in blood and plasma after a repeated oral dose at 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Time after the first adm. [h]	Time after the last adm. [h]	^{14}C-Concentration [µg eq. of inpyrfluxam/g]					
		Male			Female		
		Blood	Plasma	Blood / Plasma ratio	Blood	Plasma	Blood / Plasma ratio
24	-	<0.012	0.008 ± 0.0056	-	<0.012	0.013 ± 0.0023	-
48	-	<0.012	0.012 ± 0.0020	-	0.016 ±0.0035	0.018 ± 0.0045	0.9
96	-	0.016 ± 0.0029	0.013 ± 0.0048	1.2	0.017 ± 0.0037	0.020 ± 0.0049	0.9
312	-	0.032 ± 0.0034	0.022 ± 0.0072	1.4	0.024 ± 0.0014	0.026 ± 0.0028	0.9
312.25	0.25	0.124 ± 0.0575	0.130 ± 0.0664	1.0	0.083 ± 0.0314	0.101 ± 0.0475	0.8
312.5	0.5	0.176 ± 0.0606	0.189 ± 0.0811	0.9	0.149 ± 0.0477	0.165 ± 0.0528	0.9
313	1	0.184 ± 0.0676	0.198 ± 0.0798	0.9	0.170 ± 0.0519	0.187 ± 0.0497	0.9
314	2	0.157 ± 0.0685	0.166 ± 0.0757	0.9	0.183 ± 0.0454	0.214 ± 0.0561	0.9
316	4	0.094 ± 0.0195	0.098 ± 0.0241	1.0	0.127 ± 0.0370	0.140 ± 0.0290	0.9
320	8	0.064 ± 0.0063	0.058 ± 0.0080	1.1	0.069 ± 0.0158	0.073 ± 0.0173	0.9
324	12	0.048 ± 0.0062	0.042 ± 0.0101	1.1	0.045 ± 0.0132	0.047 ± 0.0122	1.0
336	24	0.029 ± 0.0070	0.021 ± 0.0080	1.4	0.017 ± 0.0032	0.018 ± 0.0031	1.0
360	48	0.021 ± 0.0012	0.012 ± 0.0042	1.7	<0.012	0.006 ± 0.0012	-
384	72	0.016 ± 0.0017	<0.006	-	<0.013	<0.009	-
432	120	0.014 ± 0.0017	<0.007	-	<0.012	<0.004	-
480	168	<0.012	<0.004	-	<0.012	<0.004	-
T_{max} [h] after last administration		1			2		
C_{max} [µg eq. of S-2399/g]		0.198			0.214		
$t_{1/2}$ [h]		12			9		
AUC after last administration [µg eq. of S-2399.h / g]		2.04			2.10		

Pharmacokinetic parameters were calculated from the mean values of ^{14}C-concentration in plasma

Distribution

The radioactivity concentrations in the tissues were generally low in both males and females after 7 days from the end of dosing. The highest radioactivity concentrations were noted in the liver (0.035-0.020 ppm), except for the gastrointestinal tract. The total percentages of radioactivity in tissues at 7 days after 14 consecutive administrations were 0.2% AD in males, and 0.1% AD in females; the total radioactivity concentration in the carcass was 0.1% AD in males and females. There were no remarkable sex-related differences in distribution to organs or tissues.

Table 6.1.2-4: Concentration of radioactivity in tissues in male and female rats at 7 days after 14 consecutive doses of 1 mg/kg bw of [pyrazolyl-4-^{14}C] inpyrfluxam (mean ± SD of 4 rats)

Tissue	µg eq. of inpyrfluxam/g tissue [ppm] *% of dosed ^{14}C*			
	Male		Female	
	Tissue residue	*% of dosed ^{14}C*	*Tissue residue*	*% of dosed ^{14}C*
Adrenal	<0.027	*<0.00002*	<0.020	*<0.00004*
Blood	<0.012	*<0.00543*	<0.012	*<0.00513*
Blood cell	0.026 ± 0.0018	-	<0.012	-
Plasma	<0.012	-	<0.012	-
Bone	<0.006	-	<0.006	-
Bone marrow	<0.004	-	<0.006	-
Brain	<0.009	*<0.00045*	<0.009	*<0.00061*
Caecum	0.019 ± 0.0047	*0.0 ± 0.00*	0.024 ± 0.0141	*0.0 ± 0.00*
Carcass	-	*0.1 ± 0.02*	-	*0.1 ± 0.01*
Eye	<0.009	*<0.00005*	<0.010	*<0.00007*
Fat	<0.019	-	<0.019	-
Hair and skin	0.011 ± 0.0073	-	<0.009	-
Heart	<0.009	*<0.00020*	<0.009	*<0.00022*
Kidney	<0.009	*<0.00058*	<0.009	*<0.00056*
Large intestine	<0.010	*<0.00035*	0.020 ± 0.0054	*0.0 ± 0.00*
Liver	0.035 ± 0.0025	*0.0 ± 0.01*	0.020 ± 0.0010	*0.0 ± 0.00*
Lung	<0.009	*<0.00315*	<0.009	*<0.00031*
Mandibular gland	<0.004	*<0.00005*	<0.005	*<0.00007*
Muscle	<0.010	-	<0.009	-
Ovary	-	-	<0.011	*<0.00004*
Pancreas	<0.009	*<0.00047*	<0.009	*<0.00041*
Pituitary gland	<0.141	*<0.00002*	<0.094	*<0.00004*
Sciatic nerve	<0.037	-	<0.047	-
Small intestine	<0.003	*<0.00031*	0.007 ± 0.0075	*0.0 ± 0.00*
Spinal cord	<0.009	-	<0.009	-
Spleen	<0.009	*<0.00015*	<0.010	*<0.00015*
Stomach	<0.008	*<0.00032*	0.013 ± 0.0023	*0.0 ± 0.00*
Testis	<0.009	*<0.00068*	-	-
Thymus	<0.009	*<0.00016*	<0.009	*<0.00022*
Thyroid	<0.172	*<0.00002*	<0.069	*<0.00004*

Uterus	-	-	<0.009	*<0.00016*
Caecum contents	0.010 ± 0.0091	*0.0 ± 0.00*	0.007 ± 0.0148	*0.0 ± 0.00*
Large intestine contents	0.012 ± 0.0095	*0.0 ± 0.00*	0.010 ± 0.0165	*0.0 ± 0.00*
Small intestine contents	0.010 ± 0.0060	*0.0 ± 0.00*	0.009 ± 0.0170	*0.0 ± 0.00*
Stomach contents	<0.003	*<0.00041*	<0.002	*<0.00035*
Total %	-	*0.2 ± 0.02*	-	*0.1 ± 0.01*

Metabolism

The total amounts of metabolites in urine and faeces were found to be similar between males and females on 0-3- and 11-14-days during dosing. Inpyrfluxam was extensively metabolized and a total of 12 metabolites, including two conjugates, were identified and quantified. Although, differences in the amount of some metabolites between males and females were noted, these metabolites were qualitatively similar in both males and females.

The predominant metabolites were 1′-COOH-S-2840 and N-des-Me-1′-COOH-S-2840 in urine and N-des-Me-1'-CH_2OH-S-2840, 1'-CH_2OH-3'-OH-S-2840 and glucuronide of 1'-CH_2OH-S-2399 in faeces. The parent compound was only detected in low amounts in faeces.

Table 6.1.2-5: Metabolites levels in urine and faeces in male and female rats during repeated oral doses at 1 mg/kg of [pyrazolyl-4-^{14}C] inpyrfluxam (Data from pooled sample of 4 rats)

Metabolite	% of total radioactivity in pooled urine and pooled faeces				% of the dose			
	Male		Female		Male		Female	
	0-3 days	11-14 days	0-3 days	11-14 days	0-3 days	11-14 days	0-3 days	11-14 days
Urine								
N-des-Me-1',1'-bis(CH_2OH)-S-2840	1.7	1.9	4.8	4.0	0.3	0.4	0.9	0.9
1',1'-bis(CH_2OH)-S-2840	4.6	4.0	3.3	3.5	0.7	0.9	0.6	0.8
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	1.5	0.5	3.6	4.3	0.2	0.1	0.6	1.0
glucuronide of 1'-CH_2OH-S-2840	0.8	1.1	1.1	ND	0.1	0.2	0.2	ND
N-des-Me-1'-CH_2OH-3'-OH-S-2840	3.2	2.9	2.4	4.2	0.5	0.6	0.4	0.9
N-des-Me-1'-CH_2OH-S-2840 1'-CH_2OH-3'-OH-S-2840	2.4	3.0	2.0	3.0	0.4	0.7	0.3	0.7
***N*-des-Me-1'-COOH-S-2840**	5.3	6.3	15.3	14.0	0.8	1.4	**2.7**	**3.1**
1'-COOH-S-2840	10.4	9.2	8.3	7.5	**1.6**	**2.0**	1.5	1.6
Others*	7.5	12.5	14.8	14.2	1.1	2.7	2.6	3.1
Total urine	37.3	41.4	55.5	54.6	5.7	9.1	9.9	12.1
Faeces								
N-des-Me-1',1'-bis(CH_2OH)-S-2840	2.7	2.2	3.7	3.9	0.4	0.5	0.7	0.9
1',1'-bis(CH_2OH)-S-2840	3.0	2.4	2.2	ND	0.5	0.5	0.4	ND
glucuronide of *N*-des-Me-1'-CH_2OH-S-2840	0.8	1.2	4.9	7.6	0.1	0.3	0.9	1.7

glucuronide of 1'-CH_2OH-S-2399	5.4	7.8	8.4	9.3	0.8	1.7	**1.5**	**2.0**
N-des-Me-1'-CH_2OH-3'-OH-S-2840	3.4	2.0			0.5	0.4		
***N*-des-Me-1'-CH_2OH-S-2840** **1'-CH_2OH-3'-OH-S-2840**	9.1	5.3	10.0	6.2	**1.4**	**1.2**	1.8	1.4
N-des-Me-1'-COOH-S-2840	3.8	6.9	6.0	7.8	0.6	1.5	1.1	1.7
1'-CH_2OH-S-2840	4.4	2.9	ND	1.0	0.7	0.6	ND	0.2
1'-COOH-S-2840	3.8	3.9	0.8	1.3	0.6	0.8	0.1	0.3
7'-OH-S-2399	1.8	1.4			0.3	0.3		
N-des-Me-S-2840	0.6	0.9	3.0	1.5	0.1	0.2	0.5	0.3
S-2399	0.9	1.2	0.7	ND	0.1	0.3	0.1	ND
Others**	13.4	10.4	0.0	1.2	2.0	2.3	0.0	0.3
Subtotal	53.1	48.4	39.7	39.9	8.1	10.6	7.1	8.8
acidic methanol extracts	2.6	2.7	1.7	1.8	0.4	0.6	0.3	0.4
basic methanol extracts	2.4	2.4	1.2	1.3	0.4	0.5	0.2	0.3
Unextractable	4.6	5.1	1.8	2.3	0.7	1.1	0.3	0.5
Total faeces	62.7	58.6	44.5	45.4	9.5	12.8	8.0	10.0

Data were obtained from the pooled sample of 4 rats. ND: not detected; --: not analysed; *: sum of the 28 unidentified metabolites which are not more than 0.3% of the dose in males, and of the 27 unidentified metabolites which are not more than 0.4% of the dose in females; **: sum of the 23 unidentified metabolites which are not more than 0.2% of the dose in males, and one unidentified metabolite which is not more than 0.3% of the dose in females

Conclusion

According to this GLP and guideline repeated dose ADME study, inpyrfluxam was rapidly absorbed from the GI tract with T_{max} of 1-2 hr reached following the end of dosing for 14 days. Inpyrfluxam and/or its metabolites were widely distributed. Higher levels were found in blood, plasma and liver, but overall levels after 7 days from the end of dosing were very low. These data indicate that inpyrfluxam and/or its metabolites do not accumulate.

Elimination was rapid and was essentially complete, with >90% of the AD excreted within 24 hours following the end of dosing. Elimination was predominantly via urine (33 – 51.6% of the AD) and faeces (44.8 – 61.5% of the AD). There were no sex related differences in excretion or distribution to organs or tissues.

Inpyrfluxam was extensively metabolized, and metabolites were detected in urine and faeces. In addition to the parent, a total of 12 metabolites, including two conjugates, were identified and quantified.

([redacted] 2016b)

B.6.1.2. Metabolism studies in vitro

1. Rat and human liver microsomes

Reference:	KCA 5.1.1/03
Report Title:	Comparative in vitro metabolism study of [pyrazolyl-4-^{14}C] S-2399 in rat and human liver microsomes
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. TPM-0052 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd. Japan
Substance used:	Test Material radiolabelled: [pyrazolyl-4-^{14}C] S-2399 ([pyrazolyl-4-^{14}C] inpyrfluxam) Lot/Batch: CFQ41802 Purity: ≥98.4% Test Material unlabelled: S-2399 A.S (inpyrfluxam) Lot/Batch: YT3424G Purity: 99.9%
Method of analysis:	Validated method of analysis not required
Guideline(s):	No, but compared to EFSA PPR Panel Opinion 2021
Deviations from current guideline:	N/A; some deviations from the EFSA Opinion discussed below after the second study in dog microsomes, but noted that studies conducted before Opinion was issued.
Impact of the deviation:	N/A; see text below
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Method

The in vitro metabolism of inpyrfluxam was investigated in a GLP compliant study using rat (males and females) and human (mix genders) liver microsomes. After incubation of 10 µM ^{14}C - pyrazolyl labelled inpyrfluxam with liver microsomes in the presence of 3 mM NADPH in 3 replicates, the metabolites were quantified by HPLC radio-chromatography, and identified by LC-MS analysis.

A preliminary study was conducted using 1, 10 and 100 µM in rat (male and female) liver microsomes for 0, 15, 30, 60 and 120 minutes to determine the concentration and incubation

time for the main study. Loss of linearity in inpyrfluxam depletion rate was noted at 100 µM at all time points, indicating saturation of metabolic reactions. The concentrations of formed metabolites were relatively higher at 10 µM compared to 1 µM. Also, there was a steady decrease in the depletion rate of inpyrfluxam at and after 30 min. Therefore, 10 µM concentration and 15 minutes incubation time were selected for the main study.

Concentration and homogeneity of the testing solutions were confirmed within the study. The metabolic activity of the enzyme fractions was demonstrated by the metabolization of a concurrent positive control utilising [4-^{14}C]-testosterone at 100 µM, which was incubated under identical conditions.

Results

Inpyrfluxam in buffer without microsomes was found to be stable as its peak accounted for 100% radioactivity after 15 min incubation. Therefore, any loss of parent compound or formation of metabolites was considered to be due to the microsomal enzyme activity.

Inpyrfluxam was extensively metabolised and 6 of the 10 detected metabolites were identified and quantified. The major metabolites were 1'-CH_2OH-S-2840 (36% administered radioactivity – AR in humans and 41% AR in male rats) and N-des-Me-S-2840 (20% AR in female rats and 29% AR in humans). The other four metabolites, N-des-Me-1'-CH_2OH-S-2840, 3'-OH-S-2840, 7'-OH-S-2399, and 1'-CH_2OH-3'-OH-S-2840 were detected only in minor levels (≤10% AR). Four unidentified metabolites (6% AR) were detected only in male rats.

A summary of quantitative results following incubation of 10 µM inpyrfluxam with human, and rat liver microsomes for 15 minutes is presented in Table 6.1.3-1.

Table 6.1.3-1: Quantitative results of an in vitro metabolism study (mean ± SD of 3 replicates) in human and rat liver microsomes

Metabolite	% of total radio-chromatogram			pmol/min/mg protein		
	Human	Male rat	Female rat	Human	Male rat	Female rat
1'-CH_2OH-3'-OH-S-2840	-	7 ± 0.5	-	-	47 ± 3.3	-
N-des-Me-1'-CH_2OH-S-2840	5 ± 0.1	5 ± 0.2	-	35 ± 0.9	36 ± 1.0	-
1'-CH_2OH-S-2840	**36 ± 0.6**	**41 ± 0.7**	10 ± 1.0	237 ± 4.0	274 ± 5.0	67 ± 6.6
7'-OH-S-2399	1 ± 0.1	3 ± 0.2	<LOQ	9 ± 0.9	17 ± 1.2	<LOQ
3'-OH-S-2840	2 ± 0.3	10 ± 0.6	-	10 ± 1.7	64 ± 4.1	-

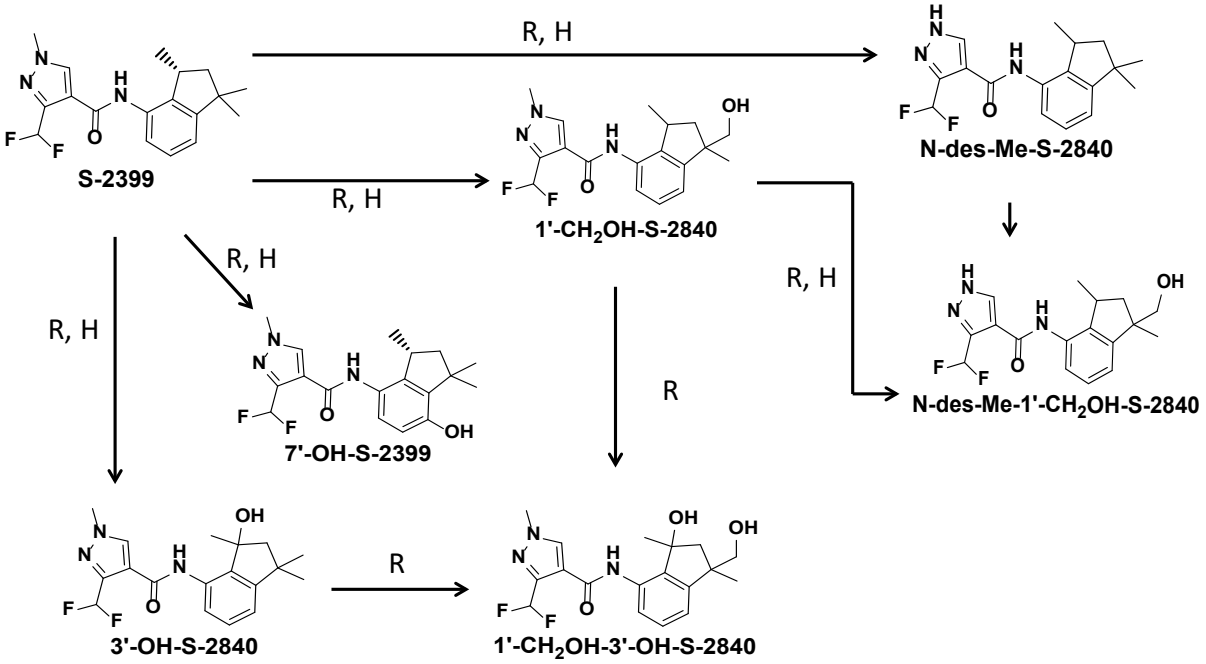
***N*-des-Me-S-2840**	**29 ± 0.3**	7 ± 0.5	**20 ± 0.5**	193 ± 2.3	46 ± 3.1	134 ± 3.1
S-2399	27 ± 0.4	21 ± 0.5	69 ± 1.1	-	-	-
others	-	6 ± 2.7*	-	-	-	-
LOQ	1			6		

LOQ: Limit of quantification; *: sum of 4 unidentified metabolites which were below 1-2% of total radio-chromatogram.

Conclusion

According to this GLP in vitro comparative metabolism study, inpyrfluxam was extensively bio-transformed after incubation with rat (male and female) and human liver microsomes. The human (mixed gender) and male rat groups showed a higher degree of biotransformation compared to the female rat microsomes. The main metabolic reactions of [pyrazolyl-4-14C] inpyrfluxam were identified as N-demethylation and oxidation of the 1′,1′-dimethyl group of the indane ring. In addition, hydroxylation at the 3′ and 7′ positions of the indane ring were identified as minor metabolic reactions. The same metabolic pathways were noted in human and rat microsomal incubations. No unique human metabolite was detected.

Figure B.6.1.3-1: Proposed metabolic pathway of inpyrfluxam in human and rat liver microsomes.



R : Rat liver microsomes
H : Human liver microsomes

-

([redacted] 2017)

2. Dog liver microsomes

Reference:	KCA 5.1.1/04
Report Title:	In vitro metabolism study of [pyrazolyl-4-^{14}C] S-2399 in dog liver microsomes
Author(s) & Year:	[redacted] (2018)
Document No, Authority registration No	Study No. TPM-0056 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd. Japan
Substance used:	Test Material radiolabelled: [pyrazolyl-4-^{14}C] S-2399 ([pyrazolyl-4-^{14}C] inpyrfluxam) Lot/Batch: CFQ41802 Purity: ≥98.4% Test Material unlabelled: S-2399 A.S (inpyrfluxam) Lot/Batch: YT3424G Purity: 99.9%
Method of analysis:	Validated method of analysis not required
Guideline(s):	No, but compared to EFSA PPR Panel Opinion 2021
Deviations from current guideline:	N/A; some deviations from the EFSA Opinion discussed below, but noted that study conducted before Opinion was issued.
Impact of the deviation:	N/A; see text below
GLP or GEP:	Yes
Acceptability:	Yes
Study relied upon:	Yes

Method

The in vitro metabolism of inpyrfluxam was further investigated in a second GLP compliant study using dog (male and female) liver microsomes. After incubation of 10 µM ^{14}C - pyrazolyl labelled inpyrfluxam with liver microsomes in the presence of 3 mM NADPH in 3 replicates, the parent and metabolites were quantified by HPLC radio-chromatography and identified by LC-MS analysis.

Concentration and homogeneity of the testing solutions were confirmed within the study. The metabolic activity of the enzyme fractions was demonstrated by the metabolization of a

concurrent positive control utilising [4-^{14}C]-testosterone at 100 µM, which was incubated under identical conditions.

Results

Inpyrfluxam in buffer without microsomes was found to be stable as its peak accounted for 100% radioactivity after 15 min incubation. Therefore, any loss of parent compound or formation of metabolites was considered to be due to the microsomal enzyme activity.

Inpyrfluxam was extensively metabolised and 6 of the 10 detected metabolites were identified and quantified. The major metabolites were N-des-Me-S-2840 (19% AR in males and 17% AR in females, which were lower than the levels measured in human microsomes in the first study but not more than 4 times lower) and 1'- CH_2OH-S-2840 (16% AR in males and 11% AR in females which were also lower than the levels measured in human microsomes in the first study but not more than 4 times lower). The other two metabolites, 3'-OH-S-2840 and 7'-OH-S-2399 were detected only in minor levels (≤10% AR).

A summary of quantitative results following incubation of 10 µM inpyrfluxam with dog liver microsomes for 15 minutes is presented in Table 6.1.3-1.

Table 6.1.3-1: Quantitative results of an in vitro metabolism study (mean ± SD of 3 replicates) in dog liver microsomes

Metabolite	% of total radio-chromatogram		pmol/min/mg protein	
	Male dog	Female dog	Male dog	Female dog
1'- CH_2OH-S-2840	16 ± 0.7	11 ± 1.0	105 ± 4.6	70 ± 6.5
7'-OH-S-2399	4 ± 0.2	5 ± 0.3	26 ± 1.4	31 ± 1.9
3'-OH-S-2840	3 ± 0.3	-	18 ± 2.1	-
***N*-des-Me-S-2840**	**19 ± 0.9**	**17 ± 1.0**	124 ± 6.1	113 ± 6.9
S-2399	59 ± 0.7	68 ± 1.7	-	-
LOQ	2	1	10	9

LOQ: Limit of quantification

Conclusion

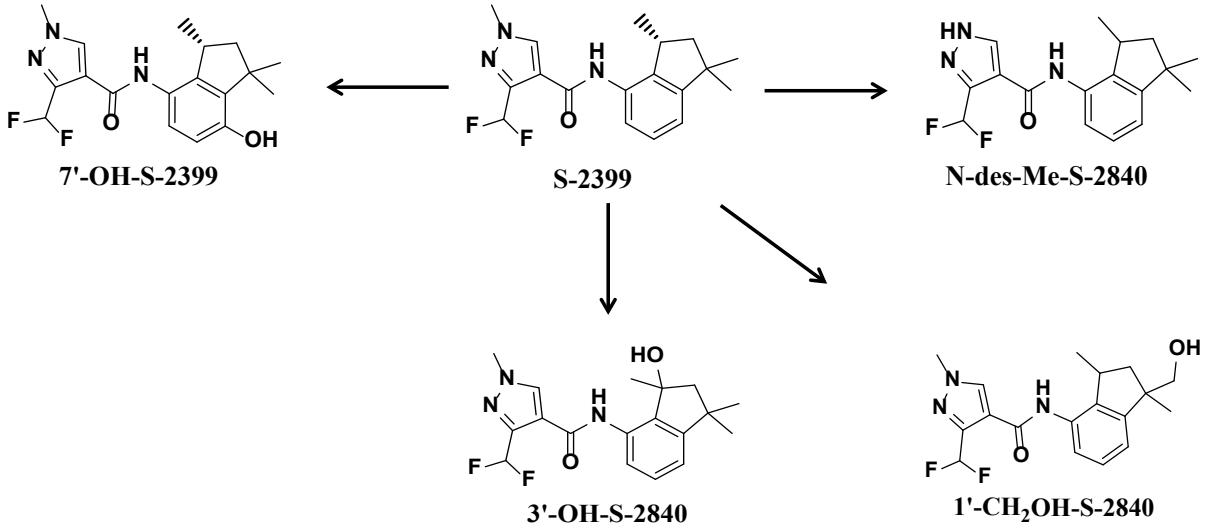
According to this GLP in vitro metabolism study, inpyrfluxam was bio-transformed after incubation with dog liver microsomes. The male group showed a higher degree of biotransformation compared to the female dog microsomes. The metabolites detected in dog microsomes were also seen in human microsomes in the previous study, although at slightly lower levels.

A metabolic pathway was proposed by the applicant with N-demethylation, oxidation of the 1',1'-dimethylgroup of the indane ring along with the minor metabolic reactions 3'and 7'-hydroxylation of the indane ring.

Both in vitro metabolism studies conducted with inpyrfluxam showed some deviations from the EFSA PPR Panel Opinion (2021) on testing and interpretation of in vitro comparative

metabolism studies. However, HSE notes that these inpyrfluxam studies were conducted before the Opinion was issued. Liver microsomes rather than hepatocytes were used. However, as in rat microsomes, similar metabolites to those seen in vivo in the rat were identified, it is considered that microsomes were sufficiently representative of the in vivo situation. Only rat and dog microsomes were tested, with no inclusion of microsomes from other species used in the toxicology package, such as the mouse and rabbit. This is not considered a major limitation as both the mouse and rabbit appear to be less sensitive to the toxicity of inpyrfluxam compared to the rat and dog.

Figure B.6.1.3-1: Proposed metabolic pathway of inpyrfluxam in dog liver microsomes.



([redacted] 2018)

B.6.1.3. Toxicokinetic information from toxicodynamic studies

Additional kinetic information on absorption is available in the 28-day study in rats, 1 year study in dogs and long-term toxicity studies in rats and mice, where proof of absorption data were generated.

In the 28-day oral (dietary) toxicity study in rats in which animals were dosed with 500, 1000, 3000 and 5000 ppm (mean substance intakes: 44.4, 85.9, 246.4 and 406.5 mg/kg bw/day for males and 47.4, 91.4, 263.0 and 377.8 mg/kg bw/day for females) plasma concentration of inpyrfluxam was measured at the end of treatment. Plasma concentration of inpyrfluxam was similar at the two lower doses (44.4 mg/kg bw/day and 85.9 mg/kg bw/day) in both males and females. The males followed a trend of dose-dependency from 85.9 mg/kg bw/day whereas no dose-dependency was observed in females (section B.6.3.1, [redacted] 2014).

In the one-year oral (gelatin capsule) toxicity study in dogs in which animals were dosed with 0, 2, 6, 30 or 160 mg/kg bw/day, concentrations of inpyrfluxam in plasma were

measured at 0 (before administration), 2, 4, 7, and 24 hours after administration at day 1, weeks 13, 26 and 52 after treatment. Inpyrfluxam was detected in the plasma of both sexes at all dose levels and all time points except at 2 mg/kg bw/day where it was lower than the limit of quantification (LOQ). A dose dependent increase in plasma levels of inpyrfluxam was noted throughout the treatment. There was no difference in the systemic exposure of male and female dogs. In addition, no accumulation after repeated oral (capsule) administration was observed in both sexes (section B.6.3.3, [redacted] 2017).

In the long-term toxicity study in rats in which females were dosed with 0, 150, 500 or 1500/1000 ppm (mean substance intake: 0, 6.77, 22.8 and 95.9 mg/kg bw/day) and males were dosed with 0, 150, 500 or 2000 ppm (mean substance intake: 0, 8.84, 30.1 and 86.4 mg/kg bw/day) over 52 weeks, plasma concentration of inpyrfluxam was analysed at 14, 26 and 51 weeks of treatment. Inpyrfluxam was detected in the plasma of females at all dose levels and in males at the high dose (at weeks 14, 26 and 51) whereas it was under the limit of quantification (LOQ) in the low and mid dose males. A dose dependent increase in plasma levels of inpyrfluxam was noted in females. At the high dose, the plasma concentrations in females were higher than in males. Furthermore, no accumulation after repeated oral administration was observed in both sexes (section B.6.5.1, [redacted] 2017).

In the long-term toxicity study in mice in which animals were dosed with 0, 700, 2000 or 7000/5000 ppm (mean substance intakes: 0, 77.1, 240 and 826 mg/kg bw/day for males and 0, 72.9, 222 and 790 mg/kg bw/day for females) over 52 weeks, plasma concentrations of inpyrfluxam were analysed after the end of the treatment. Inpyrfluxam was detected in the plasma of both sexes at the mid and high dose levels whereas it was under the limit of quantification (LOQ) at the low dose. A dose dependent increase in plasma levels of inpyrfluxam was noted in both sexes. At the high dose, the plasma concentrations in males were higher than in females (section B.6.5.2, [redacted] (2017).

No other toxicokinetic-specific information was available from the submitted toxicodynamic studies.

B.6.1.4. Absorption, distribution, metabolism and excretion by other routes

Dermal absorption of inpyrfluxam in its representative product is addressed in the product B6 document.

B.6.1.5. Toxicological consideration for the residue definition for body fluids and tissues

Body fluids: Metabolite, 1'-COOH-S-2840 is a major rat metabolite in urine. Therefore, the residue definition for body fluid should be from a toxicological point of view, 1'-COOH-S-2840 in urine. A validated method of analysis for this metabolite in urine is available.

Tissues: Metabolite, 1'-COOH-S-2840 is the most abundant metabolite in liver/kidney in the ruminant metabolism study. Therefore, the residue definition for tissues from a toxicological point of view should be 1'-COOH-S-2840 in liver/kidney. A validated method of analysis for this metabolite in liver/kidney is available.

B.6.1.6. Summary of ADME

The ADME properties of inpyrfluxam have been investigated via oral (gavage) dosing in rats. The data set consists of 2 studies using [pyrazolyl-4-^{14}C] and [phenyl-^{14}C] radiolabelled inpyrfluxam, the first following single exposure and the second following repeated exposure for 14 days. Two in vitro metabolism studies are also available, the first employing human and rat liver microsomes and the second using dog liver microsomes only. The main findings of these studies are summarised in table 6.1.6-1 below.

Table 6.1.6-1 Summary of available ADME studies

Data point/ Study Acceptability	Species / Strain/ sex	Doses	Summary
KCA 5.1.1/01 *Metabolism of S-2399 in rats* - GLP *Acceptable*	**Rat**/ Wistar Hannover rats/M & F	Males and Females: 1 and 150 mg/kg bw Single oral (gavage) dose *Radiolabel positions:* ([pyrazolyl-4-^{14}C] inpyrfluxam) and ([phenyl-^{14}C] inpyrfluxam)	12 metabolites were identified and characterised in excreta. The two major metabolites in urine were **1′-COOH-S-2840 and N-des-Me-1′-COOH-S-2840** The major metabolite in the bile was **glucuronide of 1'-CH_2OH-S-2840** Elimination was predominantly via urine and bile. Evidence of enterohepatic recirculation.
KCA 5.1.1/02 *Metabolism of S-2399 in rats (Repeated Oral Administration)* - GLP *Acceptable*	**Rat**/ Wistar Hannover rats/M & F	Males and Females: 1 mg/kg bw repeated oral (gavage) dose for 14 days *Radiolabel position:* ([pyrazolyl-4-^{14}C] inpyrfluxam)	12 metabolites were identified and characterised in excreta. No major (>10%) metabolites detected. Elimination was predominantly via urine and faeces. No accumulation
KCA 5.1.1/03 *Comparative in vitro metabolism study of [pyrazolyl-4-14C]S-2399*	**Human** (mix genders) and **rat** (male and	10 µM for 15 minutes	6 metabolites were identified and characterised. No unique human metabolite

in rat and human liver microsomes - GLP *Acceptable*	female) liver microsomes		
KCA 5.1.1/04 *In vitro metabolism study of [pyrazolyl-4-14C]S-2399 in dog liver microsomes* - GLP *Acceptable*	**Dog** (male and female) liver microsomes	10 µM for 15 minutes	6 metabolites were identified and characterised.

Overall conclusions of ADME:

Absorption

In the single oral dose study in rats, absorption of inpyrfluxam from the GI tract was more rapid at 1 mg/kg bw (plasma T_{max} of radioactivity reached at 1 h) than at 150 mg/kg bw (plasma T_{max} of radioactivity reached at 8 h). The C_{max} and AUC increased with the dose in a linear manner.

From the bile-duct experiment, it was clear that inpyrfluxam was almost completely absorbed (96%) by the oral route at the low dose of 1 mg/kg bw. An **oral absorption value of 100%** is considered appropriate at the low dose of 1 mg/kg bw. No saturation in absorption or bioaccumulation were noted at the high dose of 150 mg/kg bw.

In the repeated dose study in rats, absorption of inpyrfluxam from the GI tract was also rapid at 1 mg/kg bw, with the plasma T_{max} reached at 1 h and 2 h after administration in males and females respectively.

No data is available on inpyrfluxam with regards to **inhalation absorption**. Therefore, a default value of **100%** is proposed for inpyrfluxam. The dermal absorption of inpyrfluxam from the representative product S-2399 60 g/L EC was determined from an in vitro study in accordance with the EFSA Guidance on Dermal Absorption (EFSA Journal 2017;15(6):4873). **Dermal absorption values of 8.8% and 20%** were established for the concentrate and dilution respectively. For more detail, please refer to the volume 3CP B6.

Post hepatic systemic availability was estimated as 60% (difference between the 96% oral absorption value and the mean level of radioactivity excreted in bile during the first 6 hours (35%) and considered not to be systemically available).

Distribution

Inpyrfluxam and/or its metabolites were widely distributed, with higher levels found in blood, plasma, liver, kidney, adrenal and heart.

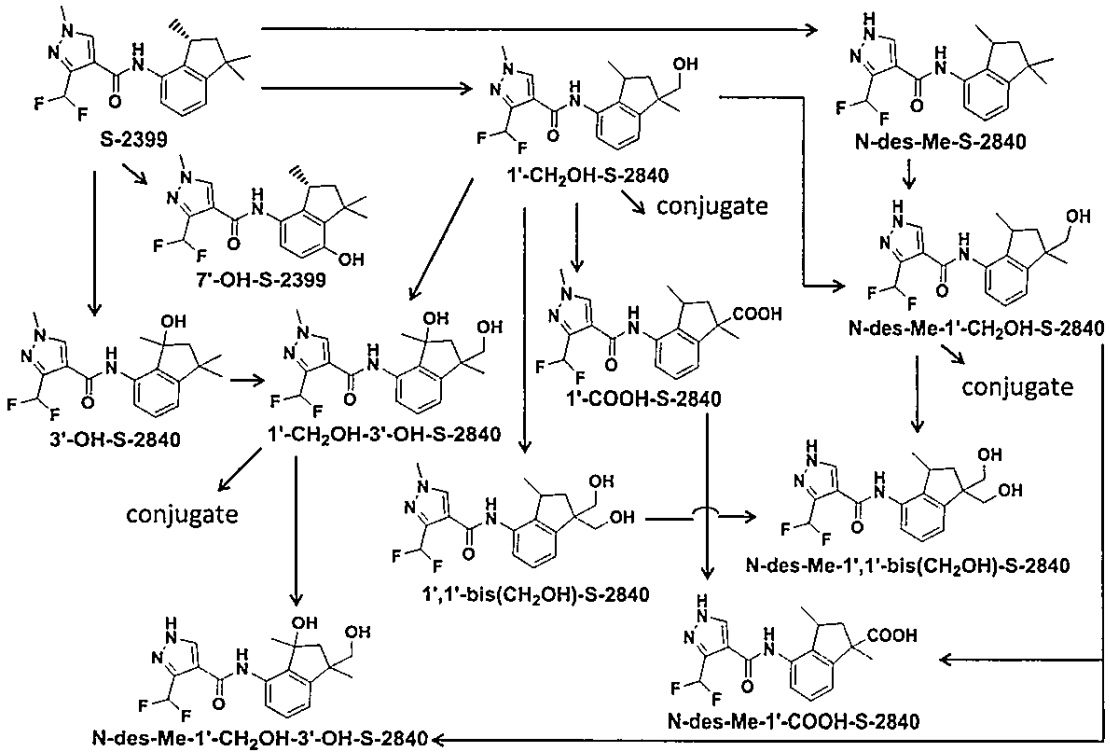
In the single oral dose study, at the low dose of 1 mg/kg bw, the plasma T_{max} of radioactivity was reached at 1 h after dosing with a C_{max} of 1.77 µg equivalents/g·h in males and 1.63 µg equivalents/g·h in females. At the high dose of 150 mg/kg bw, the plasma T_{max} of radioactivity was reached at 8 h after dosing with a C_{max} of 270 µg equivalents/g·h in males and 382 µg equivalents/g·h in females. Within 72 hours, the level of radioactivity in plasma had declined to extremely low levels, indicating that there was no retention of inpyrfluxam and/or its metabolites at the doses tested. The half-life of radioactivity remained comparable between sexes and ranged from 12 to 17 hours.

At 1 and 150 mg/kg bw, the tissue T_{max} of radioactivity was reached at 0.25 - 1 h and 1 – 8 h after dosing respectively except for the gastrointestinal tract or its content. At the low and high dose, the mean elimination half-lives of radioactivity were 2 - 8 h and 7 - 48 h respectively after dosing, in the examined tissues, except for the gastrointestinal tract or its content. At 168 h after administration of both doses, the total radioactive residue in the carcass was 0.1- 0.2% AD. No remarkable sex-related difference was observed in the tissue C_{max} values at either dose, although the C_{max} and half-lives were higher at the high dose.

In the repeated oral dose study, at 1 mg/kg bw, the plasma T_{max} of radioactivity was reached at 1 h and 2 h after dosing in males and females respectively. The radioactivity concentrations in the tissues were generally low in both males and females after 7 days from the end of dosing. The highest radioactivity concentrations were noted in the liver and GI tract. The total percentages of radioactivity in tissues 7 days after 14 consecutive administrations were 0.2% AD in males, and 0.1% AD in females. There was no evidence of bioaccumulation. In addition, no remarkable sex-related differences in distribution in organs or tissues were noted.

Metabolism

Inpyrfluxam was extensively metabolized, and in addition to the parent, a total of 12 metabolites, including two conjugates, were identified, and quantified in both single and repeat dose studies in rats. 1′-COOH-S-2840 and N-desMe-1′-COOH-S-2840 were the major metabolites in urine and the glucuronide of 1′-CH_2OH-S-2840 was a major metabolite in bile. From the in vitro metabolism studies using liver microsomes, no unique human metabolite was identified. A metabolic pathway for inpyrfluxam was proposed by the applicant with N-demethylation, oxidation of the 1',1'-dimethylgroup of the indane ring followed by further oxidation to carboxylic acid and glucuronide conjugation along with minor metabolic reactions such as 3'and 7'-hydroxylation of the indane ring.



Elimination

In the single dose study, elimination was rapid, with >67% AD excreted within 24 hours of dosing and was essentially complete (>90% AD) within 2-3 days. Elimination was predominantly via urine (49.2% – 61% AD) and bile (46.9 – 68.9% AD). Faecal excretion of radioactivity was between 39.4% and 47.9% AD, at 168 hours, of which a significant proportion was excreted via bile. There was evidence of entero-hepatic re-circulation.

In the repeated dose study, elimination was rapid and was essentially complete, with >90% AD excreted within 24 hours after the end of dosing. Elimination was predominantly via urine (33 – 51.6% AD) and faeces (44.8 – 61.5% AD).

Overall conclusion

Overall, the following conclusions regarding the toxicokinetic properties of inpyrfluxam can be made:

- Absorption of inpyrfluxam from the GI tract was rapid and practically complete.
- An overall **oral absorption value of 100%** was established. However, the **post hepatic systemic availability** was estimated **at 60%.** With regard to **inhalation absorption a default value of 100%** was proposed. **Dermal absorption values of 8.8 and 20%** were established for inpyrfluxam in the representative product for the concentrate and dilution respectively.

- Radioactivity was widely distributed throughout the body and plasma levels rapidly declined once peak plasma concentrations were reached.
- Inpyrfluxam was extensively metabolized. Up to 12 metabolites were identified and characterised in the excreta of rats, suggesting that when the parent substance is metabolised, numerous reactions take place.
- No significant bioaccumulation of radioactivity was noted in the plasma or tissue during repeat dose testing with inpyrfluxam.
- No unique human metabolites were observed in the in vitro comparative metabolism study.
- The residue definition for body fluid from a toxicological point of view should be 1'-COOH-S-2840 in urine.
- The residue definition for tissues from a toxicological point of view should be 1'-COOH-S-2840 in liver/kidney.

B.6.2. Acute Toxicity

The acute toxicity of inpyrfluxam was evaluated in multiple OECD and GLP compliant studies using both *in vivo* and *in vitro* models.

For skin sensitisation, the applicant has submitted a guinea pig maximisation test (GPMT). According to assimilated Regulation 283/2013, the local lymph node assay (LLNA) is the preferred study. The applicant has provided justification for the submission of the GPMT, and the study provides a clear result; therefore, further testing is not considered necessary.

B.6.2.1. Oral

The acute oral toxicity of inpyrfluxam was investigated in rats using two GLP compliant studies. Both studies were conducted according to OECD test guidelines (TG423 and TG425). The second study using the up and down procedure ([redacted] (2017a)) was performed to determine whether the acute oral LD_{50} for inpyrfluxam is greater than 180 mg/kg bw for the acute risk assessment of mammals in the environment. Therefore, both studies are deemed to be acceptable and reliable.

1. Acute Oral Toxicity Study of Inpyrfluxam in Rats – Toxic class method

Reference:	KCA 5.2.1/01
Report Title:	Acute Oral Toxicity Study of S-2399 Technical Grade in Rats
Author(s) & Year:	[redacted] (2015a)
Document No, Authority	Study No. 4310 [redacted]

registration No	
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 423 (2001)
Deviations from current guideline :	Yes. A minor deviation was observed where 12h light was suspended for a minute due to an accidental blackout.
Impact of the deviation:	There is no impact on the integrity of the study and the data obtained due to the deviation observed.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The acute oral toxicity of inpyrfluxam was assessed in a GLP and OECD compliant study using the toxic class method. Three female RccHan Wistar rats per dose were sequentially administered with 300 mg/kg bw, 50 mg/kg bw and 50 mg/kg bw inpyrfluxam in 0.5% w/v aqueous methylcellulose via oral gavage, depending on the observation of toxic signs and mortality according to Annex 2c of the test guideline. All the investigations required by the guideline were performed.

Results

Two of the 3 animals administered with 300 mg/kg bw of inpyrfluxam died within a day of administration. No mortality was observed in both the low dose groups (50 mg/kg bw).

All the animals administered with 300 mg/kg bw of inpyrfluxam showed a decrease in spontaneous activity and ataxic gait but the surviving animal recovered by day 1. Along with the prone or lateral position in two dead animals, loss of righting reflex was also observed in one of the dead animals. Additionally, decreased spontaneous activity was observed in one of the three animals administered with 50 mg/kg bw after 2 hours but it recovered at 4 hours post-administration. The results are summarized in the table 6.2.1-1.

Table 6.2.1-1: Acute oral toxicity of inpyrfluxam to rats

Dose (mg/kg bw)	Toxicological results*	Duration of signs	Time of death	LD_{50} (mg/kg bw)

300	2/3/3	0.5-4 h	4 h – 1 d	50 < LD_{50} < 300 mg/kg bw
50	0/1/6	2 h	NA	

* number of animals which died/number of animals with clinical signs/number of animals used;

NA: not applicable

No significant changes in bodyweight were noted in any of the surviving dosed animals.

There were no macroscopic pathological findings in any of the dosed animals.

Conclusion

Under the conditions of this GLP and OECD compliant study, the acute oral toxicity of inpyrfluxam was 50 < LD_{50} < 300 mg/kg bw in female RccHan:WIST rats. Inpyrfluxam is acutely toxic via the oral route and meets the criteria for classification for acute oral toxicity in category 3 (H301) according to Regulation 1272/2008 as it applies in GB.

[redacted] (2015a)

2. Acute Oral Toxicity Study of Inpyrfluxam in Rats – Up and down procedure

Reference:	KCA 5.2.1/02
Report Title:	Acute Oral Toxicity Study of S-2399 Technical Grade in Rats (Up and Down procedure)
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. 4374 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 425 (2008)
Deviations from current guideline :	The study utilised the dose levels of 57, 180 and 570 mg/kg bw (rather than 55, 175 and 550 mg/ kg bw) to determine whether the acute oral LD_{50} for inpyrfluxam is greater than 180 mg/kg bw for the acute risk assessment of mammals in the environment.
Impact of the deviation:	There is no impact of the deviation on the integrity of the study or the data obtained
GLP or GEP:	Yes - GLP

Acceptability:	Yes
Study relied upon:	Yes

Methods

The acute oral toxicity of inpyrfluxam was assessed in a second GLP and OECD test guideline compliant study using the Up and Down procedure (OECD TG 425, 2008). To aid in the acute risk assessment of mammals in the environment, it was necessary to determine whether the acute oral LD_{50} of inpyrfluxam is above 180 mg/kg bw or not. Therefore, 180 mg/kg bw of inpyrfluxam in 0.5% w/v aqueous methylcellulose was initially administered to a single female RccHan:WIST rat via oral gavage. Based on the observations, a further five animals were administered with either 570 mg/kg bw, 180 mg/kg or 57 mg/kg bw of inpyrfluxam. All the investigations required by the guideline were performed.

Results

No mortality was observed in animals that were administered with 57 mg/kg bw of inpyrfluxam. Two of the three animals dosed at 180 mg/kg bw and the animal dosed at 570 mg/kg bw were found dead.

Reduction in spontaneous activity and ataxic gait were observed in the animals that received 180 mg/kg bw and 570 mg/kg bw; the surviving animal dosed at 180 mg/kg bw recovered by Day 1. The animals administered with 57 mg/kg bw showed no clinical signs throughout the observation period. A summary of the results are tabulated in Table 6.2.1-2.

Table 6.2.1-2: Acute oral toxicity of inpyrfluxam to rats – Up and down procedure

Dose (mg/kg bw)	Toxicological results*	Duration of signs	Time of death	LD_{50} (mg/kg bw)
57	0/0/2	NA	NA	180 (95% confidence interval 30.08 to 735 mg/kg)
180	2/3/3	0.5-4 h	4 h	
570	1/1/1	1 h	2 h	

* number of animals which died/number of animals with clinical signs/number of animals used;
NA: not applicable

There were no changes in the body weight of the surviving animals. Body weight was decreased in some of the animals that died.

There were no macroscopic pathological findings in any of the dosed animals.

Conclusion

In this GLP and OECD compliant study, the acute oral LD_{50} value of inpyrfluxam was estimated to be 180 mg/kg bw (95% confidence interval: 30.08 mg/kg bw to 735 mg/kg bw) in female RccHan:WIST rats. In conclusion, inpyrfluxam meets the criteria for classification

for acute oral toxicity in category 3 (H301) according to Regulation 1272/2008, as it applies in GB.

[redacted] (2017a)

Overall conclusion on acute oral toxicity

The acute oral toxicity of inpyrfluxam was investigated in rats in two studies, one following the toxic class method (50 < LD_{50} < 300 mg/kg bw) and the other according to the up and down procedure (LD_{50} = 180 mg/kg bw). Both studies show that inpyrfluxam meets the criteria for classification for **acute oral toxicity, category 3 (H301)** according to Regulation 1272/2008, as it applies in GB. The second study (up and down procedure) allows refinement of the assessment to give an LD_{50} value of 180 mg/kg bw (95% confidence interval: 30.08 mg/kg bw to 735 mg/kg bw), which is consistent with the results seen in the acute toxic class study.

B.6.2.2. Dermal

The acute dermal toxicity of inpyrfluxam was investigated in rats using a GLP and OECD compliant study performed according to OECD 402.

Reference:	KCA 5.2.2/01
Report Title:	Acute Dermal Toxicity Study of S-2399 Technical Grade in Rats
Author(s) & Year:	[redacted] (2015b)
Document No, Authority registration No	Study No. 4306 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 402 (1987)
Deviations from current guideline :	From OECD 402 (2017): Two sexes were used in the study and the guideline requires 2 animals at each dose where 10 animals were tested in the present study From OECD 402 (1987): Two sexes were used in the present study
Impact of the deviation:	The deviations do not impact on the validity of the results and the methods used are still reliable to predict the acute dermal toxicity potential of the test substance.

GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD TG compliant acute dermal toxicity study, 5 male and 5 female RccHan:WIST rats were exposed to inpyrfluxam on clipped dorsal skin at 2000 mg/kg bw for 24 hours as a single dose. All the investigations required by the guideline were performed.

Results

Inpyrfluxam application did not induce mortality or change in the body weights of both male and female rats.

Appearance of a scab on the dorsal neck in one of the male rats from day 12 to 14 was deemed not to be treatment-related due to the appearance at a non-application site. There were no other clinical signs of toxicity or macroscopic pathological findings in rats after inpyrfluxam application throughout the observation period.

Conclusion

Under the conditions of this GLP and OECD compliant study, the acute dermal LD50 of inpyrfluxam was found to be >2000 mg/kg bw. Therefore, inpyrfluxam was determined to be of low acute toxic via dermal route and does not meet the criteria for classification for acute dermal toxicity according to Regulation 1272/2008 as it applies in GB.

[redacted] (2015b)

B.6.2.3. Inhalation

The acute inhalation toxicity of inpyrfluxam was evaluated in rats in a study performed according to OECD 403 (2009) under GLP conditions.

Reference:	KCA 5.2.3/01
Report Title:	Acute Inhalation Toxicity Study of S-2399 Technical Grade in Rats
Author(s) & Year:	[redacted] (2015c)
Document No, Authority registration No	Study No. 4309 [redacted]

Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Acceptable method available for aerial concentration and particle size
Guideline(s):	OECD 403 (2009)
Deviations from current guideline:	More animals were used in the study than required by the test guideline
Impact of the deviation:	This deviation has no impact on the acceptability of the study or reliability of the results obtained.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant acute inhalation toxicity study, 5 male and 5 female RccHan:WIST rats were exposed (nose only) to a mist aerosol of inpyrfluxam (in polyethylene glycol 400) at a target concentration of 2 mg/L (actual concentration 2610 mg/m^3) corresponding to the maximum attainable concentration for 4 hours according to the traditional protocol. All the investigations required by the guideline were performed.

Results

The mass median aerodynamic (MMAD) and the geometric standard deviation (GSD) of the mist aerosol particles were 3.59 µm and 2.35 µm respectively which is in accordance with the guideline.

During the exposure period, one of the female rats died. No further testing is necessary as the guideline permits the use of the limit test as a sighting study if mortality is observed at the limit concentration.

Ataxic gait and lateral position (on day 0) and decrease of spontaneous activity (on day 1) were observed in a female rat. The animal recovered from all the symptoms by day 2. Observation of wet fur in all male and female animals immediately after and 4 hours after exposure, was considered the result of being restrained in the holding tubes during exposure, and not an indication of toxicity. Results are summarised in Table 6.2.3-1.

Table 6.2.3-1: Acute inhalation toxicity of inpyrfluxam to rats

Dose (mg/L)	Toxicological results*	Duration of signs	LC_{50} (mg/L) 14 days
male rats			
2.0	0/0/5	NA	>2.0

female rats			
2.0	1/1/5	1 h – day 1	>2.0

* number of animals which died/number of animals with clinical signs/number of animals used; NA: not applicable

Reduction in body weight was observed in one female rat on days 1 and 3; this animal showed normal growth thereafter. Yellowish-white and pale focus in the right kidney was observed in a surviving female whereas the left kidney was normal. Retention of white substance in nasopharynx and trachea, along with uncollapsed lungs were observed in the dead animal. The white substance in the nasopharynx and trachea was considered to be the test substance, and the uncollapsed lung was considered to be due to retention of white substance in the trachea.

All the other animals showed no macroscopic abnormalities upon the termination of the study.

Conclusion

Based on this GLP and OECD compliant study in rats, the 4-hr LC_{50} of inpyrfluxam is > 2.0 mg/L (2.61 mg/L analytically determined), the maximum achievable concentration. Therefore, it is concluded that inpyrfluxam (aerosol) is not acutely toxic by the inhalation route and does not meet the criteria for classification for acute inhalation toxicity in accordance with Regulation 1272/2008 as it applies in GB.

[redacted] (2015c)

B.6.2.4. Skin irritation

The skin irritation potential of inpyrfluxam was investigated in a GLP and guideline (OECD 439, 2021) study.

Reference:	KCA 5.2.4/02
Report Title:	In vitro Skin Irritation Test of S-2399 TG using EpiDerm™ SIT (EPI-200)
Author(s) & Year:	[redacted] (2025a)
Document No, Authority registration No	Study No. F41-0045 Chemicals Evaluation and Research Institute, Hita (CERI Hita), Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.1%
Method of analysis:	Not required
Guideline(s):	OECD 439 (2021)

Deviations from the current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In this GLP and guideline in vitro EpiDerm™ skin irritation test, triplicate reconstructed human epidermis tissue samples were exposed to 25 mg of inpyrfluxam for 60 minutes (35 minutes in the incubator (37°C) and the remaining time at the room temperature) followed by ~42 hours post exposure. Concurrent positive (5 % sodium dodecyl sulfate) and negative control (phosphate buffered saline) samples were included.

Results

In the preliminary assays for tissue binding and chemical interference, inpyrfluxam did not show direct tissue binding or directly reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Therefore, tissue binding and chemical interference tests were not performed in the main assay.

Mean tissue viability following 60 minutes exposure with inpyrfluxam was 104.2% compared to negative control. All acceptability criteria were met as per the guideline and, the negative and positive controls showed the expected results; therefore, the assay is considered valid.

Conclusion

Under the conditions of this GLP and guideline study, the percentage viability in the Epiderm tissue model after exposure to inpyrfluxam was above 50% (established cut-off value according to the guideline for no classification). Therefore, it can be concluded that inpyrfluxam is not a skin irritant and the study can be used as a stand-alone test to conclude on hazard classification. Overall, inpyrfluxam does not meet the criteria for classification for skin irritation in accordance with Regulation 1272/2008 as it applies in GB.

[redacted] (2025a)

B.6.2.5. Eye irritation

The eye irritation potential of inpyrfluxam was investigated in a GLP and guideline (OECD 492, 2024) study.

Reference:	KCA 5.2.5/02
Report Title:	In vitro eye Irritation Test of S-2399 TG using EpiOcular™ EIT (OCL-200)
Author(s) & Year:	[redacted] (2025b)
Document No, Authority registration No	Study No. H21-0082 Chemicals Evaluation and Research Institute, Hita (CERI Hita), Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.1%
Method of analysis:	Not required
Guideline(s):	OECD 492 (2024)
Deviations from the current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In this GLP and guideline in vitro EpiOcular™ eye irritation test, duplicate reconstructed human epiocular tissue samples were exposed to 50 mg of inpyrfluxam for 6 hours followed by ~18 hours post incubation. Concurrent positive (methyl acetate) and negative control (distilled water) samples were included.

Results

In the preliminary assays for tissue binding and chemical interference, inpyrfluxam did not show direct tissue binding or directly reduce MTT. Therefore, tissue binding and chemical interference tests were not performed in the main assay.

Mean tissue viability following 60 minutes exposure with inpyrfluxam was 96.4% compared to negative control. All acceptability criteria were met as per the guideline and, the negative and positive controls showed the expected results; therefore, the assay is considered valid.

Conclusion

Under the conditions of this GLP and guideline study, the percentage viability in the EpiOcular tissue model after exposure to inpyrfluxam was above 60% (established cut-off value according to the guideline for no classification). Therefore, it can be concluded that inpyrfluxam is not an eye irritant and the study can be used as a stand-alone test to conclude on hazard classification. Overall, inpyrfluxam does not meet the criteria for classification for eye irritation in accordance with Regulation 1272/2008 as it applies in GB.

[redacted] (2025b)

B.6.2.6. Skin sensitisation

The skin sensitisation potential of inpyrfluxam was investigated in female albino Hartley guinea pigs in a GLP and OECD compliant study performed according to OECD 406 (1992). The test guideline has been updated since then, but any deviations from the updated test guideline do not affect the acceptability of the study. The study is not considered to be in contravention of Art 62 of Regulation 1107/2009 as it applies in GB as no appropriate in vitro alternatives were available at the time of commissioning. HSE note that the LLNA study is the preferred method according to the data requirements; however, as the guinea pig study provides a clear result, no further animal testing is deemed necessary.

Reference:	KCA 5.2.6/01
Report Title:	Skin sensitisation test of S-2399 Technical Grade in guinea pigs (Maximization test)
Author(s) & Year:	[redacted] (2015c)
Document No, Authority registration No	Study No. 4303/TPT-0011 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD TG 406 (1992)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes

Study relied upon:	Yes

Methods

The skin sensitisation potential of inpyrfluxam was investigated in a GLP and OECD compliant study using the Guinea Pig Maximisation Test (GPMT). In a dose finding study, the topical induction application of inpyrfluxam in acetone induced slight erythema at 50% and no skin reactions at 25%. The intradermal injection of 1% or 0.5% inpyrfluxam in corn oil induced mortality and clinical signs such as decreased spontaneous activity, prone position, ataxic gait, and tremor whereas no abnormal signs were observed with 0.2%. Based on the results from the dose finding study, in the main study twenty female albino:Hartley guinea pigs were tested with an intradermal induction concentration of 0.2%, topical induction of 50% and challenge concentration of 25% inpyrfluxam in acetone. Overall, it can be concluded that the concentrations used in the main study were maximised. Appropriate negative and positive controls were also used. All the investigations required by the guideline were performed.

Results

There was no mortality or clinical signs of toxicity in both treated or control animals. There were no skin reactions observed at induction.

Following challenge, no skin reactions were observed in the inpyrfluxam treated group with a sensitisation rate of 0%. Negative and positive control groups gave expected results.

Conclusion

Under the conditions of this GLP and OECD compliant study, inpyrfluxam did not induce sensitising effects in the skin of guinea pigs. Therefore, inpyrfluxam does not meet the criteria for classification for skin sensitisation in accordance with Regulation 1272/2008 as it applies in GB.

[redacted] (2015c)

B.6.2.7. Phototoxicity

UV absorption spectra showed that inpyrfluxam has the absorption maxima (λ_{max}) at 242 nm and 290 nm with molar absorption coefficients more than 10 L x mol^{-1} x cm^{-1} . Therefore, *in vitro* phototoxicity testing was necessary.

The phototoxicity of inpyrfluxam was investigated *in vitro* using a GLP and OECD compliant study. This study was performed according to OECD 432 (2004). The test guideline has been updated since then, but any deviations from the updated guideline do not affect the acceptability of the study or validity of the results.

Reference:	KCA 5.2.7/01
Report Title:	*In vitro* 3T3 NRU Phototoxicity Study of S-2399 Technical Grade in Cultured Mammalian Cells (Amended Final Report)
Author(s) & Year:	[redacted] M. (2016)
Document No, Authority registration No	Study No. B150567 Kashima laboratory, LSI Medience Corporation, Japan.
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 432 (2004)
Deviations from current guideline :	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	~~Yes;~~ No. The cells for the +UV assay were irradiated with solar-simulated light (generated by a Xenon arc lamp (spectral irradiance: approximately 300-800 nm). HSE notes that irradiation was not performed at the absorption maxima of inpyrfluxam. Therefore, the results of the study are invalid.
Study relied upon:	~~Yes;~~ No

Methods

In a GLP and OECD TG compliant *in vitro* 3T3 NRU phototoxicity study, Balb/3T3 (clone A31) mouse cells were exposed to various concentrations (0.586, 1.17, 2.34, 4.69, 9.38, 18.8, 37.5 and 75 µg/mL) of inpyrfluxam in the presence and absence of UVA light (+UV and -UV) along with vehicle (DMSO) and positive (chlorpromazine hydrochloride) controls. The cells for the +UV assay were irradiated with solar-simulated light (generated by a Xenon arc lamp (spectral irradiance: approximately 300-800 nm). HSE notes that irradiation was not performed at the absorption maxima of inpyrfluxam.

All the investigations required by the guideline were performed.

Results

The cell growth was not inhibited by any of the used concentrations of inpyrfluxam to more than 10% both in the presence and absence of UVA light. Therefore, an IC_{50} and photo irritation factor (PIF) were not calculated. The mean photo effect (MPE) was <0.1 (0.002).

All the acceptance criteria stipulated in the test guideline were met and the assay was deemed to be acceptable.

Conclusion

Under the conditions of this GLP and OECD compliant *in vitro* study, inpyrfluxam was concluded not to be phototoxic. However, HSE notes that irradiation was not performed at the absorption maxima of inpyrfluxam. Therefore, the results of the study are invalid, and no conclusion can be reached. Nevertheless, the study doesn't need to be repeated as guidance is lacking for phototoxicity testing at wavelengths below 320 nm.

[redacted] (2016)

B.6.2.8. Summary of acute toxicity

The acute toxicity of inpyrfluxam was investigated in multiple in vivo studies conducted via the oral, dermal and inhalation routes. In addition, an in vivo study assessing skin sensitisation, as well as in vitro studies evaluating the skin and eye irritation potential of inpyrfluxam, and phototoxicity were also available. In vivo skin and eye irritation studies were submitted; however, these were not relied upon as valid in vitro alternatives were available.

Based on the results of these studies, inpyrfluxam is acutely toxic via the oral route (LD_{50} of 180 mg/kg) and meets the criteria for classification under Reg. (EC) No. 1272/2008 as it applies in GB for **acute oral toxicity in category 3 (H301)** but has low acute toxicity when administered dermally (LD_{50} > 2000 mg/kg) and via inhalation (4-hr-LC50 > 2.61 mg/L; analytically determined) to rats. It is not a skin or eye irritant, nor a skin sensitiser. ~~and is not phototoxic.~~ No conclusion could be reached on the phototoxicity potential of inpyrfluxam.

The table below provides an overview of the available acute toxicity studies:

Table 6.2.8-1: Summary of acute toxicity studies for inpyrfluxam

Data point/ Study	Species/ Strain/tissues	Sex	Acceptable	Result	Classification according to Reg. (EC) No. 1272/2008 as it applies in GB
KCA 5.2.1/01 Acute Oral (OECD 423*; in vivo*)	Rat/ RccHan:WIST	F	Acceptable Relied upon	50 < LD_{50} < 300 mg/kg bw	Cat. 3 (H301)
KCA 5.2.1/02 Acute Oral	Rat/ RccHan:WIST	F	Acceptable Relied upon	LD_{50} 180 mg/kg bw	Cat. 3 (H301)

(OECD 425*; in vivo*)					
KCA 5.2.2/01 Acute Dermal (OECD 402*; in vivo*)	Rat/ RccHan:WIST	M&F	Acceptable Relied upon	LD_{50} >2000 mg/kg bw	Not classified
KCA 5.2.3/01 Acute Inhalation (OECD 403*;* *in vivo*)	Rat/ RccHan:WIST	M&F	Acceptable Relied upon	4-hr-LC_{50} > 2.61 mg/L analytically determined (maximal attainable concentration)	Not classified
KCA 5.2.4/02 Skin irritation (OECD 439*; in vitro*)	EpiDerm™	-	Acceptable	Not irritating	Not classified
KCA 5.2.5/02 Eye irritation (OECD 492*; in vitro*)	EpiOcular™	M	Acceptable	Not irritating	Not classified
KCA 5.2.6/01 Skin sensitisation (GPMT) (OECD 406*; in vivo*)	Guinea pig/ Hartley	F	Acceptable Relied upon	Not sensitising	Not classified
KCA 5.2.7/01 *In vitro* phototoxicity (OECD 432)	Balb/3T3 clone A31 cells/ NA	NA	Not acceptable Not relied upon	~~Not phototoxic~~ Inconclusive	N/A

M: Male

F: Female

NA: not applicable

B.6.3. Short-term Toxicity

The short-term toxicity of inpyrfluxam has been investigated via the oral route of exposure in a preliminary 28-day (range-finding) study performed in rats, 90-day studies performed in rats, mice, and dogs and 1-year study performed in dogs. A 28-day dermal exposure study in rats is also available.

B.6.3.1. Oral 28-day study

A 28-day dietary toxicity study has been conducted in rats. This study was a preliminary range-finding study conducted prior to the 90-day short-term toxicity study. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.3.1/01
Report Title:	One-month Oral Toxicity Study of S-2399 in Rats

Author(s) & Year:	[redacted] 2014)
Document No, Authority registration No	Study No. S1554 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: LS-118-101206-1 Purity: 99.2%
Method of analysis:	No validated method of analysis is available for both the dietary concentration of the active substance and the plasma kinetics. However, as this was a preliminary range finding study for the 90-day and carcinogenicity study where these validated methods of analysis are available, the lack of methods of analysis in this one month study is not critical.
Guideline(s):	No, (not required as a range finding study). The study follows the principles of OECD TG 407, with a variation in the number of animals used.
Deviations from current guideline :	N/A
Impact of the deviation:	N/A
GLP or GEP:	No, not conducted under GLP (range-finding study)
Acceptability:	Yes- as supplementary range-finding study only
Study relied upon:	Yes

Methods

Groups of 6 male and 6 female Wistar Hannover rats received inpyrfluxam by dietary administration at concentrations of 0, 500, 1000, 3000 or 5000 ppm (mean substance intakes: 0, 44.4, 85.9, 246.4 and 406.5 mg/kg bw/day for males and 0, 47.4, 91.4, 263.0 and 377.8 mg/kg bw/day for females) for one month (28 days for males and 29 days for females). The top dose was set based on a previous repeated dose 1-week oral toxicity study in rats, where a reduction in body weight with no recovery was seen in animals administered 10,000 ppm and 20,000 ppm inpyrfluxam.

Clinical observations, measurement of motor activities, bodyweight, food consumption, urinalysis, ophthalmology, haematology, blood biochemistry, organ weight, necropsy and histopathology were performed after the treatment period.

Additional groups of 4 male and 4 female rats treated under the same conditions and doses as above for 31 days were used to analyse the plasma concentration of inpyrfluxam (proof of absorption experiment).

Results

Proof of absorption

Plasma concentration of inpyrfluxam was similar at 500 and 1000 ppm in both males and females. The males followed a trend of dose-dependency from 1000 ppm whereas no dose-dependency was observed in females. These results provide evidence that the test substance was absorbed and systemically available to the rat.

Table 6.3.1-1: Plasma concentration of inpyrfluxam in the 28-day rat study

S-2399 concen-tration (µg/mL)	Sex and dose level (ppm)							
	Male				Female			
	500	1000	3000	5000	500	1000	3000	5000
	0.03 ± 0.01	0.03 ± 0.02	0.08 ± 0.03	0.12 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.09 ± 0.05	0.07 ± 0.06

Mortality and general clinical signs

There was no treatment related mortality or clinical signs of toxicity in rats, including effects on motor activity.

Body weight

From day 9 onwards, statistically significant, and dose-dependent decreases in body weights of both male and female rats were observed at 3000 and 5000 ppm. The effect on bodyweight was more severe in males compared to females. This change was accompanied by dose-dependent decreases in bodyweight gain throughout the study period. The total bodyweight gain over the study period was decreased in a dose-dependent manner (41.5% and 38.1% at the top dose in males and females respectively) but only reached statistical significance in females at 3000 and 5000 ppm. The changes in body weight and bodyweight gain at 3000 ppm and 5000 ppm are considered treatment related and adverse.

The results are summarised in table 6.3.1-2 and -3.

Table 6.3.1-2: Mean body weight (g) of rats administered inpyrfluxam in the diet for 28 days.

Day	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000
1	155	156	156	156	154	115	117	119	119	116
9	209	205	204	194* (↓7.2 %)	166** (↓20.6 %)	136	138	137	127** (↓6.6 %)	117** (↓14%)
16	250	246	246	230** (↓8.0 %)	201** (↓19.6 %)	153	155	154	144* (↓5.9 %)	133** (↓13.1 %)
22	278	271	270	253** (↓9.0 %)	227** (↓18.3 %)	165	168	166	153** (↓7.3 %)	145** (↓12.1 %)
26	291	281	279	261** (↓10.3 %)	232** (↓20.3 %)	174	173	172	157** (↓9.8 %)	151** (↓13.2 %)
29	301	290	289	270** **(↓10.3 %)**	240** **(↓20.3 %)**	178	180	176	165** **(↓7.3 %)**	155** **(↓12.9 %)**

The values are expressed as mean grams (n=6); * and **: p<0.05 and p<0.01

Table 6.3.1-3: Mean body weight gain (g) in rats administered inpyrfluxam in the diet for 28 days.

Day	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000
Bw 1	155	156	156	156	154	115	117	119	119	116
9	55	49	49	38**	12**	21	22	19	8**	1**
16	41	41	42	36	35	17	17	17	17	16
22	28	26	24	24	25	12	14	11	10	12
26	14	10	9	8**	6**	9	5	6	4*	6
29	10	9	10	9	8	4	6	4	8	4
Total wt gain	147	135 (↓8.2 %)	133 (↓9.5 %)	113# (↓23.1 %)	86# (↓41.5 %)	63	63	58 (↓7.9 %)	47** **(↓25.4 %)**	39** **(↓38.1 %)**

The values are expressed as mean grams (n=6); Bw 1: initial body weight; Total wt gain: total weight gain; * and **: p<0.05 and p<0.01; # trend without statistical significance

Food consumption and efficiency

There were no treatment-related effects in both males and females at any dose.

Ophthalmology

No treatment related abnormalities were observed in any group.

Haematology

Statistically significant decreases in mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were observed in males administered with 5000 ppm (4.9% and 3.7%, respectively) and 500 ppm (5.1% and 4.2% respectively), but not at 1000 and 3000 ppm. Given the lack of a dose-response, these changes are not considered treatment-related. In high dose males, a statistically significant increase in red blood cell count (9.8%) was also observed. However, in isolation, this finding is not considered related to treatment. Overall, there were no effects of treatment on haematology.

Table 6.3.1-4: Results of selected haematological findings in rats administered inpyrfluxam in the diet for 28-days.

Para-meter	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000
RBC ($x10^6$/µL) (mean ± SD)	7.95 ± 0.158	8.41 ± 0.294 (↑ 5.8%)	7.98 ± 0.417 (↑ 0.4%)	8.26 ± 0.351 (↑ 3.9 %)	8.73 ± 0.427** **(↑ 9.8 %)**	7.73 ± 0.391	7.66 ± 0.457 (↓0.9%)	8.00 ± 0.403 (↑ 3.5 %)	8.16 ± 0.507 (↑ 5.6 %)	8.34 ± 0.398 (↑ 7.9 %)
MCV (fl) (mean ± SD)	53.0 ± 0.53	50.3 ± 2.27* **(↓5.1%)**	53.8 ± 1.71 (↑1.5%)	53.0 ± 1.32	50.4 ± 1.67* (↓4.9%)	52.1 ± 1.89	53.4 ± 1.84 (↑ 2.5 %)	52.0 ± 1.70 (↓0.2%)	52.2 ± 0.95 (↑ 0.2%)	51.0 ± 1.51 (↓2.1%)
MCH (pg) (mean ± SD)	19.0 ± 0.46	18.2 ± 0.61* (↓4.2%)	19.1 ± 0.54 (↑ 0.5%)	19.0 ± 0.40	18.3 ± 0.50* **(↓3.7%)**	18.7 ± 0.59	19.0 ± 0.43 (↑ 1.6%)	18.7 ± 0.50	18.6 ± 0.22 (↓0.5%)	18.0 ± 0.44 (↓3.7%)

* p<0.05; ** p<0.01; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; RBC - red blood cell count

Clinical chemistry parameters

At 3000 and 5000 ppm, statistically significant and dose -dependent increases in total cholesterol (by 50% and 103.6% in males; by 100% and 115 % in females respectively), phospholipid (by 30.6 % and 66.7% in males; by 70.8% and 77.4% in females, respectively) and γ-glutamyl transpeptidase levels (by 650% and 1400% in males and by 300 % and 500% in females respectively) were recorded compared to controls. Statistically significant increases compared to controls in triglyceride (217.6% at 3000 ppm and 323.5% at 5000 ppm) were observed in females from 3000 ppm. Given the statistical significance, the magnitude of the increases and the presence of a dose-response, these changes are considered treatment related and adverse.

Changes in glucose, albumin, calcium and chloride in males and aspartate aminotransferase in females were also observed. However, due to the lack of a dose-response, they are considered to be incidental.

Overall, adverse effects on clinical-chemistry parameters indicative of liver and lipid metabolism damage were noted in both males and females from 3000 ppm.

Table 6.3.1-5: Clinical chemistry findings in findings in rats administered inpyrfluxam in the diet for 28-days.

Para-meter	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000
Alb (g/dL)	2.2 ± 0.15	2.3 ± 0.05 (↑4.5)	2.3 ± 0.05 (↑4.5)	2.3 ± 0.08 (↑4.5)	2.5 ± 0.08 ** **(↑13.6)**	2.4 ± 0.10	2.5 ± 0.12 (↑4.2)	2.4 ± 0.16	2.4 ± 0.10	2.4 ± 0.10
Gluc (mg/dL)	154 ± 12.5	141 ± 12.5 (↓8.4 %)	129 ± 13.1 ** **(↓16.2 %)**	134 ± 9.0* **(↓13.0 %)**	132 ± 16.0* **(↓14.3 %)**	121 ± 7.0	122 ± 16.5 (↑0.8 %)	113 ± 5.0 (↓6.6 %)	115 ± 8.6 (↓5.0 %)	120 ± 16.2 (↓0.8 %)
T.Chol (mg/dL)	56 ± 10.6	62 ± 5.7 (↑10.7 %)	68 ± 11.7 (↑21.4 %)	84 ± 11.2* **(↑50 %)**	114 ± 26.0* **(↑103. 6%)**	51 ± 8.6	52 ± 6.2 (↑2.0 %)	56 ± 12.7 (↑9.8 %)	102 ± 17.7* **(↑100 %)**	110 ± 26.9* **(↑115. 7%)**
PL (mg/dL)	111 ± 16.1	119 ± 10.9 (↑7.2 %)	124 ± 21.4 (↑11.7 %)	145 ± 21.1* **(↑30.6 %)**	185 ± 33.8 ** **(↑66.7 %)**	106 ± 13.1	114 ± 7.9 (↑7.5 %)	116 ± 26.4 (↑9.4 %)	181 ± 23.8* **(↑70.8 %)**	188 ± 38.0* **(↑77.4 %)**
TG (mg/dL)	64 ± 29.9	85 ± 29.6 (↑32.8 %)	71 ± 45.2 (↑10.9 %)	88 ± 28.0 (↑37.5 %)	86 ± 31.1 (↑34.4 %)	17 ± 6.0	27 ± 10.6 (↑58.8 %)	29 ± 19.1 (↑70.6 %)	54 ± 16.1 ** **(↑217. 6%)**	72 ± 16.7 ** **(↑323. 5%)**
ALP (U/L)	428 ± 120.0	391 ± 59.1 (↓8.6 %)	371 ± 41.3 (↓13.3 %)	426 ± 58.2 (↓0.5 %)	434 ± 114.0 (↑1.4 %)	196 ± 75.7	175 ± 31.6 (↓10.7 %)	248 ± 76.8 (↑26.5 %)	294 ± 60.4* **(↑50.0 %)**	270 ± 64.5# (↑37.8 %)
GGTP$^{\phi}$ (U/L)	0 ± 0.4	1 ± 0.5 (↑150 %)	1 ± 1.2 (↑150 %)	3 ± 1.0* **(↑650 %)**	6 ± 1.5** **(↑1400 %)**	1 ± 0.5	1 ± 0.5	1 ± 1.0	4 ± 1.9* **(↑300 %)**	6 ± 1.8* **(↑500 %)**
Ca (mg/dL)	10.0 ± 0.13	10.0 ± 0.18	10.2 ± 0.26 (↑2.0 %)	10.2 ± 0.15 (↑2.0 %)	10.4 ± 0.13 ** **(↑4.0 %)**	9.4 ± 1.06	9.9 ± 0.14 (↑5.3 %)	9.9 ± 0.23 (↑5.3 %)	10.1 ± 0.26 (↑7.4 %)	10.1 ± 0.27 (↑7.4 %)
Cl (mEq/L)	110 ± 1.3	109 ± 1.0	110 ± 1.6	110 ± 1.6	107 ± 1.8**	112 ± 1.2	113 ± 2.1	112 ± 1.3	111 ± 2.4	110 ± 1.4

		(↓0.9 %)			**(↓2.7 %)**		(↑0.9 %)		(↓0.9 %)	(↓1.8 %)
AST (U/L)	66 ± 6.7	64 ± 6.5 (↓3.0 %)	61 ± 3.4 (↓7.6 %)	62 ± 5.8 (↓6.1 %)	64 ± 6.8 (↓3.0 %)	70 ± 7.3	61 ± 3.8* **(↓12.9 %)**	62 ± 4.8 (↓11.4 %)	65 ± 5.8 (↓7.1 %)	58 ± 7.2** **(↓17.1 %)**

* and **: p<0.05 and p<0.01 ; # trend without statistical significance; ϕ :standard deviation (0.4) used as the control value for calculating percentage difference in males; Alb - Albumin; Gluc - Glucose, T.chol - Total cholesterol; PL - phospholipid, TG -triglycerides; ALP- alkaline phosphatase; GGTP- γ-glutamyl transpeptidase; Ca - Calcium; Cl – chloride; AST - aspartate aminotransferase

Urinalysis

There were no treatment related effects on urinalysis at any dose in either sex.

Gross pathology

At necropsy, enlarged (1 of 6 in males and 2 of 6 in females) and dark-coloured (1 of 6 in males and 1 of 6 in females) livers were observed at 5000 ppm. No treatment-related effects were observed in any of the other groups.

Organ weights

At 3000 and 5000 ppm, statistically significant and dose-dependent increases (≥ 15%)[1] in absolute liver weight were seen in females. Relative liver weights were also increased at 3000 and 5000 ppm in both sexes. Additionally, males showed statistically significant decreases in absolute and relative thymus weights at 3000 and 5000 ppm. However, in absence of any associated histopathology, these thymus weight decreases are likely to be the consequence of the reduced body weights. Absolute kidney weights were decreased in the 5000 ppm males and in females at 3000 and 5000 ppm. In the absence of any associated histopathology, the decreases in females are likely to be the consequence of the reduced body weights. In males, histopathological findings were noted. Therefore, the decrease in kidney weight at 5000 ppm in males is considered to be treatment-related and adverse. Decreases in ovary weights and uterine weights (absolute and/or relative) were also seen from 3000 ppm. These were associated with histopathological findings and therefore considered adverse.

Overall, specific and adverse effects on liver weights in both sexes and on ovary and uterus weights in females were seen from 3000 ppm and on kidney weights in males at the top dose.

Table 6.3.1-7: Absolute and relative organ weights in the 28-day rat study

[1] TOX-TAB_template_refined_with Annex

Organ	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000
Terminal body weight (g)	280 ± 13.2	267 ± 18.0 (↓4.6%)	268 ± 18.8 (↓4.3%)	250 ± 16.5* **(↓10.7%)**	222 ± 10.8 ** **(↓20.7%)**	168 ± 2.6	168 ± 5.4	164 ± 5.1 (↓2.4%)	154 ± 10.1 ** **(↓8.3%)**	145 ± 7.5** **(↓13.7%)**
Liver – abs (g)	8.41 ± 0.352	8.24 ± 0.934 (↓2.0%)	7.89 ± 0.924 (↓6.2%)	8.75 ± 0.795 (↑ 4.0%)	9.06 ± 0.609 (↑ 7.7%)	4.76 ± 0.272	4.99 ± 0.231 (↑ 4.8%)	4.89 ± 0.259 (↑ 2.7%)	5.46 ± 0.584** **(↑ 14.7%)**	5.68 ± 0.380 ** **(↑ 19.3%)**
Liver - rel	3.01 ± 0.062	3.08 ± 0.184 (↑ 2.3%)	2.95 ± 0.241 (↓2.0%)	3.50 ± 0.252 ** **(↑ 16.3%)**	4.09 ± 0.129 ** **(↑ 35.9%)**	2.83 ± 0.174	2.97 ± 0.103 (↑ 4.9%)	2.98 ± 0.131 (↑ 5.3%)	3.54 ± 0.230** **(↑ 25.1%)**	3.93 ± 0.114 ** **(↑ 38.9%)**
Kidneys – abs (g)	2.14 ± 0.312	2.00 ± 0.189 (↓6.5%)	1.93 ± 0.127 (↓9.8%)	1.92 ± 0.141 (↓10.3%)	1.71 ± 0.094 ** **(↓20.1%)**	1.35 ± 0.062	1.36 ± 0.103 (↑ 0.7%)	1.27 ± 0.067 (↓5.9%)	1.19 ± 0.060** **(↓11.9%)**	1.16 ± 0.078 ** **(↓14.1%)**
Kidneys - rel	0.77 ± 0.091	0.75 ± 0.052 (↓2.6%)	0.73 ± 0.041 (↓5.2%)	0.77 ± 0.031	0.77 ± 0.030	0.80 ± 0.034	0.81 ± 0.052 (↑ 1.3%)	0.77 ± 0.050 (↓3.8%)	0.77 ± 0.041 (↓3.8%)	0.80 ± 0.046
Thymus – abs (g)	0.57 ± 0.060	0.48 ± 0.115 (↓15.8%)	0.49 ± 0.091 (↓14.0%)	0.38 ± 0.066 ** **(↓33.3%)**	0.33 ± 0.049 ** **(↓42.1%)**	0.37 ± 0.072	0.38 ± 0.021 (↑2.7%)	0.36 ± 0.053 (↓2.7%)	0.34 ± 0.031 (↓8.1%)	0.32 ± 0.046 (↓13.5%)
Thymus – rel	0.20 ± 0.017	0.18 ± 0.035 (↓10.0%)	0.18 ± 0.027 (↓10.0%)	0.15 ± 0.021** **(↓25.0%)**	0.15 ± 0.022** **(↓25.0%)**	0.22 ± 0.044	0.23 ± 0.014 (↑ 4.5 %)	0.22 ± 0.027	0.22 ± 0.023	0.22 ± 0.029
Ovaries – abs (mg)	-					73 ± 7.4	78 ± 8.0 (↑ 6.8 %)	66 ± 10.0 (↓9.6 %)	59 ± 8.0* **(↓19.2 %)**	58 ± 11.0* **(↓20.5%)**
Ovaries - rel	-					43.2 ± 3.82	46.2 ± 3.58 (↑ 6.9 %)	40.2 ± 5.36 (↓6.9 %)	38.5 ± 5.76 (↓10.9 %)	40.1 ± 8.40 (↓7.2 %)

Uterus – abs (g)	-	0.40 ± 0.108	0.44 ± 0.154 (↑ 10.0 %)	0.42 ± 0.183 (↑ 5.0 %)	0.33 ± 0.154 (↓6.9 %)	0.27 ± 0.098# (↓32.5 %)
Uterus - rel	-	0.24 ± 0.064	0.27 ± 0.097 (↑ 12.5 %)	0.26 ± 0.107 (↑ 8.3 %)	0.21 ± 0.091 ((↓12.5 %)	0.18 ± 0.073 (↓25.0 %)

abs: absolute organ weight; rel: organ weight relative to body weight %;
* and **: p<0.05 and p<0.01
# trend without statistical significance

Histopathology

Hypertrophy (at 3000 and 5000 ppm) and smooth endoplasmic reticulum proliferation (at 5000 ppm) in the hepatocytes of male and female animals were observed, which correlated with the increased liver weights.

Hyaline droplets in the proximal tubules of the kidney were seen in males from 3000 ppm. The content of these droplets was identified as α2μ-globulin by immunohistochemistry. These findings (including the associated decreased kidney weight reported at the top dose) are male rat specific and hence not relevant to humans and are not considered further. A dose dependant increase in follicular cell hypertrophy of the thyroid was observed in males and females from 3000 ppm; this attained statistical significance at 5000 ppm. Fine vacuolisation of cortical cells in the zona fasciculata of the adrenal was observed in both males and females at 3000 and 5000 ppm. In addition, a dose dependent increase in the fine vacuolization of cortical cells in the zona glomerulosa was observed from 1000 ppm in males.

An increase in fatty infiltration of the bone marrow was observed in females at 3000 and 5000 ppm and in males at 5000 ppm. Vacuolation of the interstitial gland of the ovary and atrophy of the uterus were observed in females at 3000 and 5000 ppm.

Overall, histopathological findings were observed in the liver, thyroid, bone marrow, ovary, and uterus from 3000 ppm. In addition, the adrenal gland was affected from 1000 ppm.

Table 6.3.1-8: Selected histopathological findings in the 28-day rat study

Organ & lesion	Sex and dose level (ppm)									
	Male					Female				
	0	500	1000	3000	5000	0	500	1000	3000	5000

No. examined	6	6	6	6[1]	6	6	6[2]	6[2]	6[2]	6
Diffuse hepatocyte hypertrophy	-	-	-	3	6**	-	-	-	6**	6**
Kidney- Hyaline droplets, proximal tubular cells - slight	-	-	-	1	4*	-	-	-	-	-
Thyroid- Follicular cells hypertrophy -	1	2	2	4	6**	1	1	1	3	5*
Adrenals-Fine vacuolation, zona fasciculata - slight	-	-	-	2	5**	-	-	-	1	4*
Adrenals- Fine vacuolation, zona glomerulosa – total	1	1	4	5*	6**	-	-	-	-	-
Bone marrow-Fatty infiltration	-	1	1	1	5**	2	3	1	5*	6*
Thymus- Atrophy - slight	-	-	-	-	3	-	-	-	-	-
Thymus- Tingible macrophages, increased - slight	1	-	-	1	1	-	-	-	-	2
Ovary -Interstitial gland increase - slight	-					-	-	2	1	5**
Ovary -Vacuolation of interstitial gland - total	-					-	-	-	2	6**
Uterus- Atrophy - slight	-					-	-	-	2	2

* and **: $p<0.05$ and $p<0.01$

[1] 5 animals examined for thymus.

[2] Kidneys not examined

Conclusion

In conclusion, dietary administration of inpyrfluxam for one-month to male and female Wistar Hannover rats at dietary concentrations 500, 1000, 3000 and 5000 ppm (mean substance intakes: 44.4, 85.9, 246.4 and 406.5 mg/kg bw/day for males and 47.4, 91.4, 263.0 and 377.8 mg/kg bw/day for females) caused adverse effects relevant to humans mainly from 3000 ppm (246.4 mg/kg bw/day), including effects on body weights and body weight gains, clinical-chemistry parameters indicative of liver damage and disruption in lipid metabolism, changes in liver, ovary and uterus weights and histopathological findings of the liver, thyroid, bone marrow, ovary and uterus. In addition, the adrenal gland was affected from 1000 ppm (85.9 mg/kg bw/day).

As this was a range finding non-GLP study, no robust points of departure can be set. Based on the observed results, the top dose for the 90-day study was set at 4000 ppm.

[redacted] (2014)

B.6.3.2. Oral 90-day study

1. 90-day study in rats

A 90-day dietary toxicity study has been conducted in rats. The study was GLP complaint and performed in accordance with OECD TG 408 (1998). The test guideline was updated later and there are deviations related to endocrine and reproductive toxicity endpoints from the current test guideline. These deviations do not affect the validity of the study as the missing investigations have been addressed in mechanistic and reproductive toxicity studies.

Reference:	KCA 5.3.2/01
Report Title:	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in Rats ([redacted] 13-0069) – Final report amendment.
Author(s) & Year:	[redacted] (2016)
Document No, Authority registration No	Study No. [redacted] 13-0069 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable method of analysis available for dietary formulation
Guideline(s):	OECD TG 408 (1998)
Deviations from current guideline:	1. LDL and HDL were not measured. 3. No circulating thyroid hormones (T4, T3, TSH) and thyroid weight were measured. No measurement of prostrate, seminal vesicles with coagulating glands and coagulating glad weights; oestrus cycle and terminal vaginal cytology were not assessed.
Impact of the deviation:	1. Even though LDL and HDL were not measured, histopathological investigations of the cardiovascular system and measurements of total cholesterol and triglyceride levels are sufficient to identify any effects potentially related to these parameters. 2. The test guideline was updated later to include endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals. These deviations do not affect the validity of the study as the missing investigations have been addressed in mechanistic and reproductive toxicity studies.

GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 10 male and 10 female Wistar Hannover rats received inpyrfluxam by dietary administration at concentrations of 0, 150, 500, 2000, or 4000 ppm (mean substance intakes: 0, 9.72, 31.7, 123 and 255 mg/kg bw/day for males and 0, 11.5, 37.5, 144 and 292 mg/kg bw/day for females) over 92 consecutive days. The dose levels were selected based on a previous repeated dose 28-day oral toxicity study in rats (2014).

In accordance with OECD test guideline 408, all animals were subjected to the required investigations. Analysis of the dietary formulation was performed within the study.

Results

Mortality and general clinical signs

There was no treatment related mortality or clinical signs of toxicity in rats.

Functional observational battery (FOB) and measurement of motor activity

No treatment related effects on grip strength or motor activity were observed in rats.

Body weight

Dose-dependent decreases in body weight (up to 17% reduction in males and 19% reduction in females compared to control) and total body weight gain (up to 26% reduction in males and 39% reduction in females compared to control) were observed throughout the treatment period in both male and female rats. These changes were statistically significant in males at 4000 ppm (except weeks 5-6 for body weight gain) and in females at 2000 ppm (except week 2 and 3 for mean body weight and only in week 1 for body weight gain) and 4000 ppm (only in week 1, 5 and 6 for body weight gain).

The changes in bodyweight and body weight gains at 2000 ppm and 4000 ppm are considered treatment related and adverse. The results are summarised in table 6.3.2-1 and -2.

Table 6.3.2-1: Mean body weight (% of control) of rats administered inpyrfluxam in the diet for 90 days.

Week	Sex and dose level (ppm)							
	Male				Female			
	150	500	2000	4000	150	500	2000	4000
1	102	99	98	87**	100	100	95*	87**
2	100	98	97	87**	101	99	96	86**
3	100	97	96	88**	101	100	95	85**
4	100	96	95	88**	101	98	93*	85**
5	101	96	95	87**	100	98	91**	84**
6	101	97	95	86**	101	97	90**	82**
7	102	97	96	86**	102	96	90**	82**
8	102	97	95	85**	102	97	90**	82**
9	102	97	95	84**	101	96	89**	81**
10	101	97	95	84**	101	96	89**	82**
11	101	97	95	83**	102	95	89**	82**
12	101	97	95	83**	102	96	89**	81**
13	101	97	95	83** **(↓17%)**	102	96	90** **(↓10%)**	81** **(↓19%)**

The values are expressed as % of control (n=10); * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.3.2-2: Mean body weight gain (% of control) of rats administered inpyrfluxam in the diet for 90 days.

Week	Sex and dose level (ppm)							
	Male				Female			
	150	500	2000	4000	150	500	2000	4000
0-1	107	98	91	43**	100	100	67**	5**
1-2	98	91	93	89*	111	100	105	84
5-6	117	117	100	67	120	80	70	60*
7-8	115	100	92	54*	100	140	80	100
9-10	75	100	100	67*	100	125	75	125
10-11	92	100	83	58*	125	25	75	50
0-13	101	95	92	74** **(↓26%)**	104	92	79** **(↓21%)**	61** **(↓39%)**

The values are expressed as % of control (n=10); * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Food consumption and efficiency

There was a dose dependent reduction in food consumption throughout the treatment period in both males and females from 2000 ppm. The effect was more severe in females (statistically significant reduction – up to 17% compared to control from 2000 ppm) than males. The effect on food consumption in females correlates with the reduction in bodyweight and it is considered as treatment related and adverse.

Table 6.3.2-3: Summary of food consumption (% of control) of rats administered inpyrfluxam in the diet for 90 days.

Week	Sex and dose level (ppm)							
	Male				Female			
	150	500	2000	4000	150	500	2000	4000
1	102	100	88**	56**	99	96	65**	42**
2	103	99	95	84**	97	97	92	84**
3	103	97	97	88**	99	101	93	87**
4	100	92	93	94	102	98	92	88*
5	103	94	92	96	101	94	87*	79**
6	108	99	94	88*	103	94	86**	82**
7	106	97	93	88*	95	94	86**	82**
8	107	96	90	92	103	93	86	77**
9	104	99	93	86**	104	94	85**	83**
10	104	100	96	91*	103	93	83**	88*
11	102	101	94	91	107	92	88*	82**
12	102	99	94	89*	104	93	86**	82**
13	106	99	96	98	97	93	83** **(17%)**	83** **(17%)**

The values are expressed as % of control (n=10); * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Ophthalmology

No treatment related abnormalities were observed.

Haematology

Statistically significant and dose dependent decreases in mean corpuscular volume (MCV - 4% at high dose) and mean corpuscular haemoglobin (MCH - 4% at high dose) were observed in females administered 4000 ppm compared to control. The decreases in MCV and MCH were not considered as adverse given the low magnitude and because there were no significant changes in other indicators of anaemia (haematocrit, haemoglobin concentration, and erythrocyte count).

An increase in prothrombin time (PT) was observed in both males and females at the top dose, attaining statistical significance in females (7% increase compared to control). This effect is considered treatment related and adverse and may be related to the liver toxicity observed (see below).

At 500 ppm, statistically significant increases in white blood cell count (41%), lymphocytes (41%) and neutrophils (42%) were also observed. These incidental changes were considered not to be of toxicological significance due to the lack of a dose-response.

Overall, adverse effects on coagulation parameters (prothrombin time) were noted at the top dose.

Table 6.3.2-4: Summary of significant changes at haematology [mean ± SD (% of controls)] in rats administered inpyrfluxam in the diet for 90 days.

Para-meter	Sex and dose level (ppm)									
	Male					Female				
	0	150	500	2000	4000	0	150	500	2000	4000
MCV (fL)	48.6 ± 1.3	49.2 ± 1.9 (101)	48.4 ± 1.3 (100)	50.0 ± 1.7 (103)	48.2 ± 1.4 (99)	52.9 ± 1.5	53.3 ± 1.8 (101)	53.1 ± 1.1 (100)	52.0 ± 1.9 (98)	51.0 ± 1.6* (96) **(↓4%)**
MCH (pg)	16.9 ± 0.4	17.1 ± 0.6 (101)	16.9 ± 0.3 (100)	17.2 ± 0.4 (102)	16.7 ± 0.5 (99)	18.3 ± 0.5	18.5 ± 0.6 (101)	18.3 ± 0.3 (100)	17.9 ± 0.4 (98)	17.5 ± 0.6** (96) **(↓4%)**
PT (sec)	15.5 ± 1.9	16.0 ± 1.8 (103)	15.7 ± 2.7 (101)	16.3 ± 2.6 (105)	18.0 ± 3.3 (116)	10.2 ± 0.3	10.3 ± 0.3 (101)	10.2 ± 0.3 (100)	10.4 ± 0.5 (102)	10.9 ± 0.5** (107) **(↑ 7%)**
WBC (10^3/µL)	3.97 ± 1.22	4.41 ± 1.29 (111)	5.60 ± 1.26* (141) **(↑41%)**	4.63 ± 1.33 (117)	4.13 ± 0.35 (104)	2.57 ± 0.39	2.37 ± 0.31 (92)	2.61 ± 0.37 (102)	2.47 ± 0.56 (96)	2.51 ± 0.45 (98)
Differential leukocyte count										
Lymphocytes (10^3/µL)	3.29 ± 1.05	3.66 ± 1.05 (111)	4.64 ± 1.16* (141) **(↑41%)**	3.84 ± 1.20 (117)	3.35 ± 0.38 (102)	2.10 ± 0.46	1.89 ± 0.35 (90)	2.06 ± 0.42 (98)	1.97 ± 0.48 (94)	1.93 ± 0.46 (92)
Neutrophils (10^3/µL)	0.53 ± 0.14	0.58 ± 0.21 (109)	0.75 ± 0.21* (142) **(↑42%)**	0.65 ± 0.17 (123)	0.63 ± 0.20 (119)	0.38 ± 0.15	0.37 ± 0.15 (97)	0.45 ± 0.19 (118)	0.39 ± 0.19 (103)	0.48 ± 0.25 (126)

The values are expressed as mean ± SD (% of controls); * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Clinical chemistry parameters

Statistically significant and dose dependent increases in γ-glutamyl transpeptidase (GGTP) (at 2000 and 4000 ppm by 114% and 414% in males; by 238% and 438% in females respectively) and alkaline phosphatase (ALP) (by 34% at 2000 ppm and 28% (not statistically significant) at 4000 ppm in females) were observed compared to control. Given the statistical significance, dose dependency and the magnitude of the increases, these alterations were considered as treatment related and adverse.

A decrease in total bilirubin (by 43% and 43% at 2000 and 4000 ppm in females) was not considered to be toxicologically significant given the direction of change (decrease rather than increase). However, decreases in glucose (by 14% at 4000 ppm in females), and increases in phosphorous (by 14% at 4000 ppm in males) were considered treatment related and adverse.

Overall, adverse effects on clinical chemistry parameters indicative of liver damage were noted in both males and females from 2000 ppm.

Table 6.3.2-5: Summary of significant changes at clinical chemistry [mean ± SD (% of controls)] in rats administered inpyrfluxam in the diet for 90 days.

Para-meter	Sex and dose level (ppm)									
	Male					Female				
	0	150	500	2000	4000	0	150	500	2000	4000
ALP (U/L)	249 ± 39	222 ± 25 (89)	216 ± 36 (87)	216 ± 21 (87)	217 ± 48 (87)	80 ± 16	94 ± 25 (118)	86 ± 23 (108)	107 ± 24* (**134**) (**↑34%**)	102 ± 22 (128)# (**↑28%**)
GGTP (U/L)	0.7 ± 0.1	0.7 ± 0.1 (100)	0.7 ± 0.1 (100)	1.5 ± 0.5** (**214**) (**↑114%**)	3.6 ± 0.9** (**514**) (**↑414%**)	0.8 ± 0.2	0.9 ± 0.5 (113)	0.8 ± 0.1 (100)	2.7 ± 0.7** (**338**) (**↑238%**)	5.1 ± 1.1** (**638**) (**↑538%**)
Gluc (mg/dL)	182 ± 22	180 ± 12 (99)	205 ± 28 (113)	172 ± 18 (95)	168 ± 12 (92)	148 ± 14	145 ± 16 (98)	138 ± 14 (93)	135 ± 12 (91)	128 ± 13* (**86**) (**↓24%**)
T.Bil (mg/dL)	0.06 ± 0.01	0.06 ± 0.01 (100)	0.06 ± 0.01 (100)	0.06 ± 0.01 (100)	0.06 ± 0.01 (100)	0.07 ± 0.02	0.07 ± 0.01 (100)	0.07 ± 0.01 (100)	0.04 ± 0.01** (**57**) (**↓43%**)	0.04 ± 0.01** (**57**) (**↓43%**)
P (mg/dL)	4.9 ± 0.5	5.1 ± 0.4 (104)	5.2 ± 0.7 (106)	5.5 ± 0.8 (112)	5.6 ± 0.8* (**114**) (**↑14%**)	4.3 ± 0.8	4.4 ± 0.8 (102)	4.3 ± 0.6 (100)	4.2 ± 0.9 (98)	4.6 ± 0.6 (107)
K (mEq/L)	3.58 ± 0.17	3.62 ± 0.20 (101)	3.66 ± 0.18 (102)	3.71 ± 0.14 (104)	3.69 ± 0.17 (103)	3.28 ± 0.25	3.32 ± 0.35 (101)	3.33 ± 0.17 (102)	3.57 ± 0.21* (**109**) (**↑9%**)	3.56 ± 0.22 (109)

The values are expressed as mean ± SD (% of controls); * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); # trend without statistical significance; Gluc - Glucose, T.Bil - Total bilirubin; P – Phopshorous; ALP- alkaline phosphatase; GGTP- γ-glutamyl transpeptidase; K- Potassium

Urinalysis

At 4000 ppm, a statistically significant decrease in the urine pH was observed in both sexes. This effect is considered treatment-related and adverse.

Gross pathology

At necropsy, dark-coloured (3 of 10 in males and 7 of 10 in females) livers were observed at 4000 ppm.

Organ weights

At 2000 and 4000 ppm, statistically significant and dose-dependent increase (> 10%) in relative liver weight was observed in both males and females. This correlated with the coagulation, clinical chemistry and histopathological changes (see below) observed at these doses. Therefore, it was considered as treatment related and adverse.

A decrease (by 13%) in absolute kidney weights was observed in females at the top dose. In the absence of any associated histopathological changes, the decrease in females is likely to be the consequence of the reduced body weights. In males, a statistically significant increase (by 14%) in relative kidney weight was noted at the top dose. This was associated with histopathological findings. Therefore, the decrease in kidney weight at 4000 ppm in males was considered to be treatment-related and adverse.

In both males and females, a reduction (> 10%) in absolute adrenal weights (statistically significant in females alone) was noted at the top dose. A dose-dependent increase (> 10%) in relative ovary weight was observed from 2000 ppm (statistically significant at 4000 ppm). The effects on the weights of the adrenals at the top dose and of the ovaries are considered as treatment related and adverse due to the dose dependency and associated histopathological changes.

Dose dependent increases in relative brain weight (statistically significant in males - at 4000 ppm and in females -at 2000 and 4000 ppm); increases in relative heart, testes, and epididymis weights (statistically significant at 4000 ppm) in males; decreases in absolute spleen weight (statistically significant at 4000 ppm in both sexes) were also observed. These changes in organ weights without related histopathological changes in the brain, heart, spleen, testes, and epididymis in the 2000 and/or 4000 ppm groups were considered likely to be due to the decreased final body weight.

Overall, specific and adverse increases in liver weight were seen in both sexes from 2000 ppm. In addition, adverse effects on adrenal weights in both sexes, kidney weights in males and ovary weights in females were seen at 4000 ppm.

Table 6.3.2-6: Summary of significant changes in absolute and relative organ weights in rats administered inpyrfluxam in the diet for 90 days.

Organ	Sex and dose level (ppm)									
	Male					Female				
	0	150	500	2000	4000	0	150	500	2000	4000

Terminal body weight (g)	404 ± 35	407 ± 15 (101)	394 ± 27 (98)	384 ± 32 (95)	336 ± 17** **(83)** **(↓17%)**	234 ± 11	239 ± 11 (102)	224 ± 15 (96)	211 ± 15** (90) **(↓10%)**	193 ± 12** (82) **(↓18%)**
Brain - rel	0.49 ± 0.04	0.50 ± 0.02 (102)	0.50 ± 0.03 (102)	0.52 ± 0.04 (106)	0.58 ± 0.02** (118) **(↑18%)**	0.77 ± 0.03	0.78 ± 0.04 (101)	0.82 ± 0.05 (106)	0.86 ± 0.05** (112) **(↑12%)**	0.94 ± 0.05** (122) **(↑22%)**
Heart – abs (mg)	1014 ± 90	1095 ± 95 (108)	1050 ± 90 (104)	1018 ± 80 (100)	951 ± 61 (94)	712 ± 35	723 ± 52 (102)	673 ± 54 (95)	678 ± 53 (95)	626 ± 32** **(88)** **(↓12%)**
Heart - rel	0.25 ± 0.02	0.27 ± 0.02 (108)	0.27 ± 0.01 (108)	0.27 ± 0.03 (108)	0.28 ± 0.02** **(112)** **(↑12%)**	0.30 ± 0.02	0.30 ± 0.02 (100)	0.30 ± 0.02 (100)	0.32 ± 0.02 (107)	0.33 ± 0.02 (110)
Liver – abs (g)	9.79 ± 1.21	10.28 ± 0.42 (105)	10.26 ± 0.83 (105)	10.30 ± 1.12 (105)	10.09 ± 0.68 (103)	5.89 ± 0.51	6.16 ± 0.52 (105)	5.80 ± 0.50 (98)	6.28 ± 0.46 (107)	6.57 ± 0.47* **(112)** **(↑12%)**
Liver - rel	2.42 ± 0.14	2.53 ± 0.16 (105)	2.60 ± 0.12* (107)	2.69 ± 0.19** **(111)** **(↑11%)**	3.00 ± 0.11** **(124)** **(↑24%)**	2.51 ± 0.21	2.58 ± 0.16 (103)	2.59 ± 0.07 (103)	2.98 ± 0.18** **(119)** **(↑19%)**	3.42 ± 0.28** **(136)** **(↑36%)**
Kidneys – abs (mg)	2286 ± 194	2463 ± 144 (108)	2397 ± 181 (105)	2280 ± 192 (100)	2175 ± 145 (95)	1537 ± 95	1706 ± 224 (111)	1565 ± 181 (102)	1446 ± 149 (94)	1332 ± 54* **(87)** **(↓13%)**
Kidneys - rel	0.57 ± 0.02	0.61 ± 0.05 (107)	0.61 ± 0.05 (107)	0.60 ± 0.60 (105)	0.65 ± 0.03** **(114)** **(↑14%)**	0.66 ± 0.04	0.71 ± 0.08 (108)	0.70 ± 0.06 (106)	0.68 ± 0.06 (103)	0.69 ± 0.03 (105)
Spleen – abs (mg)	592 ± 75	611 ± 72 (103)	621 ± 92 (105)	594 ± 70 (100)	506 ± 20* **(85)** **(↓15%)**	444 ± 56	483 ± 57 (109)	429 ± 60 (97)	434 ± 48 (98)	377 ± 55* **(85)** **(↓15%)**
Adrenals – abs (mg)	70.7 ± 8.3	74.7 ± 6.4 (106)	67.4 ± 8.5 (95)	64.6 ± 7.2 (91)	62.8 ± 5.6 (89)	75.7 ± 7.6	75.9 ± 8.5 (100)	78.9 ± 10.1 (104)	68.6 ± 6.6 (91)	65.1 ± 6.2* **(86)** **(↓14%)**
Testes – rel	0.86 ± 0.08	0.87 ± 0.06 (101)	0.88 ± 0.05 (102)	0.88 ± 0.07 (102)	1.02 ± 0.02** **(119)** **(↑19%)**	-				
Epididymides – rel	0.30 ± 0.03	0.31 ± 0.02 (103)	0.31 ± 0.04 (103)	0.32 ± 0.04 (107)	0.35 ± 0.02** **(117)** **(↑17%)**	-				

Ovaries - rel	-	0.044 ± 0.010	0.044 ± 0.007 (100)	0.047 ± 0.008 (107)	0.051 ± 0.009 (116)	0.056 ± 0.006* **(127)** **(↑27%)**

rel: organ weight relative to body weight %; abs: absolute organ weight
* and **: p≤0.05 and p≤0.01 (Dunnett's multiple comparison test)

Histopathology

Hypertrophy in the hepatocytes of male and female animals were observed at 2000 and 4000 ppm. Hyaline droplets in the proximal tubules of the kidney were seen in males from 2000 ppm. The content of these droplets was identified as α2μ-globulin by immunohistochemistry. These findings (including the associated increased kidney weight reported at the top dose) are male rat specific and hence not relevant to humans and are not considered further. A dose dependent increase in follicular epithelial cell hypertrophy of the thyroid was observed in females from 2000 ppm (reaching statistical significance at 4000 ppm). At 2000 and 4000 ppm, fine vacuolisation of cortical cells of the adrenal gland was observed in both males and females. Vacuolation of the interstitial gland of the ovary was observed in females at 2000 and 4000 ppm.

Overall, histopathological findings were observed in the liver, kidney, thyroid, and ovary from 2000 ppm. These results correlate with the findings of the 28- day short term toxicity study in rats ([redacted] 2014).

Table 6.3.2-7: Selected histopathological findings in rats administered inpyrfluxam in the diet for 90 days.

Organ & lesion	Sex and dose level (ppm)									
	Male					Female				
	0	150	500	2000	4000	0	150	500	2000	4000
Liver Diffuse hepatocyte hypertrophy	0	0	0	4	10**	0	0	0	7**	9**
Kidney Increased deposition of hyaline droplets in proximal tubular cells	0	0	0	2	8**	0	0	0	0	0
Thyroid Follicular cells hypertrophy	1	2	2	2	2	0	1	0	4	7**
Adrenal Increased cortical cell vacuolation of zona fasciculata	1	0	0	1	5	0	0	0	3	5*
Ovary Interstitial gland vacuolation	-					1	2	2	7*	8**

* and **: p≤0.05 and p≤0.01 (Fisher's exact probability test); No of organs examined: 10.

Conclusion

In conclusion, in a GLP and guideline compliant study, dietary administration of inpyrfluxam for 90 days to male and female Wistar Hannover rats at dietary concentrations of 150, 500, 2000, or 4000 ppm (mean substance intakes: 9.72, 31.7, 123 and 255 mg/kg bw/day for males and 11.5, 37.5, 144 and 292 mg/kg bw/day for females) caused adverse effects relevant to humans from 2000 ppm (123 mg/kg bw/day), including effects on body weights, body weight gains and food consumption, effects on liver weight (with associated hypertrophy), coagulation and clinical-chemistry parameters indicative of liver damage, and histopathological findings of the liver, thyroid, adrenal and ovary.

Overall, a NOAEL of 500 ppm (31.7 mg/kg bw/day) can be established from this study based on the effects observed on body weight, body weight gain and food consumption, liver toxicity and histopathological findings of the adrenal glands, thyroid and ovary at the LOAEL of 2000 ppm (123 mg/kg bw/day).

[redacted] (2016)

2. 90-day study in mice

A 90-day dietary toxicity study has been conducted in mice. The study was GLP complaint and performed in accordance with OECD TG 408 (1998). The test guideline was updated later and there are deviations from the current test guideline. These deviations do not affect the validity of the study as the missing investigations have been addressed in mechanistic and reproductive toxicity studies.

Reference:	KCA 5.3.2/02
Report Title:	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in mice.
Author(s) & Year:	[redacted] (2016a)
Document No, Authority registration No	Study No. [redacted] 13-0068 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable method of analysis available for dietary formulation
Guideline(s):	OECD TG 408 (1998); the guideline was updated in 2018
Deviations from current guideline:	1. Endocrine and reproductive toxicity - No measurement of thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) and thyroid gland weight. - No measurement of prostate, seminal vesicles with coagulating glands and pituitary gland weight. - Oestrus cycle was not assessed.

	2. Other parameters - No measurement of low-density lipoproteins (LDL) and high-density lipoproteins (HDL). - Ophthalmic examinations and FOBs were not performed. - No measure of blood clotting time/potential. - Clinical chemistry - no sodium or potassium measured.
Impact of the deviation:	1. The test guideline was updated later to include endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals. These deviations do not affect the validity of the study as the missing investigations have been addressed in mechanistic and reproductive toxicity studies. 2. This study was conducted as a dose finding study for the subsequent carcinogenicity study; the missing investigations have been addressed in the long-term carcinogenicity study. Therefore, the observed deviations do not affect the validity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 10 male and 10 female [redacted] mice received inpyrfluxam by dietary administration at concentrations of 0, 200, 800, 3500, or 7000 ppm (mean substance intakes: 0, 27.2, 111, 491, 973 mg/kg bw/day for males and 0, 31.7, 130, 559 and 1097 mg/kg bw/day for females) over 91 and 92 consecutive days respectively. The dose levels were selected based on a previous repeated dose one-month oral toxicity study in mice (not submitted) where the maximum tolerated dose level was not achieved even at 7000 ppm (corresponding to 1000 mg/kg bw/day). Therefore, the high dose was selected at the limit dose (1000 mg/kg bw/day) stated in test guideline.

In accordance with OECD test guideline 408, all animals were subjected to the required investigations with the exception of the deviations mentioned above. Analysis of the dietary formulation was performed within the study.

Results

Mortality and general clinical signs

There was no treatment related mortality or clinical signs of toxicity in mice.

Body weight, food consumption and efficiency

There were no treatment related changes in body weight, body weight gain, food consumption and food efficiency in either sex.

Haematology

No treatment related haematological changes were observed in either sex.

Clinical chemistry parameters
At 3500 and 7000 ppm, dose dependent increases in globulin levels (by 10% and 12% in males respectively; by 10% and 14% in females respectively) and decreases in albumin (by 8% and 9% in females) were observed. These changes led to a dose dependent reduction in the albumin/globulin (A/G) ratio at 3500 and 7000 ppm in both males (by 9% and 13% respectively) and females (by 15% and 20% respectively). The effects on albumin and globulin were statistically significant in both sexes with an exception in males at 3500 ppm. Additionally, there was a dose dependent increase in total cholesterol in both sexes which attained statistical significance in females at 7000 ppm (reduction by 24%). These effects were considered as treatment related and adverse.

A decrease in total bilirubin (by 34% and 34% at 3500 and 7000 ppm in males; 23% at 7000 ppm in females) was not considered to be toxicologically significant. Changes in glucose and calcium levels in males were considered as incidental due to low values in the concurrent control compared to the historical control mean and the values in the treated groups within the historical control data.

Overall, adverse effects on clinical chemistry parameters indicative of liver damage were noted from 3500 ppm in both males and females.

Table 6.3.2-7: Summary of significant changes at clinical chemistry [mean ± SD (% of controls)] in mice administered inpyrfluxam in the diet for 90 days.

Para-meter [HC; n=125]	Sex and dose level (ppm)									
	Male					Female				
	0	200	800	3500	7000	0	200	800	3500	7000
Alb (g/dL)	2.73 ± 0.21	2.80 ± 0.18 (103)	2.72 ± 0.15 (100)	2.72 ± 0.14 (100)	2.66 ± 0.22 (97)	3.04 ± 0.18	3.06 ± 0.16 (101)	3.01 ± 0.18 (99)	2.80 ± 0.14** **(92) (↓8%)**	2.76 ± 0.10** **(91) (↓9%)**
Glob (g/dL)	1.86 ± 0.12	1.84 ± 0.16 (99)	1.90 ± 0.15 (102)	2.04 ± 0.15* **(110) (↑10%)**	2.09 ± 0.18** **(112) (↑12%)**	1.43 ± 0.06	1.47 ± 0.15 (103)	1.14 ± 0.11 (99)	1.57 ± 0.15 (110)	1.63 ± 0.05** **(114) (↑14%)**

A/G	1.48 ± 0.10	1.53 ± 0.15 (103)	1.44 ± 0.13 (97)	1.34 ± 0.12 (91)	1.29 ± 0.15** **(87) (↓13%)**	2.13 ± 0.15	2.10 ± 0.15 (99)	2.14 ± 0.12 (100)	1.80 ± 0.19** **(85) (↓15%)**	1.70 ± 0.09** **(80) (↓20%)**
Gluc (mg/dL) [235 ± 28]	208 ± 31	246 ± 28** **(118) (↑18%)**	227 ± 23 (109) **(↑9%)**	246 ± 25** **(118) (↑18%)**	238 ± 16* **(114) (↑14%)**	209 ± 28	221 ± 19 (106)	218 ± 17 (104)	212 ± 20 (101)	209 ± 22 (100)
T.Chol (mg/dL)	111 ± 26	129 ± 30 (116)	122 ± 16 (110)	127 ± 21 (114)	135 ± 28 (122)	86 ± 15	83 ± 15 (97)	89 ± 16 (103)	93 ± 23 (108)	107 ± 19* **(124) (↑24%)**
T.Bil (mg/dL)	0.09 ± 0.02	0.07 ± 0.01 (78)	0.07 ± 0.01 (78)	0.05 ± 0.01** **(56) (↓34%)**	0.05 ± 0.01** **(56) (↓34%)**	0.06 ± 0.01	0.07 ± 0.01 (117)	0.07 ± 0.02 (117)	0.05 ± 0.01 (83)	0.04 ± 0.01** **(67) (↓23%)**
Ca (mg/dL) [8.6 ± 0.4]	8.4 ± 0.3	8.4 ± 0.1 (100)	8.6 ± 0.4 (102)	8.7 ± 0.2* **(104) (↑4%)**	8.7 ± 0.1* **(104) (↑4%)**	8.7 ± 0.3	8.6 ± 0.3 (99)	8.7 ± 0.2 (100)	8.8 ± 0.2 (101)	8.7 ± 0.2 (100)

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); Alb - Albumin; Glob – Globulin, A/G- Albumin/Globulin ratio; Gluc - Glucose, T.Bil- Total bilirubin; T.chol - Total cholesterol; Ca - Calcium;

Gross pathology

At necropsy, dark-coloured (4 of 10 in males and 9 of 10 in females) and enlarged (3 of 10 in males and 2 of 10 in females) livers were observed at 7000 ppm.

Organ weights

At 3500 and 7000 ppm, dose-dependent increases in absolute (16% and 25% in males respectively; 7% and 24% in females respectively) and relative (18% and 33% in males respectively; 11% and 29% in females respectively) liver weights were observed. These effects were statistically significant in both sexes, except in the females at 3500 ppm. The observed effects correlate with the clinical chemistry and histopathological changes observed. Therefore, the increases in liver weight are considered as treatment related and adverse.

Table 6.3.2-8: Summary of significant changes in absolute and relative organ weights in mice administered inpyrfluxam in the diet for 90 days.

Organ	Sex and dose level (ppm)									
	Male					Female				
	0	200	800	3500	7000	0	200	800	3500	7000
Terminal body weight (g)	43.6 ± 2.6	46.1 ± 3.7 (106)	45.8 ± 4.8 (105)	42.8 ± 2.7 (98)	40.9 ± 2.4 (94)	34.1 ± 2.9	33.6 ± 3.1 (99)	34.4 ± 2.0 (101)	32.9 ± 2.4 (96)	32.7 ± 2.9 (96)

Liver – abs (g)	2.31 ± 0.27	2.50 ± 0.22 (108)	2.52 ± 0.30 (109)	2.67 ± 0.19** (116) **(↑16%)**	2.89 ± 0.25** (125) **(↑25%)**	1.82 ± 0.20	1.76 ± 0.25 (97)	1.83 ± 0.17 (101)	1.95 ± 0.28 (107)	2.26 ± 0.35** (124) **(↑24%)**
Liver - rel	5.29 ± 0.44	5.42 ± 0.26 (102)	5.51 ± 0.31 (104)	6.24 ± 0.25** (118) **(↑18%)**	7.06 ± 0.44** (133) **(↑33%)**	5.34 ± 0.41	5.23 ± 0.35 (98)	5.31 ± 0.40 (99)	5.91 ± 0.48* (111) **(↑11%)**	6.90 ± 0.49** (129) **(↑29%)**

rel: organ weight relative to body weight %; abs: absolute organ weight
* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Histopathology

Hypertrophy in the hepatocytes of male and female animals was observed at 3500 and 7000 ppm. Centrilobular hepatocyte fatty change was also noted in males from 3500 ppm. These effects are considered as treatment related and adverse.

An increase in follicular epithelial cell hypertrophy of the thyroid was observed in males and females at 7000 ppm (reaching statistical significance only in males) and is considered as treatment related and adverse.

Overall, histopathological findings were observed in the liver from 3500 ppm and in the thyroid at high dose in both males and females.

Table 6.3.2-9: Selected histopathological findings in mice administered inpyrfluxam in the diet for 90 days.

Organ & lesion	Sex and dose level (ppm)									
	Male					Female				
	0	200	800	3500	7000	0	200	800	3500	7000
Liver: Centrilobular hepatocyte fatty change	0	0	0	3	4	0	0	0	0	0
Centrilobular hepatocyte hypertrophy	0	0	0	8**	10**	0	0	0	0	0
Diffuse hepatocyte hypertrophy	0	0	0	0	0	0	0	0	4	10**
Thyroid Follicular cell hypertrophy	0	0	1	0	5*	0	0	0	0	1

* and **: p≤0.05 and p≤0.01 (Fisher's exact probability test).) No of organs examined: 10.

Conclusion

In conclusion, dietary administration of inpyrfluxam for 90 days to male and female [redacted] mice at dietary concentrations 0, 200, 800, 3500, or 7000 ppm (mean substance intakes: 0, 27.2, 111, 491, 973 mg/kg bw/day for males and 0, 31.7, 130, 559 and 1097 mg/kg bw/day for females) caused adverse effects from 3500 ppm (491 mg/kg bw/day), including effects on liver weight, clinical-chemistry parameters indicative of liver damage and histopathological changes in the liver. Furthermore, adverse histopathological effects were observed in the thyroid at 7000 ppm in both sexes.

Overall, a NOAEL of 800 ppm (111 mg/kg bw/day) can be established from this study based-on the effects observed in the liver at the LOAEL of 3500 ppm (491 mg/kg bw/day).

[redacted] (2016a)

3. 90-day study in dogs

A 90-day dietary toxicity study has been conducted in dogs. The study was GLP complaint and performed in accordance with OECD TG 409 (1998).

Reference:	KCA 5.3.2/03
Report Title:	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in Dogs
Author(s) & Year:	[redacted] (2016)
Document No, Authority registration No	Study No. [redacted] 13-0106 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	OECD TG 409 (1998)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes

Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 4 male and 4 female Beagle dogs received inpyrfluxam in gelatin capsules at 0, 40, 160 or 700/500 mg/kg bw/day for 13 weeks. The high dose level was changed from 700 mg/kg bw/day to 500 mg/kg bw/day at week 4 (considered as week 1 for the dose level of 500 mg/kg bw/day), because the male and female dogs in the group showed considerable decreases in body weight and food consumption in addition to vomiting of feed until week 2. Temporal discontinuation of the treatment was applied at week 3. Therefore, the top dose group was administered inpyrfluxam at 500 mg/kg bw/day for 13 weeks corresponding to week 16 from treatment initiation.

The dose levels were selected based on a previous repeated dose one-month oral toxicity study in dogs (not submitted) where reduction in body weight, body weight gain and effects on the liver were observed in animals administered 500 mg/kg bw/day and 1000 mg/kg bw/day inpyrfluxam.

In accordance with OECD test guideline 409, all animals were subjected to the required investigations.

Results

Mortality and general clinical signs

In the 700/500 mg/kg bw/d group, one male (No.16 at week 9) and two females (No. 33 at week 8 and No.34 at week 9) were killed in extremis (ke), due to the decrease in body weight, food consumption and/or deteriorated general condition. Just before unscheduled kill decreased spontaneous motor activity, reddish urine, yellow-coloured eyes, and/or mucosa of oral cavity were observed in these animals. No mortality in other dose groups of either sex was noted.

At the high dose, two males (one at weeks 6, 8, and 10; one (no.16, ke) at weeks 4-9) and two females (one (no.34, ke) at week 3 and 8; one at week 16) showed staggering gait. One of the males (no.16, ke) also showed torticollis and ananastasia (at week 4), convulsion (at week 5), reduction in spontaneous motor activity and increased skin and fur changes (at week 8). There was an increase in the incidence of vomiting in both males and females from 160 mg/kg bw/day.

Overall, there were treatment related effects on mortality and clinical signs of toxicity at the top dose, and increased incidence of vomiting from the mid dose in both sexes.

Body weight

There was an adverse decrease (> 10%) in mean body weight at the high dose throughout the treatment period in both sexes which attained statistical significance in males at week 8.

Table 6.3.2-10: Mean body weight (% of control) of dogs administered inpyrfluxam in gelatin capsules for 90 days.

Week	Sex and dose level (mg/kg/day)					
	Male			Female		
	40	160	700/500	40	160	700/500
0	100	101	100	100	100	100
1	99	98	90	100	96	91
2	99	98	83	103	98	88
3	99	97	88	104	98	93
4	100	98	86	104	99	89
5	102	101	84	104	99	89
6	102	101	84	105	100	85
7	102	101	82	105	99	78
8	102	101	80*	106	100	79
9	102	102	82	106	100	89ex
10	102	102	84	106	101	88ex
11	102	103	84	107	102	86ex
12	102	103	86	108	102	84ex
13	102	103	84	104	101	86ex

*: p≤0.05 (Dunnett's test or Dunnett-type test); ex: excluded from statistical evaluation because of insufficient numbers

Food consumption

There was significant reduction in food consumption throughout the treatment period in both males (at weeks 2, 4, 7 to 9, 12, and 13) and females (at weeks 1, 2, 4 to 8) at the high dose. At the mid dose, males also showed decrease in food consumption from weeks 2 to 4, which was mainly due to the reduced food consumption by a single male. Only the effects on food consumption seen at the top dose are therefore considered adverse.

Table 6.3.2-11: Summary of food consumption (% of control) of dogs administered inpyrfluxam in gelatin capsule for 90 days.

Week	Sex and dose level (mg/kg/day)					
	Male			Female		
	40	160	700/500	40	160	700/500
BT	100	100	100	100	100	100
1	100	97	86	100	99	87*
2	100	89	45*	100	100	53*
3	100	90	97wd	97	100	98wd
4	100	91	78*	95	100	68*

5	107	100	58	100	100	62*
6	102	97	76	99	100	61*
7	100	97	68*	96	100	47*
8	100	96	68*	98	100	37*
9	100	96	66*	99	100	56
10	100	97	87	98	100	80ex
11	100	100	88	99	100	67ex
12	100	100	90**	95	100	74ex
13	100	98	85**	95	100	92ex

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); wd: excluded from statistical evaluation because of withdrawal period; ex: excluded from statistical evaluation because of insufficient numbers

Ophthalmology

No treatment related abnormalities were observed in any group.

Haematology

Statistically significant increases in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were observed in males even from the lowest dose; however, in the absence of a dose-response, these are considered unrelated to treatment. At week 4, there was a dose dependent and substantial decrease in reticulocyte count in both sexes from the mid dose (statistically significant only in high dose females). These effects, indicative of anaemia are considered as treatment related and adverse.

Changes were also seen in prothrombin time (PT), activated partial thromboplastin time and lymphocytes, but in the absence of a dose-response, these were not considered as treatment related.

Overall, haematological changes relating to anaemia were observed from 160 mg/kg bw/day in both sexes.

Table 6.3.2-12: Summary of significant changes at haematology [mean ± SD (% of controls)] in dogs administered inpyrfluxam in gelatin capsules for 90 days.

Parameter	Week	Sex and dose level (mg/kg/day) (N=4 unless otherwise noted)							
		Male				Female			
		0	40	160	700 / 500	0	40	160	700 / 500
MCV (fL)	13	63.6 ±1.7	65.4 ± 1.2 (103)	67.3 ± 2.0* **(106) ↑6%**	65.5 ± 2.4 (103) (N=3)	63.8 ± 2.2	66.6 ± 1.9 (104)	64.8 ± 1.8 (102)	66ex (103) (N=2)
MCH (pg)	4	22.2 ± 0.5	22.6 ± 0.2 (102)	23.1 ± 0.2** **(104) ↑4%**	22.5 ± 0.3 (101)	22.4 ± 0.9	23.0 ± 0.6 (103)	22.7 ± 0.5 (101)	22.8 ± 0.4 (102)
	8	21.7 ± 0.6	22.4 ± 0.4 (103)	22.8 ± 0.3* **(105) ↑5%**	22.3 ± 0.6 (103)	22.1 ± 0.8	22.9 ± 0.7 (104)	22.5 ± 0.6 (102)	22.7 ± 0.5 (103)
	13	21.6 ± 0.5	22.4 ± 0.3* **(104) ↑4%**	23.1 ± 0.2** **(107) ↑7%**	22.7 ± 0.6* **(105) ↑5%** (N=3)	21.9 ± 0.8	22.9 ± 0.6 (105)	22.5 ± 0.6 (103)	22.8ex (104) (N=2)
Retics (10^9/L)	4	55.5 ± 12	63.6 ± 19.6 (115)	41.7 ± 26.2 (75)	28.6 ± 10.9 (52)	51.5 ± 14.7	52.3 ± 12.6 (102)	38.6 ± 4.6 (75)	28.0 ± 11.8* **(54) ↓46%**
PT (sec)	BT	8.5 ± 0.0	9.6 ± 1.4 (113)#	8.9 ± 0.4 (105)	8.9 ± 0.5 (105)	9.2 ± 0.4	8.7 ± 0.2 (95)	8.6 ± 0.1* (93)	8.6 ± 0.2* (93)
	4	8.7 ± 0.2	10.2 ± 2.1 (117)#	9.8 ± 0.6* **(113) ↑13**	9.6 ± 0.5 (110)	9.2 ± 0.6	8.8 ± 0.2 (96)	9.1 ± 0.4 (99)	9.2 ± 0.5 (100)
	8	8.6 ± 0.2	10.0 ± 2.0 (116)#	9.6 ± 0.4 (112)	9.9 ± 0.8* **(115) ↑15%**	9.1 ± 0.4	8.8 ± 0.2 (97)	9.2 ± 0.4 (101)	11.4 ± 2.6 (125)
	13	8.6 ± 0.2	10.0 ± 1.9 (116)#	9.6 ± 0.3* **(112) ↑12%**	9.6 ± 0.3 (112) (N=3)	9.0 ± 2.0	8.8 ± 0.2 (98)	9.0 ± 0.3 (100)	9.3ex (103) (N=2)
APTT (sec)	4	13.8 ± 0.1	13.4 ± 0.2 (97)	12.9 ± 0.9* **(93) ↓7**	13.2 ± 0.4* **(96) ↓4%**	13.9 ± 0.4	13.9 ± 0.6 (100)	12.9 ± 0.3* **(93) ↓7%**	13.5 ± 0.3 (97)
	13	14.1 ± 0.3	13.4 ± 0.6 (95)	13.5 ± 0.2 (96)	13.0 ± 0.4** **(92) ↓8%** (N=3)	13.9 ± 0.3	13.9 ± 0.2 (100)	13.1 ± 0.4** **(94) ↓6%**	13.3ex (96) (N=2)
Differential leukocyte count									
Lymphocytes (10^3/µL)	13	3.47 ± 0.71	3.86 ± 0.38 (111)	5.09 ± 0.78* **(147) ↑47%**	4.51 ± 1.06 (130) (N=3)	4.38 ± 0.94	4.22 ± 0.37 (96)	4.10 ± 0.65 (94)	3.87ex (88) (N=2)

BT: Before initiation of treatment; * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); ex: excluded from statistical evaluation because of insufficient sample numbers; #: one animal excluded from the statistical evaluation as BT-value higher than the HCD+3SD

Clinical chemistry parameters

Both males and females showed statistically significant increases in alkaline phosphatase (ALP) (by >100%) and γ-glutamyl transpeptidase (GGTP) (by >50%) and in alanine aminotransferase (ALT) (by >50%) at 160 mg/kg bw/ day and 700/500 mg/kg bw/day. In addition, decreases in total cholesterol (TC) were seen in both sexes from the mid dose. These effects are considered as treatment-related and adverse. Changes were also seen in the levels of triglycerides, aspartate aminotransferase (AST), calcium, phosphorus, sodium, creatinine, glucose and total bilirubin. However, due to the lack of a dose-response or inconsistency at different timepoints, these changes are considered unrelated to treatment.

Dose-dependent decreases in albumin, globulin, A/G ratio and total protein were seen consistently at week 8 and 13 from the mid dose in both sexes. These effects are considered treatment-related and adverse.

Overall, adverse effects on clinical chemistry parameters indicative of liver damage were noted from the mid dose in both males and females.

Table 6.3.2-13: Summary of significant changes at clinical chemistry [mean ± SD (% of controls)] in dogs administered inpyrfluxam in gelatin capsules for 90 days.

Parameter	Week	Sex and dose level (mg/kg/day) (N=4 unless otherwise noted)							
		Male				Female			
		0	40	160	700 / 500	0	40	160	700 / 500
ALP (U/L)	4	481 ± 101	631 ± 75 (131)	1322 ± 756** **(275) ↑175%**	603 ± 233 (125)	413 ± 41	609 ± 77 (147)	843 ± 222* (204)	769 ± 244 (186)
	8	442 ± 97	651 ± 115 (147)	1512 ± 825** **(342) ↑242%**	1245 ± 612* **(282) ↑182**	365 ± 70	722 ± 236 (198)	1008 ± 409* **(276) ↑176%**	2865 ± 2684* **(785) ↑685** (N=3)

	13	379 ± 58	718 ± 195 (189)	1368 ± 439** **(361)** **↑261%**	1330 ± 880 * **(351)** **↑251%** (N = 3)	317 ± 84	720 ± 276 (227)	1101 ± 482* **(347)** **↑247%**	2153ex (679) (N=2)
	16	-	-	-	1254 ± 628 (331) (N = 3)	-	-	-	2519ex (795) (N=2)
HC data at week 13		N = 132	278 ± 67 Mean ± 3SD: 479			N = 132	235 ± 64 Mean ± 3SD: 426		

AST (U/L)	8	28 ± 2	28 ± 3 (100)	33 ± 14 (118)	81 ± 104 (289)	31 ± 4	31 ± 3 (100)	29 ± 4 (94)	172 ± 227 (555) (N=3)
	13	31 ± 3	30 ± 3 (97)	34 ± 9 (110)	34 ± 3 (110) (N = 3)	28 ± 4	30 ± 2 (107)	27 ± 5 (96)	47ex (168) (N=2)
	16	-	-	-	34 ± 4 (110) (N = 3)	-	-	-	48ex (171) (N=2)
ALT (U/L)	BT	27 ± 3	42 ± 9* **(157) ↑43%**	29 ± 4 (107)	35 ± 5 (130)	32 ± 5	29 ± 5 (91)	38 ± 3 (119)	34 ± 7 (106)

	4	30 ± 5	35 ± 5 (117)	36 ± 24 (120)	65 ± 86 (217)	33 ± 7	31 ± 6 (94)	38 ± 12 (115)	41 ± 19 (124)
	8	29 ± 4	38 ± 7 (131)	79 ± 102 (272)	254 ± 417 (876)	35 ± 4	31 ± 6 (89)	46 ± 14 (131)	292 ± 439 (834) (N=3)
	13	32 ± 5	40 ± 6 (125)	65 ± 54 (203)	60 ± 41 (188) (N=3)	35 ± 5	36 ± 7 (103)	53 ± 30 (151)	124ex (354) (N=2)
	16	-	-	-	53 ± 24 (166) (N=3)	-	-	-	100ex (286) (N=2)

GGTP (U/L)	BT	3.7 ± 0.3	3.7 ± 0.3 (100)	4.0 ± 0.5 (108)	4.5 ± 0.5* **(122)** ↑**22%**	3.8 ± 0.2	3.3 ± 0.4 (87)	3.5 ± 0.5 (92)	3.9 ± 0.5 (103)
	4	3.3 ± 0.3	3.8 ± 0.1 (115)	5.9 ± 1.8* **(179)** ↑**79**	7.0 ± 2.1** **(212)** ↑**112%**	3.1 ± 0.4	3.3 ± 0.3 (106)	5.1 ± 0.7** **(165)** ↑**65**	5.4 ± 1.1** **(174)** ↑**174%**

	8	3.1 ± 0.5	4.1 ± 0.2 (132)	6.2 ± 1.7* **(200) ↑100%**	**11.8 ± 6.3** (381) **↑281%**	3.6 ± 0.5	3.4 ± 0.4 (94)	5.4 ± 0.8 (150)	11.8 ± 5.5* **(328) ↑228** (N=3)
	13	3.9 ± 0.5	5.0 ± 0.3 (128)	7.3 ± 1.4** **(187) ↑87%**	**9.0 ± 1.5** (231) **↑131%** (N=3)	4.0 ± 0.4	4.1 ± 0.6 (103)	7.6 ± 1.4** **(190) ↑90%**	14ex (350) (N=2)

	16	-	-	-	8.6 ± 1.5 (221) (N = 3)	-	-	-	11.0ex (275) (N=2)
HC data at week 13		N = 132	3.4 ± 0.7 Mean ± 3SD: 5.6			Not reported			
Creat (mg/dL)	8	0.60 ± 0.03	0.62 ± 0.05 (103)	0.60 ± 0.03 (100)	0.52 ± 0.03* **(87) ↓13%**	0.62 ± 0.09	0.64 ± 0.07 (103)	0.60 ± 0.04 (97)	0.60 ± 0.14 (97)
TP (g/dL)	4	5.54 ± 0.27	5.14 ± 0.14 (93)	4.91 ± 0.28** **(89) ↓11**	5.35 ± 0.10 (97)	5.48 ± 0.24	5.32 ± 0.08 (97)	4.85 ± 0.28* **(89)**	5.24 ± 0.42 (96)

	8	5.82 ± 0.41	5.39 ± 0.22 (93)	4.86 ± 0.36** **(84) ↓16%**	4.86 ± 0.32** **(84) ↓16%**	5.52 ± 0.17	5.30 ± 0.12 (96)	4.84 ± 0.32* **(88) ↓12%**	4.74 ± 0.57* **(86) ↓14%** (N=3)
	13	6.02 ± 0.42	5.60 ± 0.08 (93)	4.82 ± 0.43** **(80) ↓20%**	4.84 ± 0.20** **(80) ↓20%** (N=3)	5.86 ± 0.17	5.55 ± 0.19 (95)	5.17 ± 0.33** **(88) ↓12%**	4.71ex (80) (N=2)

	16	-	-	-	5.11 ± 0.06 (85) (N=3)	-	-	-	4.96ex (85) (N=2)
Alb (g/dL)	4	2.96 ± 0.08	2.80 ± 0.07 (95)	2.58 ± 0.13** **(87)** **↓13%**	2.78 ± 0.12 (94)	3.10 ± 0.10	2.96 ± 0.09 (95)	2.61 ± 0.17** **(84)** **↓16%**	2.78 ± 0.17* **(90)** **↓10%**
	8	2.94 ± 0.23	2.85 ± 0.06 (97)	2.36 ± 0.26** **(80)** **↓20%**	2.39 ± 0.15** **(81)** **↓19%**	3.09 ± 0.08	2.82 ± 0.08 (91)	2.42 ± 0.24* **(78)** **↓12%**	2.17 ± 0.55* **(70)** **↓30%** (N=3)

	13	3.00 ± 0.17	2.85 ± 0.09 (95)	2.37 ± 0.25** **(79) ↓21%**	2.28 ± 0.11** **(76) ↓24%** (N=3)	3.19 ± 0.12	2.98 ± 0.10 (93)	2.53 ± 0.27** **(79) ↓21**	2.18ex (68) (N=2)
	16	-	-	-	2.31 ± 0.13 (77) (N=3)	-	-	-	2.20ex (69) (N=2)

Glob (g/dL)	8	2.89 ± 0.29	2.54 ± 0.21 (88)	2.50 ± 0.11 (87)	2.46 ± 0.17* **(85) ↓15%**	2.44 ± 0.22	2.49 ± 0.12 (102)	2.42 ± 0.37 (99)	2.57 ± 0.14 (105) (N=3)
	13	3.02 ± 0.46	2.75 ± 0.14 (91)	2.45 ± 0.19* **(81) ↓19%**	2.56 ± 0.15 (85) (N=3)	2.68 ± 0.28	2.57 ± 0.10 (96)	2.64 ± 0.15 (99)	2.53ex (94) (N=2)
A/G	8	1.03 ± 0.11	1.13 ± 0.10 (110)	0.94 ± 0.08 (91)	0.97 ± 0.01 (94)	1.28 ± 0.15	1.13 ± 0.07 (88)	1.02 ± 0.22 (80)	0.84 ± 0.21* (66) (N=3)

	13	1.01 ± 0.17	1.04 ± 0.08 (103)	0.97 ± 0.05 (93)	0.89 ± 0.07 (87) (N=3)	1.20 ± 0.17	1.16 ± 0.03 (97)	0.96 ± 0.10* **(80) ↓20%**	0.87ex (73) (N=2)
	16	-	-	-	0.83 ± 0.10 (82) (N=3)	-	-	-	0.81ex (68) (N=2)
Gluc (mg/dL)	4	102 ± 7	100 ± 4 (98)	89 ± 5** **(87) ↓13%**	**92 ± 4** (90) ↓10%**	104 ± 4	102 ± 5 (98)	96 ± 4 (92)	95 ± 4* **(91) ↓9%**

	8	99 ± 2	100 ± 3 (101)	89 ± 2** **(90) ↓9%**	89 ± 2 **(90) ↓9%**	101 ± 4	100 ± 4 (99)	95 ± 5 (94)	91 ± 8 (90) (N=3)
	13	100 ± 2	103 ± 8 (103)	93 ± 3 (93)	87 ± 5 * **(87) ↓13%** (N = 3)	101 ± 5	100 ± 8 (99)	97 ± 2 (96)	93ex (92) (N=2)
	16	-	-	-	88 ± 8 (88) (N = 3)	-	-	-	91ex (90) (N=2)

T.Chol (mg/dL)	BT	140 ± 27	143 ± 20 (102)	146 ± 18 (104)	141 ± 32 (101)	117 ± 19	142 ± 20 (121)	134 ± 14 (115)	154 ± 17* **(132) ↑32%**
	4	132 ± 19	134 ± 28 (102)	101 ± 26 (77)	109 ± 33 (83)	123 ± 20	127 ± 16 (103)	91 ± 16 (74)	109 ± 21 (89)
	8	130 ± 22	139 ± 38 (107)	89 ± 31 (68)	53 ± 33* **(41) ↓59%**	114 ± 19	126 ± 15 (111)	95 ± 15 (83)	64 ± 46 (56) (N=3)

	13	121 ± 26	139 ± 39 (115)	84 ± 32 (69)	56 ± 15* **(46) ↓54%** (N=3)	114 ± 22	126 ± 19 (111)	96 ± 12 (84)	59ex(52) (N=2)
	16	-	-	-	70 ± 12 (58) (N=3)	-	-	-	57 (50) (N=2)
TG (mg/dL)	8	25 ± 12	39 ± 11 (156)	31 ± 14 (124)	20 ± 10 (8000)	14 ± 4	28 ± 8* **(200) ↑100%**	23 ± 4 (164)	16 ± 7 (114) (N=3)

T.Bil (mg/dL)	8	0.06 ± 0.02	0.05 ± 0.01 (83)	0.04 ± 0.01 (67)	0.14 ± 0.20 (233)	0.07 ± 0.02	0.05 ± 0.01 (71)	0.05 ± 0.01 (71)	0.35 ± 0.51 (500) (N=3)
	13	0.07 ± 0.02	0.06 ± 0.01 (86)	0.04 ± 0.00 (57)	0.04 ± 0.01 (57)	0.08 ± 0.01	0.06 ± 0.01* **(75) ↓25**	0.05 ± 0.01** **(63) ↓37**	0.05ex (63) (N=2)
Ca (mg/dL)	4	10.6 ± 0.0	10.3 ± 0.2 (97)	9.9 ± 0.2* **(93) ↓7%**	10.5 ± 0.4 (99)	10.2 ± 0.3	10.3 ± 0.1 (101)	9.8 ± 0.3 (96)	10.0 ± 0.4 (98)

	8	10.6 ± 0.2	10.3 ± 0.3 (97)	9.8 ± 0.3* **(92) ↓8%**	9.8 ± 0.4* **(92) ↓8%**	10.4 ± 0.3	10.4 ± 0.0 (100)	9.8 ± 0.2 (94)	9.2 ± 0.7* **(88)↓12%** (N=3)
	13	10.6 ± 0.3	10.3 ± 0.2 (97)	9.7 ± 0.2** **(92) ↓8%**	9.5 ± 0.2** **(90) ↓10%** (N=3)	10.5 ± 0.3	10.4 ± 0.3 (99)	9.9 ± 0.3 (94)	9.4ex (90) (N=2)

	16	-	-	-	9.5 ± 0.1 (90) (N=3)	-	-	-	9.3 (89) (N=2)
P (mg/dL)	4	6.1 ± 0.5	6.1 ± 0.3 (100)	5.9 ± 0.4 (97)	6.0 ± 0.3 (98)	5.6 ± 0.3	6.3 ± 0.3* **(113) ↑13%**	6.0 ± 0.3 (107)	5.6 ± 0.4 (100)
Na (mEq/L)	8	147.1 ± 0.8	147.6 ± 1.0 (100)	147.9 ± 1.6 (101)	149.3 ± 0.6* **(101) ↑1%**	148.8 ± 1.2	148.5 ± 1.1 (100)	148.3 ± 0.7 (100)	145.1 ± 3.6 (98)

~: for week 16 data, ratio is to control at week 13; BT: Before initiation of treatment; * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); ex: excluded from statistical evaluation because of insufficient sample numbers.

Urinalysis

At the top dose, males showed decreased urine pH at weeks 4 and 8 (statistically significant at week 8). This effect is considered treatment-related and adverse.

Gross pathology

At necropsy, dark-coloured livers at 700/500 mg/kg bw/day (in all the males and one female) and 160 mg/kg bw/day (in three males and all the females) were observed. Enlarged livers were noted at 700/500 mg/kg bw/day (in one male and one female) and 160 mg/kg bw/day (in three males and two females). Calculi (in one male and one female at 700/500 mg/kg bw/day) and biliary sludge (in one male at 160 mg/kg bw/day) in the gallbladder were also recorded. All these effects indicative of liver and gall bladder damage are considered as treatment related and adverse.

Animals killed in extremis at 700/500 mg/kg bw/day showed additional gross pathology findings indicative of the extreme toxicity of the 700 mg/kg bw/day dose.

Overall, treatment related, and adverse gross pathological effects were observed in the liver and gall bladder in both the sexes from 160 mg/kg bw/day.

Organ weights

Dose dependent and statistically significant increases in absolute (up to 53% in males and 41% in females) and relative (up to 69% in males and 57% in females) weights of the liver were noted from 160 mg/kg bw/day. Although an increase (20-26%) was also seen in males at the low dose, this was not associated with changes in clinical-chemistry parameters and histopathological findings beyond hypertrophy. Therefore, only the liver weight increases seen in both sexes from the mid dose are considered adverse.

A decrease in absolute heart weight was seen in both sexes at the top dose. However, in the absence of associated histopathology, this was considered just the secondary consequence of the decreased body weights. Statistically significant decreases in absolute (by 52% and 52% in both mid and high dose) and relative (52% in mid dose) prostrate weights were observed in males. Again, in the absence of associated histopathology, these decreases were not considered adverse.

Animals killed in extremis at 700/500 mg/kg bw/day showed additional organ weight changes (thymus, spleen, testes, epididymis), indicative of the extreme toxicity of the 700 mg/kg bw/day dose.

Overall, there were treatment related and adverse effects on liver weight from the mid dose. Additional organ weights were affected at the top dose, but these were the consequence of the decreased body weights.

Table 6.3.2-14: Summary of significant changes in absolute and relative organ weights in dogs administered inpyrfluxam in gelatin capsule for 90 days.

Organ	Sex and dose level (mg/kg/day)							
	Male				Female			
	0 (N=4)	40 (N=4)	160 (N=4)	700 / 500 (N=3)	0 (N=4)	40 (N=4)	160 (N=4)	700 / 500 (N=2)
Terminal body weight (kg)	9.9 ± 0.6	10.1 ± 1.0 (102)	10.2 ± 1.1 (103)	8.7 ± 1.0 (88)	9.2 ± 0.8	9.6 ± 0.3 (104)	9.3 ± 0.9 (101)	8.2ex (89)
Liver – abs (g)	243 ± 12	313 ± 16* **(129) ↑29%**	371 ± 18** **(153) ↑53%**	363 ± 57** **(149) ↑49%**	241 ± 34	290 ± 21 (120)	339 ± 59* **(141) ↑41%**	338ex (140)
Liver - rel	2.47 ± 0.18	3.11 ± 0.21* **(126) ↑26%**	3.69 ± 0.47** **(149) ↑49%**	4.18 ± 0.28** **(169) ↑69%**	2.63 ± 0.31	3.02 ± 0.13 (115)	3.64 ± 0.28** **(138) ↑38%**	4.14ex (157)
Heart – abs (g)	74.9 ± 2.8	76.8 ± 5.0 (103)	70.4 ± 4.6 (94)	64.8 ± 6.9* **(87) ↓13%**	71.4 ± 7.1	70.6 ± 3.7 (99)	69.2 ± 2.9 (97)	59.6ex (83)
Prostate – abs (g)	6.0 ± 1.8	5.6 ± 1.7 (93)	2.9 ± 0.8* **(48) ↓52%**	2.9 ± 0.4* **(48) ↓52%**	-			
Prostate – rel	0.060 ± 0.016	0.055 ± 0.018 (92)	0.029 ± 0.011* **(48) ↓52%**	0.034 ± 0.008 (57)	-			

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); ex: excluded from statistical evaluation because of insufficient sample numbers.

Histopathology

Liver

In the liver, hypertrophy was seen in both sexes from the low dose, but it is considered adverse from the mid dose, as only from this dose it correlates with changes in clinical-chemistry parameters and other histopathological findings. Indeed, cytoplasmic eosinophilic inclusion bodies were seen in top dose males and from the mid dose in females. Diffuse single cell necrosis was noted in top dose females and at the mid dose in males. Brown pigment deposition in the Kupffer cells was observed from the mid dose in males and at the top dose in females. In addition, extrahepatic bile duct inflammation was seen in top dose females. Overall, multiple adverse histopathological findings were seen in the liver from the mid dose in both sexes.

Gall bladder

Calculi in the gallbladder were seen from the mid dose in males and at the top dose in males.

Kidney
There was hypertrophy and cytoplasmic eosinophilic inclusion body in the proximal tubular cells of the kidneys in males from the mid dose. In addition, proximal tubular cell vacuolation was observed at the top dose in both sexes. Overall, histopathological findings of the kidney were seen from the mid dose.

Other organs
Splenic congestion was noted in top dose males and females; thyroid follicular cell hypertrophy was observed in one mid dose female and one top dose male; zona fasciculata cell vacuolation of the adrenal gland was seen from the mid dose in males and at the top dose in females; optic nerve fibre degeneration was noted at the top dose in males (3 vs 0 in controls) and from the mid dose in females (1 and 4 at the mid and high dose vs 0 in controls). These findings are considered as treatment related and adverse. Historical control data (HCD) from the testing facility (nineteen 90-day studies conducted from 2006 to 2023) showed that optic nerve fibre degeneration was observed only twice (one in males and one in females) among 76 control males and 76 control females, indicating that it is a rare finding. This supports the conclusion that the increased incidence of optic nerve fibre degeneration in top-dose males and females, as well as mid-dose females, is treatment-related.

Additional histopathological findings were noted in various other organs only in the animals killed in the extremis, indicative of the extreme toxicity of the 700 mg/kg bw/day dose.

Overall, histopathological findings were observed in the liver, gall bladder, kidney, thyroid, adrenal and optic nerve from the mid dose. In addition, the spleen was affected at the top dose and many other organs showed findings at 700 mg/kg bw/day in the animals killed in extremis.

Table 6.3.2-15: Selected histopathological findings in dogs administered inpyrfluxam in gelatin capsule for 90 days.

Organ & lesion	Fate	Sex and dose level (mg/kg/day)							
		Male				Female			
		0	40	160	700 / 500	0	40	160	700 / 500
Liver									
Diffuse hepatocyte hypertrophy	All	0/4	3/4	**4/4***	3/4	0/4	3/4	**4/4***	2/4
	Tk	0/4	3/4	**4/4***	**3/3***	0/4	3/4	**4/4***	2/2ex
	Ke	-	-	-	0/1	-	-	-	0/2
Cytoplasmic eosinophilic inclusion body	All	0/4	0/4	0/4	3/4	0/4	0/4	1/4	1/4
	Tk	0/4	0/4	0/4	**3/3***	0/4	0/4	1/4	1/2ex
	Ke	-	-	-	0/1	-	-	-	0/2
Diffuse single cell necrosis	All	0/4	0/4	1/4	0/4	0/4	0/4	0/4	3/4
	Tk	0/4	0/4	1/4	0/3	0/4	0/4	0/4	1/2ex
	Ke	-	-	-	0/1	-	-	-	2/2
	All	0/4	0/4	1/4	1/4	0/4	0/4	0/4	2/4

Brown pigment deposition in Kupffer cell	Tk	0/4	0/4	1/4	0/3	0/4	0/4	0/4	0/2ex
	Ke	-	-	-	1/1	-	-	-	2/2
Extrahepatic bile duct inflammation	All	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	Tk	0/4	0/4	0/4	0/3	0/4	0/4	0/4	1/2ex
	ke	-	-	-	0/1	-	-	-	0/2
Gall bladder									
Calculi in gallbladder	All	0/4	0/4	1/4	1/4	0/4	0/4	0/4	2/4
	tk	0/4	0/4	1/4	0/3	0/4	0/4	0/4	0/2ex
	ke	-	-	-	1/1	-	-	-	2/2
Kidney									
Proximal tubular cell hypertrophy	All	0/4	0/4	3/4	2/4	0/4	0/4	0/4	0/4
	tk	0/4	0/4	3/4	2/3	0/4	0/4	0/4	0/2ex
	ke	-	-	-	0/1	-	-	-	0/2
Cytoplasmic eosinophilic inclusion body in proximal tubular cell	All	0/4	0/4	1/4	2/4	0/4	0/4	0/4	0/4
	tk	0/4	0/4	1/4	2/3	0/4	0/4	0/4	0/2ex
	ke	-	-	-	0/1	-	-	-	0/2
Proximal tubular cell vacuolation	All	0/4	1/4	0/4	2/4	0/4	0/4	0/4	1/4
	tk	0/4	1/4	0/4	1/3	0/4	0/4	0/4	0/2ex
	ke	-	-	-	1/1	-	-	-	1/2
Spleen									
Congestion	All	0/4	0/4	0/4	2/4	0/4	0/4	0/4	2/4
	tk	0/4	0/4	0/4	1/3	0/4	0/4	0/4	0/2ex
	ke	-	-	-	1/1	-	-	-	2/2
Thyroid									
Follicular cell hypertrophy	All	0/4	0/4	0/4	1/4	0/4	0/4	1/4	0/4
	tk	0/4	0/4	0/4	1/3	0/4	0/4	1/4	0/2ex
	ke	-	-	-	0/1	-	-	-	0/2
Adrenal									
Zona fasciculata cell vacuolation	All	0/4	0/4	2/4	2/4	0/4	0/4	0/4	2/4
	tk	0/4	0/4	2/4	2/3	0/4	0/4	0/4	2/2ex
	ke	-	-	-	0/1	-	-	-	0/2
Eye									
Optic nerve fibre degeneration	All	0/4	0/4	0/4	3/4	0/4	0/4	1/4	**4/4***
	tk	0/4	0/4	0/4	2/3	0/4	0/4	1/4	2/2ex
	ke	-	-	-	1/1	-	-	-	2/2

All: all animals; tk: terminal kill; ke: killed *in extremis*; ex: excluded from statistical evaluation because of insufficient sample numbers; *: p≤0.05 (Fisher's exact probability test)

Conclusion

In conclusion, in a GLP and guideline compliant study, administration of inpyrfluxam in gelatin capsules to beagle dogs at 0, 40, 160 or 700/500 mg/kg bw/day for 13 weeks caused adverse effects from the mid dose of 160 mg/kg bw/day, including vomiting, signs of anaemia, changes in clinical-chemistry parameters indicative of liver damage, increased liver weight and histopathological findings in the liver, gall bladder, kidney, thyroid, adrenal and optic nerve. The top dose was highly toxic with additional signs of toxicity, such as

mortality, clinical signs of toxicity, effects on body weight and food consumption, changes in urinalysis parameters and additional effects on other organs.

Overall, a NOAEL of 40 mg/kg bw/day can be established from this study based on vomiting, signs of anaemia, changes in clinical-chemistry indicative of liver damage, increased liver weight and histopathological findings in the liver, gall bladder, kidney, thyroid, adrenal and optic nerve at the LOAEL of 160 mg/kg bw/day.

[redacted] (2016)

B.6.3.3. One year study in dogs

A one-year dietary toxicity study has been conducted in dogs. The study was GLP complaint and performed in accordance with OECD TG 452 (2009). Although the one-year dog study is no longer required by Regulation 283/2013 as it applies in GB, this study has been accepted and evaluated as it represents adverse data (lower NOAEL identified).

Reference:	KCA 5.3.2/04
Report Title:	S-2399 Technical Grade: Repeated Dose 1-Year Oral Toxicity Study in Dogs
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. [redacted] 14-0096 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable method of analysis available for plasma kinetics
Guideline(s):	OECD TG 452 (2009)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 4 male and 4 female Beagle dogs received inpyrfluxam in gelatin capsule at 0, 2, 6, 30 or 160 mg/kg bw/day for 52 weeks.

The dose levels were selected based on a previous repeated dose 90-day oral toxicity study in dogs where the administration of 700/500 mg/kg bw/day inpyrfluxam resulted in severe toxicity including mortality, clinical signs of toxicity, effects on body weight and food consumption, changes in urinalysis parameters, signs of anaemia, changes in clinical-chemistry indicative of liver damage, and additional effects on various organs.

In accordance with OECD test guideline 452, all animals were subjected to the required investigations. In addition, concentrations of inpyrfluxam in plasma were measured at 0 (before administration), 2, 4, 7, and 24 hours after administration at day 1, weeks 13, 26 and 52 after treatment.

Results

Toxicokinetics

Inpyrfluxam was detected in the plasma of animals of both sexes at all dose levels and all time points except at 2 mg/kg bw/day where it was lower than the limit of quantification (LOQ). A dose dependent increase in plasma levels of inpyrfluxam was noted throughout the treatment. There was no difference in the systemic exposure of male and female dogs. In addition, no accumulation after repeated oral (capsule) administration was observed in both sexes.

Table 6.3.3-1: Summary of toxicokinetic parameters in plasma of dogs administered inpyrfluxam in gelatin capsules for 52 weeks.

Dose level mg/kg/day	Male				Female			
	2	6	30	160	2	6	30	160
Day 1								
T_{max} (hr)	-	2	4	2	-	7	2	7
C_{max} (µg/mL)	<LOQ	0.009	0.652	0.917	<LOQ	0.096	0.252	0.794
AUC_{0-t} (hr*µg/mL)	na	0.048	6.100	11.682	na	0.158	1.222	11.303
Week 13								
T_{max} (hr)	-	2	2	7	-	2	4	4
C_{max} (µg/mL)	<LOQ	0.050	0.293	0.936	<LOQ	0.006	0.034	0.555
AUC_{0-t} (hr*µg/mL)	na	0.114	1.777	11.091	na	0.031	0.148	5.482
Week 26								
T_{max} (hr)	-	2	7	7	-	2	4	4
C_{max} (µg/mL)	<LOQ	0.041	0.159	1.060	<LOQ	0.062	0.028	0.892
AUC_{0-t} (hr*µg/mL)	na	0.101	2.114	12.877	na	0.182	0.125	10.553
Week 52								

T_{max} (hr)	-	2	7	7	-	2	2	4
C_{max} (µg/mL)	<LOQ	0.036	0.262	0.721	<LOQ	0.042	0.148	1.040
AUC_{0-t} (hr*µg/mL)	na	0.130	2.899	8.589	na	0.210	0.626	10.737

na: not applicable; LOQ: limit of quantitation = 0.004 µg/mLTmax: Time to reach the maximum plasma concentration; Cmax: Maximum plasma concentration; AUC0-t: Area under the curve from the time of dosing (Dosing_time) to the time of the last quantifiable plasma concentration.

Mortality and general clinical signs

There was no mortality in any group in both sexes.

Increased incidence of vomiting of the feed was observed at 160 mg/kg bw/day in both sexes and only in one female at 30 mg/kg bw/day. The frequency of vomiting in females increased at 30 mg/kg bw/day (12 incidences vs 3 in controls) and 160 mg/kg bw/day (37 incidences vs 3 in controls), and in males at the high dose of 160 mg/kg bw/day (34 incidences vs 2 in controls) compared to controls. Therefore, adverse clinical signs of toxicity were seen from 30 mg/kg bw/day.

There were no effects of treatment on body weight, food consumption, ophthalmology, haematology, or urinalysis.

Clinical chemistry parameters

At 160 mg/kg bw/day, there were significant increases in alkaline phosphatase (ALP) (by >100%), alanine aminotransferase (ALT) (by >50%) and γ-glutamyl transpeptidase (GGTP) (by >75%) in both sexes. At 30 mg/kg bw/day, increasing trends of ALP (by >60%) and GGTP (by >50%) were observed in males. At the high dose, significant decreases or decreasing trends in albumin (Alb), albumin/globulin ratio (A/G) (by >40%), total cholesterol (T. Chol) (by >10%) and calcium (Ca) (by >5%) were noted in males. High dose females also showed decreased levels of potassium (K) (by >10%), triglycerides (by >50%) and total cholesterol (T.chol) (by >10%). All the observed changes were considered as treatment related and adverse except for the changes in triglyceride levels in high dose females due to the lack of a dose response.

Overall, adverse effects on clinical chemistry parameters indicative of liver damage were noted in males from 30 mg/kg bw/day and in females at 160 mg/kg bw/day.

Table 6.3.3-2: Summary of significant changes at clinical chemistry [mean ± SD (% of controls)] in dogs administered inpyrfluxam in gelatin capsules for 52 weeks.

Para-meter	Week	Male					Female				
		0	2	6	30	160	0	2	6	30	160
ALP (U/L)	13	364 ± 61	298 ± 51 (82)	420 ± 78 (115)	595 ± 142 (163)	860 ± 281** **(236)**	283 ± 55	248 ± 96 (88)	353 ± 107 (125)	547 ± 190 (193)	753 ± 296** **(266)**

						↑136%					**↑166%**
	26	265 ± 29	231 ± 76 (87)	325 ± 26 (123)	694 ± 206** **(262) ↑162%**	799 ± 203** **(302) ↑202%**	220 ± 48	187 ± 111 (85)	295 ± 108 (134)	534 ± 269 (243)	596 ± 278* **(271) ↑171%**
	52	233 ± 38	213 ± 76 (91)	323 ± 35 (139)	766 ± 241** **(262) ↑162%**	1003 ± 294** **(430) ↑330%**	180 ± 25	176 ± 98 (98)	370 ± 166 (206)	534 ± 357 (297)	600 ± 430 (333)
ALT (U/L)	13	34 ± 11	30 ± 8 (88)	30 ± 7 (88)	33 ± 7 (97)	86 ± 83 (253)	31 ± 5	30 ± 3 (97)	32 ± 7 (103)	33 ± 6 (106)	47 ± 8** **(152) ↑52%**
	26	38 ± 12	32 ± 7 (84)	31 ± 8 (82)	36 ± 7 (95)	50 ± 21 (132)	27 ± 5	28 ± 4 (104)	31 ± 10 (115)	30 ± 7 (111)	42 ± 11 (156)
	52	38 ± 13	35 ± 9 (92)	34 ± 6 (89)	37 ± 10 (97)	57 ± 9* **(150) ↑50%**	26 ± 6	27 ± 5 (104)	29 ± 7 (112)	30 ± 6 (115)	48 ± 14** **(185) ↑85%**
GGTP (U/L)	13	3.1 ± 0.4	3.7 ± 0.6 (119)	4.0 ± 1.0 (129)	4.9 ± 0.4* **(158) ↑58%**	5.5 ± 1.2** **(177) ↑77%**	3.7 ± 0.4	3.8 ± 1.1 (103)	3.7 ± 0.5 (100)	4.0 ± 0.2 (108)	6.0 ± 1.0** **(162) ↑62%**
	26	3.3 ± 0.5	4.2 ± 0.8 (127)	4.2 ± 0.9 (127)	5.4 ± 0.8* **(164) ↑64%**	6.2 ± 1.8** **(188) ↑88%**	3.7 ± 0.4	4.3 ± 0.8 (116)	3.8 ± 0.4 (103)	4.2 ± 0.7 (114)	6.0 ± 1.3** **(162) ↑62%**
	52	3.2 ± 0.5	3.9 ± 0.8 (122)	4.0 ± 1.0 (125)	5.0 ± 0.6 (156)	6.1 ± 2.0** **(191) ↑91%**	3.8 ± 0.5	3.5 ± 0.6 (92)	3.6 ± 0.4 (95)	4.4 ± 1.4 (116)	6.6 ± 1.2** **(174) ↑74%**
Alb (g/dL)	13	2.97 ± 0.07	2.98 ± 0.20 (100)	2.92 ± 0.20 (98)	2.77 ± 0.16 (93)	2.24 ± 0.25** **(75) ↓25%**	2.96 ± 0.20	3.05 ± 0.06 (103)	3.02 ± 0.07 (102)	2.81 ± 0.07 (95)	2.83 ± 0.32 (96)
	26	2.94 ± 0.04	2.99 ± 0.25 (102)	2.92 ± 0.27 (99)	2.72 ± 0.26 (93)	2.20 ± 0.16** **(75)**	2.92 ± 0.21	2.97 ± 0.04 (102)	3.00 ± 0.13 (103)	2.77 ± 0.13 (95)	2.87 ± 0.32 (98)
	52	2.94 ± 0.10	2.90 ± 0.23 (99)	2.86 ± 0.29 (97)	2.54 ± 0.20 (86)	2.06 ± 0.17** **(70) %**	2.92 ± 0.28	2.95 ± 0.05 (101)	2.90 ± 0.14 (99)	2.73 ± 0.06 (93)	2.92 ± 0.34 (100)
Glob (g/dL)	13	3.00 ± 0.34	3.00 ± 0.14 (100)	2.94 ± 0.47 (98)	3.04 ± 0.56 (101)	2.91 ± 0.39 (97)	3.02 ± 0.60	2.71 ± 0.41 (90)	3.04 ± 0.39 (101)	3.17 ± 0.19 (105)	2.77 ± 0.28 (92)
	26	3.41 ± 0.51	3.36 ± 0.21 (99)	3.23 ± 0.80 (95)	3.59 ± 0.47 (105)	3.35 ± 0.39 (98)	3.27 ± 0.62	2.89 ± 0.30 (88)	3.32 ± 0.40 (102)	3.42 ± 0.10 (105)	3.04 ± 0.26 (93)

	52	3.40 ± 0.21	3.60 ± 0.17 (106)	3.59 ± 0.75 (106)	4.45 ± 0.42* **(131) ↑31%**	3.65 ± 0.33 (107)	3.54 ± 0.72	3.48 ± 0.38 (98)	3.82 ± 0.23 (108)	3.82 ± 0.37 (108)	3.44 ± 0.42 (97)
A/G ratio	13	1.00 ± 0.11	1.00 ± 0.10 (100)	1.02 ± 0.25 (102)	0.94 ± 0.17 (94)	0.77 ± 0.04 (77)	1.02 ± 0.25	1.14 ± 0.17 (112)	1.01 ± 0.16 (99)	0.89 ± 0.07 (87)	1.03 ± 0.15 (101)
	26	0.88 ± 0.14	0.90 ± 0.12 (102)	0.97 ± 0.34 (110)	0.77 ± 0.14 (88)	0.66 ± 0.04 (75)	0.93 ± 0.22	1.04 ± 0.11 (112)	0.92 ± 0.13 (99)	0.81 ± 0.05 (87)	0.95 ± 0.13 (102)
	52	0.87 ± 0.07	0.81 ± 0.09 (93)	0.83 ± 0.25 (95)	0.57 ± 0.07* **(66) ↓44%**	0.57 ± 0.09* **(66) ↓44%**	0.86 ± 0.26	0.86 ± 0.09 (100)	0.76 ± 0.08 (88)	0.72 ± 0.09 (84)	0.86 ± 0.17 (100)
T. Chol (mg/dL)	13	123 ± 18	124 ± 9 (101)	134 ± 30 (109)	132 ± 23 (107)	86 ± 41 (70)	118 ± 7	125 ± 35 (106)	120 ± 6 (102)	129 ± 19 (109)	104 ± 28 (88)
	26	105 ± 11	112 ± 7 (107)	123 ± 27 (117)	121 ± 9 (115)	81 ± 25 (77)	127 ± 15	147 ± 37 (116)	120 ± 17 (94)	131 ± 16 (103)	109 22 (86)
	52	99 ± 8	106 ± 6 (107)	115 ± 27 (116)	122 ± 4 (123)	82 ± 19 (83)	158 ± 30	150 ± 33 (95)	120 ± 14 (76)	150 ± 42 (95)	107 ± 22 (68)
TG (mg/dL)	13	25 ± 13	23 ± 5 (92)	26 ± 6 (104)	46 ± 12* **(184) ↑84%**	22 ± 8 (88)	26 ± 9	33 ± 12 (127)	29 ± 8 (112)	**45 ± 6* (173) ↑73%**	30 ± 5 (115)
	26	23 ± 15	22 ± 3 (96)	27 ± 3 (117)	40 ± 9* **(174) ↑74%**	22 ± 3 (96)	35 ± 6	36 ± 11 (103)	32 ± 8 (91)	37 ± 11 (106)	21 ± 4 (60)
	52	25 ± 11	21 ± 4 (84)	31 ± 4 (124)	44 ± 14* **(176) ↑76%**	24 ± 5 (96)	44 ± 8	59 ± 12 (134)	31 ± 8 (70)	40 ± 12 (91)	19 ± 5** **(43) ↓57%**
Ca (mg/dL)	13	10.2 ± 0.3	10.4 ± 0.5 (102)	10.3 ± 0.3 (101)	10.4 ± 0.3 (102)	9.6 ± 0.3 (94)	10.2 ± 0.3	10.2 ± 0.2 (100)	10.1 ± 0.2 (99)	10.0 ± 0.2 (98)	10.2 ± 0.4 (100)
	26	9.8 ± 0.1	10.1 ± 0.5 (103)	10.1 ± 0.3 (103)	9.9 0.3 (101)	9.2 ± 0.1* **(94) ↓6%**	10.0 ± 0.3	10.1 ± 0.3 (101)	10.0 ± 0.2 (100)	9.9 ± 0.2 (99)	10.0 ± 0.5 (100)
	52	9.6 ± 0.2	9.9 ± 0.5 (103)	9.9 ± 0.3 (103)	9.7 ± 0.3 (101)	9.0 ± 0.1* **(94) ↓6%**	9.9 ± 0.3	10.1 ± 0.2 (102)	9.8 ± 0.3 (99)	9.8 ± 0.2 (99)	10.0 ± 0.6 (101)
K (mEq/L)	52	4.15 ± 0.19	4.16 ± 0.30 (100)	4.01 ± 0.14 (97)	4.18 ± 0.27 (101)	3.99 ± 0.26 (96)	4.29 ± 0.14	4.23 ± 0.20 (99)	4.01 ± 0.25 (93)	3.95 ± 0.21 (92)	3.78 ± 0.16** **(88) ↓12%**

*: p≤0.05; **: p≤0.01 (Dunnett's test or Dunnett-type test)

Gross pathology

There was an increased incidence of dark coloured in males and enlarged liver in both sexes at 160 mg/kg bw/day. These changes were considered as treatment related and adverse.

Organ weights

At 30 mg/kg bw/day and 160 mg/kg bw/day, there were adverse increases (by >15%) in absolute and relative liver weights in both sexes. At 160 mg/kg bw/day, the females showed significant increases (by 31%) in relative adrenal weight.

Overall, adverse effects were observed in liver weights from 30 mg/kg bw/day in both sexes and in adrenal weights at 160 mg/kg bw/day in females.

Table 6.3.3-3: Summary of significant changes in absolute and relative organ weights in dogs administered inpyrfluxam in gelatin capsules for 52 weeks.

Dose level mg/kg/day	Male					Female				
	0	2	6	30	160	0	2	6	30	160
Liver										
Absolute (g)	274 ± 29	272 ± 18 (99)	316 ± 46 (115)	361 ± 36* **(132) ↑32%**	410 ± 73** **(150) ↑50%**	280 ± 32	312 ± 31 (111)	293 ± 23 (105)	322 ± 43 (115)	367 ± 67* **(131) ↑31%**
Relative (%)	2.35 ± 0.29	2.24 ± 0.15 (95)	2.63 ± 0.19 (112)	2.92 ± 0.28* **(124) ↑24%**	3.61 ± 0.43** **(154) ↑54%**	2.52 ± 0.43	2.77 ± 0.46 (110)	2.60 ± 0.33 (103)	3.01 ± 0.47 (119)	3.54 ± 0.50* **(140) ↑40%**
Adrenals										
Absolute (mg)	998 ± 57	1027 ± 109 (103)	1056 ± 30 (106)	1180 ± 198 (118)	1097 ± 176 (110)	1116 ± 130	1049 ± 164 (94)	1196 ± 61 (107)	979 ± 93 (88)	1351 ± 146 (121)
Relative (%)	0.0086 ± 0.0013	0.0084 ± 0.0005 (98)	0.0089 ± 0.0010 (103)	0.0096 ± 0.0021 (112)	0.0097 ± 0.0010 (113)	0.0100 ± 0.0013	0.0093 ± 0.0019 (93)	0.0106 ± 0.0009 (106)	0.0091 ± 0.0003 (91)	0.0131 ± 0.0012** **(131) ↑31%**

*: p≤0.05; **: p≤0.01 (Dunnett's test or Dunnett-type test)

Histopathology

At 160 mg/kg bw/day, diffuse hepatocyte hypertrophy and cytoplasmic eosinophilic inclusion body were observed in both sexes. Males also showed diffuse hepatocyte hypertrophy at 30 mg/kg bw/day. From 30 mg/kg bw/day, zona fasciculata cell vacuolation was noted in the adrenal glands of both sexes. These changes in the liver and adrenal gland were considered as treatment related and adverse.

At 30 mg/kg bw/day, one male showed cytoplasmic eosinophilic inclusion bodies in the proximal tubular cells of the kidney. This effect was considered incidental due to the lack of a dose response. At 160 mg/kg bw/day, there was an increased occurrence of optic nerve fibre degeneration in females compared to controls (2 vs 1 in controls). This finding was considered treatment-related and adverse. Historical control data (HCD) from the testing facility (nineteen 1-year studies conducted from 2006 to 2023) showed that optic nerve fibre degeneration was not observed in any of the 76 control males and 76 control females, supporting the conclusion that this finding is treatment related.

Overall, histopathological findings were observed in the liver and adrenal gland from 30 mg/kg bw/day and in the optic nerve at the top dose (females only).

Table 6.3.3-4: Selected histopathological findings in dogs administered inpyrfluxam in gelatin capsule for 90 days.

Organ & lesion	Sex and dose level (mg/kg/day)									
	Male					Female				
	0	2	6	30	160	0	2	6	30	160
Liver										
Diffuse hepatocyte hypertrophy	0 / 4	0 / 4	0 / 4	1 / 4	3 / 4	0 / 4	0 / 4	0 / 4	0 / 4	3 / 4
Cytoplasmic eosinophilic inclusion body	0 / 4	0 / 4	0 / 4	0 / 4	1 / 4	0 / 4	0 / 4	0 / 4	0 / 4	1 / 4
Kidney										
Cytoplasmic eosinophilic inclusion body in proximal tubular cell	0 / 4	0 / 4	0 / 4	1 / 4	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4
Adrenal										
Zona fasciculata cell vacuolation	0 / 4	0 / 4	0 / 4	2 / 4	1 / 4	0 / 4	0 / 4	0 / 4	1 / 4	2 / 4
Eye										
Optic nerve fibre degeneration	1 / 4	0 / 4	0 / 4	1 / 4	1 / 4	1 / 4	0 / 4	1 / 4	1 / 4	2 / 4

Conclusion

In conclusion, in a GLP and guideline compliant study, administration of inpyrfluxam in gelatin capsules at 0, 2, 6, 30 or 160 mg/kg bw/day to beagle dogs for 52 weeks caused adverse effects from 30 mg/kg bw/day, including increased incidence and frequency of vomiting, clinical chemistry parameters indicative of liver ~~damage~~ changes (ALP by >60%, GGTP by >50%), increased liver weights (>15%), and histopathological findings of the liver (diffuse hepatocyte hypertrophy) and adrenal gland (zona fasciculata cell vacuolation). In addition, increased degeneration of the optic nerve was noted at 160 mg/kg bw/day in females.

Overall, a NOAEL of 6 mg/kg bw/day can be established from this study based on vomiting, changes in clinical chemistry indicative of liver damage, increased liver weights, and histopathological findings of the liver and adrenal gland at the LOAEL of 30 mg/kg bw/day.

[redacted] (2017)

B.6.3.4. Other routes

28-day inhalation toxicity (rodents)

No study available.

90-day inhalation toxicity (rodents)

No study available.

28-day dermal toxicity (rodents)

A 28-day dermal toxicity study has been conducted in rats. The study was GLP complaint and performed in accordance with OECD TG 410 (1981).

Reference:	KCA 5.3.3/01
Report Title:	A 28-Day Repeated Dose Dermal Toxicity Study of S-2399 Technical Grade in Rats
Author(s) & Year:	[redacted] (2015)
Document No, Authority registration No	Study No. P140742 [redacted]

Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	OECD TG 410 (1981)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 10 male and 10 female Sprague Dawley [redacted] rats were exposed to inpyrfluxam via 6-hour occlusive dermal applications at 0, 100, 300 or 1000 mg/kg bw/day for 28 days.

The dose levels were selected based on the previous acute dermal toxicity study where no treatment related changes were noted up to 2000 mg/kg bw. Therefore, a dose level of 1000 mg/kg bw/day as recommended by the test guideline was selected as the maximum dose for this study.

In accordance with OECD test guideline 410, all animals were subjected to the required investigations.

Results

There were no effects of treatment on mortality, clinical signs of toxicity, body weights, food consumption, ophthalmology or urinalysis.

Haematology

A significant increase in MCH observed in females from the mid dose was not considered as treatment related as the changes were within the laboratory historical control data. No other adverse haematological changes were observed in either sex.

Clinical chemistry

A significant decrease in sodium (Na) observed in females from the mid dose was not considered as treatment related as the changes were within the laboratory historical control data. No other adverse clinical chemistry parameters were observed in any of the groups in either sex.

Gross pathology

Yellowish patch in the epididymis at 300 mg/kg bw/day and white patch in the liver at 100 mg/kg bw/day were observed in one single male each. These changes were not considered as adverse due to the lack of a dose response.

Organ weights

At 1000 mg/kg bw/day, an increase in relative heart weight was observed in males. This change was not considered as treatment related or adverse as the value was within the laboratory historical control data.

At 100 mg/kg bw/day and 300 mg/kg bw/day, there were increases in absolute and relative ovary weights in females. Females also showed increased relative brain weight at 100 mg/kg bw/day. These changes in ovary and brain were not considered as adverse due to the lack of a dose response.

Histopathology

One of the males showed spermatic granuloma in the epididymis at 300 mg/kg bw/day and another male showed massive necrosis of hepatocyte in the liver at 100 mg/kg bw/day. These effects correlated with the observed gross pathological changes and were not considered as treatment related or adverse due to the lack of a dose-response.

Conclusion

In conclusion, in a GLP and guideline compliant study, exposure of Sprague Dawley [redacted] rats to inpyrfluxam via 6-hour occlusive dermal application at 0, 100, 300 or 1000 mg/kg bw/day for 28 days did not cause any adverse effects. Therefore, a NOAEL of 1000 mg/kg bw/day (highest tested dose) can be established from this study.

[redacted] (2015)

90-day dermal toxicity (rodents)

No study available.

As the NOAEL in the 28-day dermal toxicity study in rats was determined to be 1000 mg/kg/day, no further study is necessary.

B.6.3.5. Summary of short-term toxicity

The short-term toxicity of inpyrfluxam has been investigated via the oral route of exposure in a preliminary 28-day (range-finding) study performed in rats, 90-day studies performed in rats, mice, and dogs and 1-year study performed in dogs. A 28-day dermal exposure study in rats is also available.

With the exception of the 28-day range-finding oral study in rats, all the remaining studies were conducted in compliance with GLP and OECD test guidelines. The main findings are summarised in table below.

Table 6.3.5-1: Summary of short-term toxicity studies for inpyrfluxam

Data point/ Study *Acceptability*	Species / Strain/ sex	Doses	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects at LOAEL
KCA 5.3.1/01 *One-month Oral Toxicity* - not GLP or OECD compliant - Range finding study *Acceptable as a range-finding study*	**Rat**/ Wistar Hannover rats/ M&F	0, 500, 1000, 3000 and 5000 ppm <u>Mean substance intakes</u> Males: 0, 44.4, 85.9, 246.4 and 406.5 mg/kg bw/day Females: 0, 47.4, 91.4, 263.0 and 377.8 mg/kg bw/day	Not set as range-finding study	Not set as range-finding study	3000 ppm: ↓ bw (10.7%* in M & 8.3%** in F) ↑ total cholesterol (50%* in M &100% in F), phospholipid (30.6% in M & 70.8% in F), γ-glutamyl transpeptidase (650%* in M and 300%* in F), triglyceride (217.6%** in F) ↑ relative liver weight (16.3%** in M & 25.1%** in F), absolute liver weight (14.7%** in F), ↓ absolute kidney weight (11.9%** in F), absolute thymus weight (33.3%** in M), relative thymus weight (25%** in M), absolute ovary weight (19.2%* in F) <u>*Histopathological findings*</u> Liver: ↑ hepatocellular hypertrophy (3/6 in M & 6/6** in F) Kidney: ↑ tubular hyaline droplets in 1/6 in M - not relevant to humans Thyroid: ↑ follicular cell hypertrophy (4/6 in M & 5/6* in F)

					Adrenal gland: glomerular fine vacuolation (5/6** in M) Bone marrow: fatty infiltration (5/6* in F) Ovary: Vacuolation of the interstitial gland (2/6 in F) Uterus: Slight atrophy (2/6 in F)
KCA 5.3.2/01 *90-Day Oral Toxicity* OECD TG 408 (1998) *Acceptable*	**Rat**/ Wistar Hannover/ M&F	0, 150, 500, 2000, or 4000 ppm <u>Mean substance intakes</u> Males: 0, 9.72, 31.7, 123 and 255 mg/kg bw/day Females: and 11.5, 37.5, 144 and 292 mg/kg bw/day	500 ppm 31.7 mg/kg bw/day	2000 ppm 123 mg/kg bw/day	↓ bw (10%** in F) ↓ bw gain (21%** in F) ↓ food consumption (17%** in F) ↑ γ-glutamyl transpeptidase (114%** in M and 238%** in F), Alkaline phosphatase (34%** in F) ↑ relative liver weight (11%** in M & 19%** in F) <u>*Histopathological findings*</u> Liver: ↑ diffuse hepatocellular hypertrophy (4/10** in M & 7/10** in F) Kidney: ↑ tubular hyaline droplets in 2/10 in M - not relevant to humans Thyroid: ↑ follicular cell hypertrophy (4/10 in F) Adrenal gland: ↑ cortical cell vacuolation (3/10 in F) Ovary: ↑ vacuolation of the interstitial gland (7/10* in F)
KCA 5.3.2/02 *90-Day Oral Toxicity* OECD TG 408 (1998) *Acceptable*	**Mice**/ [redacted] / M&F	0, 200, 800, 3500, or 7000 ppm <u>Mean substance intakes</u> Males: 0, 27.2, 111, 491, 973 mg/kg bw/day	800 ppm 111 mg/kg bw/day	3500 ppm 491 mg/kg bw/day	↑ globulin (10%* in M & 10% in F) ↓ albumin (8%** in F), albumin/globulin ratio (9% in M & 15%** in F) ↑ relative liver weight (18%** in M & 11%** in F), absolute liver weight (16%** in M & 7% in F) <u>*Histopathological findings*</u> Liver: ↑ diffuse hepatocellular hypertrophy (4/10 in F), Centrilobular hepatocyte hypertrophy (8/10** in M), Centrilobular hepatocyte fatty change (3/10 in M)

		Females: and 0, 31.7, 130, 559 and 1097 mg/kg bw/day			
KCA 5.3.2/03 *90-Day Oral Toxicity* OECD TG 409 (1998) *Acceptable*	**Dogs**/ Beagle / M&F	0, 40, 160 or 700/500 mg/kg bw/day (Due to severe toxicity at 700 mg/kg bw/day at week 2, the animals were not treated at week 3. At week 4 (week 1 for this group), the high dose was reduced to 500 mg/kg bw/day)	40 mg/kg bw/day	160 mg/kg bw/day	↓ reticulocyte count (25%) in M&F, albumin (21%** in M & F), globulin (19%* in M) albumin/globulin ratio (13% in M & 20%** in F) ↑ alkaline phosphatase (ALP) (261%** in M& 247%** in F) and γ-glutamyl transpeptidase (GGTP) (87%** in M& 90%** in F) and in alanine aminotransferase (ALT) (103% in M & 51% in M), total cholesterol (31% in M & 16% in F) Enlarged liver in M (3/4) & F (2/4), biliary sludge in gall bladder (1/4 in M) ↑ relative liver weight (49%** in M & 38%** in F), absolute liver weight (53%** in M and 41%** in F) <u>*Histopathological findings*</u> Liver: ↑ diffuse hepatocellular hypertrophy (3/4 in M and 4/4* in F), cytoplasmic eosinophilic inclusion bodies (/4 in F), Brown pigment deposition in the Kupffer cells (1/4 in M) Gall bladder: calculi (1/4 in M) Kidney: hypertrophy (3/4 in M) and cytoplasmic eosinophilic inclusion body (1/4 in M) in the proximal tubular cells Thyroid: Follicular cell hypertrophy (1/4 in F) Adrenal gland: zona fasciculata cell vacuolation (2/4 in M) Eye: optic nerve fibre degeneration (1/4 in F)

KCA 5.3.2/04 *One-year Oral Toxicity* OECD TG 452 (2009) *Acceptable*	**Dogs**/ Beagle / M&F	0, 2, 6, 30 or 160 mg/kg bw/day	6 mg/kg bw/day	30 mg/kg bw/day	↑ ALP (by >60%) and GGTP (by >50%) in M ↓ Albumin/globulin ratio (44%) in M ↑ absolute (32%*) and relative (24%*) liver weights in M <u>*Histopathological findings*</u> Liver: ↑ diffuse hepatocellular hypertrophy (1/4 in M), Adrenal gland: zona fasciculata cell vacuolation (2/4 in M & 1/4 in F) Eye: optic nerve fibre degeneration (1/4 in F)
KCA 5.3.3/01 28-day dermal toxicity OECD TG 410 (1981) *Acceptable*	**Rats**/ Sprague Dawley [redacted] M&F	0, 100, 300 or 1000 mg/kg bw/day	1000 mg/kg bw/day	Not established	No adverse effects

* and **: p≤0.05 and p≤0.01 (Fisher's exact probability test)

Rat

In rats, the main target organs of toxicity were the liver, adrenal glands, thyroid, and ovary.

Adverse (≥15%) increased liver weights with hypertrophy were seen from 123 mg/kg bw/day in the 90-day study. These effects were associated with alterations in coagulation (eg. prothrombin time) and clinical-chemistry parameters (eg. increased total cholesterol, γ-glutamyl transpeptidase etc) indicative of liver damage.

Increased cortical cell vacuolation in the adrenal gland of females was seen from 144 mg/kg bw/day in the 90-day study. Increased thyroid follicular hypertrophy was noted in females from 144 mg/ kg bw/day in the 90-day study.

In addition to decrease in weight, vacuolation of the interstitial gland was noted in the ovaries of females from 144 mg/kg bw/day in the 90-day study.

In addition to toxic effects in these organs, decreases in body weight and/or body weight gain and food consumption were also observed from around 123 mg/kg bw/day in the 90-day study.

Mouse

In mice, the main target organs of toxicity were the liver and thyroid.

Adverse (≥15%) increased liver weights with hypertrophy and fatty changes were seen from 491 mg/kg bw/day in the 90-day study. These effects were associated with alterations in albumin globulin ratio.

In addition, follicular cell hypertrophy of the thyroid was observed at the top dose of 973 mg/kg bw/day in the 90-day study.

Dog

In dogs, the main target organs of toxicity were the liver, gall bladder, kidney, adrenal glands, thyroid and the optic nerve.

Adverse (≥15%) increased liver weights with hypertrophy, eosinophilic inclusion bodies and brown pigmentation of Kupffer cells (the latter only in the 90-day) were seen from 160 mg/kg bw/day in the 90-day study and from 30 mg/kg bw/day in the one-year study. These effects were associated with alterations in clinical-chemistry parameters indicative of liver damage (eg. increased total cholesterol, γ-glutamyl transpeptidase etc).

In the adrenal gland, zona fasciculata cell vacuolation was seen in males from 160 mg/kg bw/day in the 90-day study and from 30 mg/kg bw/day in both sexes in the one-year study.

In the 90-day study, proximal tubular hypertrophy and cytoplasmic eosinophilic inclusion bodies were seen in the kidneys of males from 160 mg/kg bw/day. In addition, proximal tubular cell vacuolation was observed at the top dose of 700/500 mg/kg bw/day in both sexes.

Calculi in the gallbladder were seen from 160 mg/kg bw/day in males and at 700/500 mg/kg bw/day in females in the 90-day study.

Thyroid follicular cell hypertrophy was observed in one female at 160 mg/kg bw/day and one male at 700/500 mg/kg bw/day in the 90-day study.

In females, increased degeneration of the optic nerve was noted from 160 mg/kg bw/day in the 90-day study and at the top dose of 160 mg/kg bw/day in the one-year study.

In addition to these effects, vomiting was reported from 160 mg/kg bw/day in the 90-day study and from 30 mg/kg bw/day in the one-year study and signs of anaemia were seen from 160 mg/kg bw/day in the 90-day study. The most sensitive NOAEL for the short-term toxicity of inpyrfluxam was **6 mg/kg bw/day** (with a respective LOAEL of 30 mg/kg bw/day) taken from the 12-month dog study. At the LOAEL, there were adverse (>15%) increases in liver weights with hypertrophy, clinical chemistry parameters indicative of liver damage (ALP, GGTP, A/G ratio) and zona fasciculata cell vacuolation of the adrenal gland in both sexes.

Overall conclusion

Overall, therefore, repeated-dose toxicity has been adequately investigated in studies in rats, mice and dogs. The critical target organs were the liver and thyroid in all three species, with the adrenal gland affected in rats and dogs, the ovary in rats and the gall bladder, kidney, and optic nerve in dogs. The most sensitive species appears to be the dog, followed by the rat. The mouse was the least sensitive species to the short-term toxicity of inpyrfluxam.

B.6.4. Genotoxicity

The genotoxicity of inpyrfluxam was investigated using a standard package of GLP and OECD compliant in vitro and in vivo studies. In vitro bacterial reverse mutation and mammalian gene mutation tests were used to investigate the point mutation potential whereas the clastogenic potential was assessed using the in vitro chromosomal aberration test. The in vivo mouse micronucleus test was used to assess the clastogenic and aneugenic potential of inpyrfluxam. All the tests were performed according to the older versions of the test guidelines. The observed deviations from the current guidelines are considered to be minor with no impact on the outcome and validity of the studies. Therefore, all the studies are relied upon to draw conclusions on the genotoxic potential of inpyrfluxam.

B.6.4.1. In vitro studies

Inpyrfluxam was evaluated for its mutagenic potential using the bacterial reverse mutation test (OECD 471, 1997) and the in vitro mammalian gene mutation test (OECD 476, 1997). The clastogenic potential was investigated using the in vitro chromosomal aberration test (OECD 473, 1997). All these test guidelines were updated later but there were no significant deviations from the current guidelines.

1) Reverse mutation test of inpyrfluxam in bacterial systems

Reference:	KCA 5.4.1/01
Report Title:	Amended Final report: Reverse mutation test of S-2399 Technical Grade in bacterial systems
Author(s) & Year:	[redacted] (2014a/2017)
Document No, Authority registration No	Study No. 4289 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material: Inpyrfluxam (S-2399 Technical Grade) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required

Guideline(s):	OECD 471 (1997)
Deviations from current guideline:	For the WP2*uvr*A strain, AF-2 was used as positive control without S9. AF-2 is the positive control recommended in the test guideline for the strains with plasmids whereas WP2*uvr*A do not contain any plasmids (Sugiyama et al., 2016[2]). No justification available in the study report.
Impact of the deviation:	The justification provided by the applicant is acceptable with reference to a published literature for the use of AF-2 as the positive control for the strain WP2*uvr*A[2].Therefore, the observed deviation does not impact the integrity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The mutagenic potential of inpyrfluxam was investigated in an OECD and GLP compliant bacterial reverse mutation test using *Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (strain WP2uvrA). The test was performed following the pre-incubation method in the presence and absence of S9. Inpyrfluxam in DMSO was tested in the presence and absence of S9 from 313 - 5000 µg/plate for WP2uvrA and TA98 strains, and 19.5 - 625 µg/plate for the remaining strains. Concentrations were chosen based on the results from a range-finding assay. The positive controls used are listed in table 6.4.1-1 below.

Table 6.4.1-1: Positive controls for reverse mutation test - pre-incubation method

Test strain	Chemical	Dose (µg/plate)
Without S9 mix		
TA100	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	0.01
TA1535	sodium azide (SA)	0.50
WP2*uvr*A	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	0.01
TA98	2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)	0.10
TA1537	9-Aminoacridine (9-AA)	80.00
With S9 mix		
TA100	2-aminoanthracene	1.00
TA1535	2-aminoanthracene	2.00
WP2*uvr*A	2-aminoanthracene	10.00
TA98	2-aminoanthracene	0.50
TA1537	2-aminoanthracene	2.00

[2] The strains recommended for use in the bacterial reverse mutation test (OECD guideline 471) can be certified as non-genetically modified organisms (biomedcentral.com)

[2] Negative and positive control ranges in the bacterial reverse mutation test: JEMS/BMS collaborative study - PMC (nih.gov).

Results

Range finding assay

Precipitation of inpyrfluxam was observed at and above 1500 µg/plate in the presence or absence of S9. No cytotoxicity was observed in strains WP2uvrA and TA98 at any of the tested concentrations, while concentrations of 500 µg/plate and above were cytotoxic to strains TA100, TA1535, and TA1537.

The results are summarized in table 6.4.1 -2. No increase in revertant colonies were seen in any strain with or without metabolic activation.

Table 6.4.1-2: Reverse mutation test of inpyrfluxam - Range finding assay

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	TA98	TA1537	WP2*uvrA*
Without metabolic activation					
Vehicle control	82 ± 9.8	7 ± 3.0	14 ± 2.9	8 ± 2.6	21 ± 7.1
1.50	95 ± 14.0	7 ± 1.5	22 ± 2.1	6 ± 3.8	19 ± 2.0
5	94 ± 5.0	7 ± 2.6	18 ± 4.9	5 ± 0.6	19 ± 5.2
15	91 ± 10.1	8 ± 3.6	18 ± 2.6	4 ± 0.6	16 ± 6.1
50	88 ± 8.2	3 ± 1.0	19 ± 0.6	6 ± 0.6	21 ± 4.9
150	94 ± 6.8	8 ± 4.4	18 ± 3.6	7 ± 1.7	22 ± 2.1
500	74 ± 15.0 [b]	7 ± 4.2 [b]	19 ± 3.2	4 ± 0.6 [b]	21 ± 7.5
1500 [a]	65 ± 10.6 [b]	6 ± 1.7 [b]	14 ± 2.6	4 ± 1.5 [b]	20 ± 1.5
5000 [a]	69 ± 3.2 [b]	7 ± 2.6 [b]	18 ± 2.6	5 ± 1.0 [b]	17 ± 3.1
Positive control	591 ± 25.1	302 ± 19.9	313 ± 10.8	328 ± 45.6	98 ± 8.7
With metabolic activation					
Vehicle control	90 ± 4.0	7 ± 3.2	26 ± 6.4	16 ± 7.2	21 ± 5.5
1.50	91 ± 7.9	7 ± 3.5	28 ± 8.7	16 ± 5.0	27 ± 11.0
5	90 ± 6.8	8 ± 3.6	25 ± 8.0	14 ± 1.5	28 ± 1.5
15	83 ± 5.9	6 ± 5.9	32 ± 1.7	15 ± 1.5	21 ± 7.0
50	104 ± 5.5	8 ± 2.5	25 ± 7.8	15 ± 2.6	22 ± 2.6
150	103 ± 13.1	8 ± 3.5	29 ± 4.6	12 ± 6.8	21 ± 3.2
500	89 ± 16.0 [b]	8 ± 3.0 [b]	31 ± 8.0	14 ± 3.2 [b]	27 ± 11.0
1500 [a]	78 ± 9.0 [b]	4 ± 1.0 [b]	30 ± 7.6	6 ± 3.8 [b]	21 ± 9.3
5000 [a]	30 ± 2.6 [b]	2 ± 1.7 [b]	24 ± 1.7	4 ± 1.2 [b]	18 ± 3.8
Positive control	726 ± 4.5	205 ± 8.4	259 ± 4.2	133 ± 12.1	324 ± 10.7

[a] Precipitation of the test substance was observed; [b] toxic effect was observed; The results were shown as Mean ± SD (n=3).

Confirmatory assay

There was no statistically significant, concentration dependent increase in the number of revertant bacterial colonies observed after inpyrfluxam treatment in the presence or absence of S9. Positive controls showed expected results.

The results are summarized in the table 6.4.1-3

Table 6.4.1-3: Reverse mutation test of inpyrfluxam - pre-incubation method

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	TA98	TA1537	WP2*uvrA*
Without metabolic activation					
Vehicle control	86 ± 5.7	7 ± 2.6	15 ± 2.1	7 ± 4.0	16 ± 3.6
19.5	97 ± 5.8	9 ± 2.1	-	4 ± 2.3	-
39.1	85 ± 12.0	6 ± 4.6	-	6 ± 4.0	-
78.1	102 ± 7.2	8 ± 2.5	-	6 ± 4.0	-
156	92 ± 8.1	4 ± 1.5	-	5 ± 2.1	-
313	75 ± 11.4 [b]	5 ± 2.1 [b]	16 ± 7.0	4 ± 0.6 [b]	16 ± 5.0
625 [a]	72 ± 1.5 [b]	7 ± 4.0 [b]	18 ± 4.0	5 ± 1.0 [b]	16 ± 6.8
1250 [a]	-	-	12 ± 0.6	-	17 ± 3.1
2500 [a]	-	-	15 ± 4.7	-	19 ± 1.7
5000 [a]	-	-	15 ± 1.7	-	20 ± 2.1
Positive control	602 ± 11.0	355 ± 22.9	404 ± 19.1	399 ± 19.5	110 ± 20.2
With metabolic activation					
Vehicle control	86 ± 14.6	8 ± 3.5	22 ± 3.6	12 ± 3.2	19 ± 2.6
19.5	89 ± 2.6	6 ± 4.2	-	14 ± 0.6	-
39.1	96 ± 9.5	9 ± 4.7	-	17 ± 3.5	-
78.1	87 ± 4.4	7 ± 3.0	-	11 ± 3.6	-
156	91 ± 12.0	9 ± 3.8	-	13 ± 3.1	-
313	90 ± 9.2	6 ± 3.8	23 ± 0.0	13 ± 1.2	20 ± 1.5
625	100 ± 5.3 [b]	6 ± 1.2 [b]	22 ± 5.3	10 ± 3.1 [b]	19 ± 3.5
1250 [a]	-	-	27 ± 7.4	-	26 ± 9.5
2500 [a]	-	-	24 ± 2.5	-	19 ± 4.9
5000 [a]	-	-	20 ± 3.2	-	16 ± 4.7
Positive control	676 ± 8.4	194 ± 11.3	245 ± 10.5	138 ± 8.5	398 ± 21.9

[a] Precipitation of the test substance was observed; [b] toxic effect was observed; - not tested; The results were shown as Mean ± SD (n=3).

Conclusion

Under the conditions of this GLP and OECD compliant bacterial reverse mutation assay, inpyrfluxam did not induce an increase in the revertant colonies of *S. typhimurium* and *E. coli* strains with or without metabolic activation up to the limit concentration or concentrations causing precipitation or cytotoxicity. Therefore, it is concluded that inpyrfluxam is not mutagenic in bacteria. HSE notes the use of AF-2 as the positive control for WP2uvrA strain without S9 which is not recommended by the test guideline for a plasmid containing strain. The justification provided by the applicant by refencing the publication by Kao et al. (2018)[2] for the use of AF-2 as positive control for WP2uvrA is considered acceptable.

[redacted] (2014a/2017)

2. *In vitro* chromosomal aberration test for inpyrfluxam in Chinese hamster lung cells (CHL/IU)

Reference:	KCA 5.4.1/02
Report Title:	*In vitro* chromosomal aberration test on S-2399 Technical Grade in Chinese hamster lung cells (CHL/IU)
Author(s) & Year:	[redacted] (2014b)
Document No, Authority registration No	Study No. 4288 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material: Inpyrfluxam (S-2399 Technical Grade) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 473 (1997)
Deviations from current guideline:	1. Only 200 cells were scored for chromosomal aberration instead of 300 cells as specified in OECD 472 (2016), however only 200 cells are required as per the TG in place at the time the study was performed. 2. Cell culture conditions such as pH and osmolarity were not measured in determining the highest test chemical concentration.
Impact of the deviation:	1. Scoring of 200 cells instead of 300 cells reduces the sensitivity. However, HSE notes that the results were clearly negative. This assures that scoring of 100 more cells would not have produced a different outcome. 2. pH and osmolarity are less important when a clear cytotoxic effect is demonstrated at the highest concentration. Therefore, the observed deviations have no impact on the integrity and validity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The clastogenic potential of inpyrfluxam in the presence and absence of metabolic activation (S9) was investigated in a GLP and OECD compliant study using Chinese hamster lung (CHL/IU) cells. Cytotoxicity tests were performed to choose/limit the relevant concentrations

for the chromosomal aberration tests. Relative increase in cell count (RICC) was used as the measure of cytotoxicity. The lowest concentration at which the RICC was 50% or lower was chosen as the maximum concentration for the chromosomal aberration test. In the initial chromosomal aberration test (Experiment I), CHU/IL cells were exposed to inpyrfluxam in DMSO at concentrations of 42.5, 85.0 and 170 µg/mL with S9 and 32.5, 65.0, 130 µg/mL without S9 for 6 h followed by an 18 h recovery period. In the second experiment (Experiment II), cells were treated continuously with the test substance for 24 h in the absence of S9 at 0.188, 0.375 and 1.50 µg/mL and similarly as in experiment 1 in the presence of S9. All the investigations required by the guideline were performed and appropriate controls were used.

Results

Cytotoxicity test

A preliminary cytotoxicity test was performed to determine the concentrations to be used for the chromosomal aberration tests. In the preliminary cytotoxicity test, precipitation was observed at and above 425 µg/mL both in the presence and absence of S9. After 24h treatment, all the tested concentrations in the absence of S9 induced cytotoxicity (RICC of 50±5%), whereas after 6h treatment, the cytotoxic effect was observed only at and above 213 µg/mL in the presence and absence of S9.

Therefore, an additional cytotoxicity test was performed using lower concentrations (100-200 µg/mL for 6h treatment and 13.3 - 0.0519 µg/mL for 24h treatment). In the absence of S9, the concentrations at and above 1.66 µg/mL were cytotoxic after 24h and the concentrations at and above 140 µg/mL were cytotoxic after 6h. In the presence of S9, 180 and 200 µg/mL were observed to be cytotoxic. The results are summarised in the tables 6.4.1-5 and 6.4.1-6.

Table 6.4.1-5: Preliminary cytotoxicity test of inpyrfluxam in Chinese hamster lung (CHL/IU) cells

Concentration (µg/mL)	% Relative increase in cell count (RICC) compared to control		
	6 h treatment		24 h treatment
	-S9	+S9	-S9
Vehicle control	100	100	100
13.3	80.1	94.2	22.3
26.6	78.3	82.7	20.8
53.1	70.4	77.0	12.5
106	61.0	65.4	2.9
213	-44.6	-42.1	-48.7
425 [P]	-46.0	-47.1	-49.4
850 [P]	-46.2	-47.3	-49.4
1700 [P]	-46.1	-47.5	-49.3
3400 [P]	-31.8	-4.3	-47.0

[P] precipitate was observed at the beginning and at the end of treatment

Table 6.4.1-6: Additional cytotoxicity test of inpyrfluxam in Chinese hamster lung (CHL/IU) cells

Concentration (µg/mL)	% Relative increase in cell count (RICC) compared to control		
	6 h treatment		24 h treatment
	-S9	+S9	-S9
Vehicle control	100	100	100
0.0519	-	-	103.4
0.104	-	-	107.5
0.208	-	-	103.3
0.415	-	-	93.7
0.830	-	-	60.8
1.66	-	-	33.6
3.32	-	-	26.8
6.64	-	-	20.7
13.3	-	-	18.7
100	61.9	71.9	-
120	56.6	71.7	-
140	31.3	69.2	-
160	-29.8	58.1	-
180	-43.2	26.3	-
200	-44.1	-30.0	-

- not tested

Chromosomal aberration test

No statistically significant or biologically relevant increase in structural alterations (excluding gaps) or polyploid and endoreduplicated cells was observed in the treated groups compared to controls in the presence and absence of metabolic activation in experiment I and II up to an appropriate level of cytotoxicity. In experiment II (24 hour treatment without S9), there was a slight increase in structural alterations (excluding gaps) at the top concentration of 1.5 µg/mL (2.4 vs 0 in controls). However, this was observed at a highly cytotoxic concentration. On this basis, the increase was not considered to be biologically relevant. All negative and positive controls gave expected results which were within the laboratory HCD. Tables 6.4.1-7 to 6.4.1-10 summarise the results of the chromosomal aberration tests performed. The positive and negative HCD are summarised in tables 6.4.1-11 and 6.4.1-12.

Table 6.4.1-7: Experiment I - Cytogenetic assay of inpyrfluxam in CHL/IU cells without metabolic activation (-S9) – 6 h treatment and 18 h recovery.

Dose group (µg/mL)	Number of cells scored	Relative increase in cell count (%)	Polyploid and endorepduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Vehicle control	200	100	1.0	0.5	0.0
32.5	200	86.7	0.0	1.0	1.0
65	200	78.0	0.5	1.0	0.5
130	200	49.3	2.5	0.5	0.0

Positive control (MMC)	200	84.1	1.0	20.0	20.0

Table 6.4.1-8: Experiment I - Cytogenetic assay of inpyrfluxam in CHL/IU cells with metabolic activation (+S9) - 6 h treatment and 18 h recovery.

Dose group (µg/mL)	Number of cells scored	Relative increase in cell count (%)	Polyploid and endorepduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Vehicle control	200	100	0.5	1.0	1.0
42.5	200	81.6	0.0	0.5	0.0
85	200	76.2	0.0	2.5	1.0
170	200	48.3	1.0	1.0	0.5
Positive control (CP)	200	59.4	0.0	26.0	25.0

Table 6.4.1-9 : Experiment II - Cytogenetic assay of inpyrfluxam in CHL/IU cells without metabolic activation (-S9) - 24 h treatment

Dose group (µg/mL)	Number of cells scored	Relative increase in cell count (%)	Polyploid and endorepduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Vehicle control	200	100	0.0	0.5	0.0
0.188**	200	91.0	0.0	0.5	0.5
0.375	200	90.4	0.5	0.0	0.0
0.750	200	64.2	1.0	1.5	1.0
1.5*	167	44.3	0.0	2.4	2.4
Positive control (MMC)	200	109.7	0.0	15.5	15.5

***** Not enough cells to count due to cytotoxicity.

** Additional group scored due to the cytotoxicity at the highest (1.5 µg/mL) concentration.

Table 6.4.1-10 : Experiment II - Cytogenetic assay of inpyrfluxam in CHL/IU cells with metabolic activation (+S9) - 6 h treatment and 18 h recovery

Dose group (µg/mL)	Number of cells scored	Relative increase in cell count (%)	Polyploid and endorepduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Vehicle control	200	100	0.0	0.5	0.0
42.5	200	69.8	1.0	1.5	1.5
85	200	61.6	0.0	0.5	0.0
170	200	33.7	0.0	2.0	1.0

Positive control (CP)	200	46.4	0.5	24.0	23.5

Table 6.4.1-11: Historical control data for CHL/IU cells in negative control groups

Total no. of groups	Aberrant cells per 100 scored	% mean ± SD	Range of means (%)	
			Min	Max
204	Structural aberrations including gaps	1.5 ± 0.92	0	4
204	Structural aberrations excluding gaps	0.98 ± 0.80	0	3
204	Numerical aberrations	0.37 ± 0.47	0	2

The above values were calculated from the data obtained from January 2002 to December 2013 including screening studies. Two hundred cells from each group were scored. The applicant states that the values with and without S9 and at 24 hours were not separated because there was no substantive difference.

Table 6.4.1-12: Historical control data for CHL/IU cells in positive control groups

chemical (Conc. µg/mL)	metabolic activation	Culture [a] (h)	Total no. of groups	% mean ± SD	Range of means (%)	
					min	max
MMC (0.06)	-	6-18	59	29.6 ± 10.2	15.5	56.5
CP (10)	+	6-18	91	43.3 ± 20.4	18.5	92
MMC (0.02)	-	24-0	42	20.0 ± 5.3	13.5	32.5

The above values were calculated from the data obtained from January 2002 to December 2013. Two hundred cells from each group were scored. [a] Treatment period – recovery period

Conclusion

In this GLP and OECD compliant in vitro chromosomal aberration test, inpyrfluxam did not induce structural aberrations in the chromosomes of CHL/IU cells up to cytotoxic concentrations. Therefore, it is concluded that inpyrfluxam is not a clastogen under the tested conditions.

[redacted] (2014b)

3. In vitro gene mutation test for inpyrfluxam in Chinese hamster lung fibroblast cells (V79)

Reference:	KCA 5.4.1/03
Report Title:	S-2399 TG: Gene Mutation Assay in Chinese Hamster V79 Cells In Vitro (V79/HPRT)
Author(s) & Year:	[redacted] (2014)

Document No, Authority registration No	Study No. 1601100 Harlan Cytotest Cell Research GmbH, Germany
Substance used:	Test Material: Inpyrfluxam (S-2399 Technical Grade) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 476 (1997)
Deviations from current guideline:	None
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The potential of inpyrfluxam to induce gene mutations at the HPRT locus was investigated in the presence and absence of metabolic activation (S9) in a GLP and OECD compliant study using cultured Chinese hamster V79 cells. The appropriate concentrations for the gene mutation assay were selected based on the relative survival (RS) (measured as cloning efficiency) and precipitation of inpyrfluxam. The lowest non-precipitating concentration that had a cloning efficiency of 10-20% of solvent and/or negative control was set as the highest concentration for the gene mutation assay. In the first experiment, the cells were exposed to inpyrfluxam in DMSO at 13-78 µg/mL for 4 h with S9 and at 6.5-39 µg/mL without S9. The second experiment was performed with a treatment time of 4 h at concentrations ranging from 6.5-65 µg/mL with S9 and 24 h at 13-78 µg/mL without S9. All the investigations required by the guideline were performed and appropriate controls were used.

Results

Preliminary cytotoxicity assay

A preliminary cytotoxicity assay was performed at concentrations ranging from 26.1-3334 µg/mL to set the top concentrations for the gene mutation assay. In all the treatment groups, precipitation was observed at and above 104.2 µg/mL and cytotoxicity (RS<10-20%) was observed at and above 52.1 µg/mL both in the presence and absence of S9. The results are presented in table 6.4.1-13.

Table 6.4.1-13: Preliminary cytotoxicity test of inpyrfluxam using Chinese hamster V79 cells

Concentration (µg/mL)	S9 mix	Duration of treatment	Cell seeded I/II	Mean number of colonies per flask (n=2)	CE% absolute	CE% relative	Precipitation
Solvent control	-	4 h	503	307	61.0	100.0	No
26.1	-	4 h	503	251	49.8	81.6	No
52.1	-	4 h	503	0	0.0	0.0	No
104.2	-	4 h	503	0	0.0	0.0	Yes
208.4	-	4 h	503	0	0.0	0.0	Yes
416.8	-	4 h	503	0	0.0	0.0	Yes
833.5	-	4 h	503	0	0.0	0.0	Yes
1667.0	-	4 h	503	0	0.0	0.0	Yes
3334.0	-	4 h	503	84	16.6	27.2	Yes
Solvent control	+	4 h	503	237	47.0	100.0	No
26.1	+	4 h	503	255	50.7	107.8	No
52.1	+	4 h	503	109	21.7	46.1	No
104.2	+	4 h	503	0	0.0	0.0	Yes
208.4	+	4 h	503	0	0.0	0.0	Yes
416.8	+	4 h	503	0	0.0	0.0	Yes
833.5	+	4 h	503	0	0.0	0.0	Yes
1667.0	+	4 h	503	0	0.0	0.0	Yes
3334.0	+	4 h	503	122	24.2	51.4	Yes
Solvent control	-	24 h	503	380	75.4	100.0	No
26.1	-	24 h	503	359	71.3	94.5	No
52.1	-	24 h	503	293	58.3	77.2	No
104.2	-	24 h	503	0	0.0	0.0	Yes
208.4	-	24 h	503	0	0.0	0.0	Yes
416.8	-	24 h	503	0	0.0	0.0	Yes
833.5	-	24 h	503	0	0.0	0.0	Yes
1667.0	-	24 h	503	0	0.0	0.0	Yes
3334.0	-	24 h	503	165	32.7	43.3	Yes

CE- cloning efficiency

Chromosomal aberration test

Inpyrfluxam treatment did not produce any biologically relevant, reproducible (between cultures) or concentration-related increases in the number of mutant colonies of V79 cells in the presence or absence of metabolic activation compared to the concurrent negative control. The results observed at 65 µg/mL (4.9-fold increase compared to solvent control) in Exp II (4h) with S9 (in culture II) was not considered biologically relevant based upon excessive cytotoxicity (RS =7.7%). The slight increase (3.4-fold compared to solvent control) at 78 µg/mL in Exp II (24 hr) without S9 (in culture II) was also considered of no biological relevance due to the relatively low number of mutant colonies in the solvent control and lack of reproducibility in the duplicate culture (culture I). In addition, the mutant colony numbers at these two concentrations were within the laboratory negative control (solvent) (HCD).

Positive and negative controls produced the expected mutant colonies, and these were within the laboratory HCD ranges. Results from the mutation assays are presented in table 6.4.1-14 and the HCD are summarised in 6.4.1-15.

Table 6.4.1-14: Summary of results of Experiment I and II for gene mutations at the HPRT locus in Chinese hamster V79 cells treated with inpyrfluxam.

Conc. µg/mL	S9 mix	Rel. clon. eff. I (survival) %	Rel. clon. eff. II (viability) %	Mutant colonies / 10^6 cells	Induction factor	Rel. clon. eff. I (survival) %	Rel. clon. eff. II (viability) %	Mutant colonies / 10^6 cells	Induction factor
Exp. I / 4 h treatment									
		Culture I				**Culture II**			
Neg C	-	91.5	102.2	6.8	0.4	99.8	99.0	11.9	0.9
Solv C	-	100.0	100.0	16.2	1.0	100.0	100.0	12.5	1.0
3.3	-	90.0	#			99.1	#		
6.5	-	90.7	100.4	16.9	1.0	103.0	99.9	11.4	0.9
13.0	-	85.4	98.2	12.4	0.8	97.2	97.9	13.5	1.1
26.0	-	55.1	98.1	9.5	0.6	60.8	100.0	10.7	0.9
32.5	-	20.1	102.3	12.4	0.8	13.9	100.5	9.6	0.8
39.0	-	1.2	103.0	5.1	0.3	0.8	97.5	9.4	0.7
45.5	-	0.0	##			0.0	##		
52.0	-	##				##			
Pos C	-	74.2	95.6	207.5	12.8	90.2	101.8	186.9	14.9
Exp. I / 4 h treatment									
		Culture I				**Culture II**			
Neg C	+	95.3	90.5	15.8	0.8	98.1	122.0	13.5	1.0
Solv C	+	100.0	100.0	19.4	1.0	100.0	100.0	13.3	1.0
6.5	+	90.2	#			95.3	#		
13.0	+	82.5	105.4	10.1	0.5	99.5	111.6	4.3	0.3
26.0	+	90.0	104.6	19.4	1.0	94.5	101.7	15.0	1.1
52.0	+	83.3	101.1	8.2	0.4	84.8	124.2	24.2	1.8
65.0	+	54.8	91.1	13.8	0.7	65.0	119.1	16.5	1.2
78.0	+	19.2	95.5	13.3	0.7	22.1	155.8	24.0	1.8
91.0 P	+	1.3	##			2.2	##		
104.0 P	+	0.0	##			0.0	##		
Pos C	+	78.2	89.7	350.7	18.1	87.3	145.4	158.4	11.9
Exp. II / 24 h treatment									
		Culture I				**Culture II**			
Neg C	-	100.6	108.5	20.5	1.6	125.3	126.0	9.7	1.7
Solv C	-	100.0	100.0	12.9	1.0	100.0	100.0	5.6	1.0
6.5	-	92.2	#			115.8	#		
13.0	-	96.7	94.1	7.1	0.6	100.5	127.5	6.4	1.1
26.0	-	75.4	104.9	9.0	0.7	102.0	107.1	14.3	2.6
52.0	-	80.3	89.2	15.9	1.2	38.9	105.9	9.1	1.6
65.0	-	66.6	92.3	5.2	0.4	32.8	110.5	7.0	1.3
78.0	-	10.7	114.0	8.9	0.7	22.6	67.0	**19.0**	**3.4**
91.0	-	7.0	##			3.8	##		
104.0	-	0.0	##			0.0	##		

Pos C	-	68.8	91.6	197.0	15.3	116.1	90.2	288.1	51.8
Exp. II / 4 h treatment									
		Culture I				**Culture II**			
Neg C	+	102.7	79.0	39.2	2.4	101.1	111.0	31.8	1.9
Solv C	+	100.0	100.0	16.2	1.0	100.0	100.0	16.8	1.0
6.5	+	92.7	99.1	10.9	0.7	91.0	106.1	15.9	0.9
13.0	+	92.3	105.3	7.9	0.5	79.7	114.3	12.6	0.7
26.0	+	92.3	78.9	12.8	0.8	88.0	105.5	10.2	0.6
52.0	+	40.7	98.9	12.3	0.8	48.2	96.6	19.3	1.1
65.0	+	3.3	68.9	14.9	0.9	7.7	75.3	**82.8**	**4.9**
78.0	+	0.0	##			0.0	##		
91.0	+	0.0	##			0.0	##		
104.0 P	+	0.0	##			0.0	##		
Pos C	+	90.6	101.6	211.1	13.1	93.1	107.9	225.4	13.4

Rel. clon. eff.: relative cloning efficiency; Cloning efficiency I (survival): cloning efficiency determined immediately after treatment to measure toxicity; cloning efficiency II (viability): cloning efficiency determined after the expression period to measure viability of cells without selective agent); Neg C: negative control – medium; Solv C: solvent control – DMSO; Pos C: positive control – EMS without S9 mix or DMBA with S9 mix; P: precipitation visible to the naked eye at the end of treatment; #: culture was not continued as only four analysable concentrations were required; ##: culture was not continued due to exceedingly severe cytotoxic effects

Table 6.4.1-15: Historical control data for the gene mutations at the HPRT locus in Chinese hamster V79 cells

Number of mutant colonies per 10^6 cells		
without metabolic activation (4 h treatment time)		
	Positive control EMS 150 µg/mL	Solvent control (medium, acetone, water, DMSO, ethanol, THF)
Range	54.8-889.0	1.6-42.8
mean value	137	14.9
standard deviation	92.3	7.8
number of studies	82	82
without metabolic activation (4 h treatment time)		
	Positive control DMBA 1.1 µg/mL	Solvent control (medium, acetone, water, DMSO, ethanol, THF)
Range	77.7-2042.6	3.4-44.2
mean value	568.7	14.3
standard deviation	301.3	7.1
number of studies	82	82
without metabolic activation (24 h treatment time)		
	Positive control EMS 150 µg/mL	Solvent control (medium, acetone, water, DMSO, ethanol, THF)
Range	108.5-786.1	2.4-41.8
mean value	332.6	14.1
standard deviation	125.9	7.4
number of studies	81	81

The above values were calculated from the data obtained from 2012 to 2013.

Conclusion

In this GLP and OECD compliant *in vitro* mammalian gene mutation study, inpyrfluxam did not induce any biologically relevant, reproducible or concentration dependant increase in mutant colonies up to cytotoxic concentrations. Therefore, inpyrfluxam is non-mutagenic under the tested conditions.

[redacted] (2014)

Overall conclusion for in vitro genotoxicity tests

Overall, inpyrfluxam did not induce mutagenicity in bacteria and mammalian cells or clastogenicity in mammalian cells in a battery of valid GLP and guideline in vitro studies.

B.6.4.2. In vivo studies in somatic cells

The potential of inpyrfluxam to induce clastogenicity and/or aneugenicity (micronuclei formation) was investigated using a GLP and OECD compliant study. The study was conducted in erythrocytes of mice bone marrow according to the previous version of OECD 474 (1997). There were no significant deviations from the currently available updated guideline.

Reference:	KCA 5.4.2/01
Report Title:	*Micronucleus Test on S-2399 Technical Grade in CD-1 Mice*
Author(s) & Year:	[redacted] (2015)
Document No, Authority registration No	Study No. 4290 [redacted]
Substance used:	Test Material: Inpyrfluxam (S-2399 Technical Grade) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	N/A
Guideline(s):	OECD 474 (1997)
Deviations from current guideline:	1. A single administration was performed with no scientific justification provided. The guideline requires a scientific justification for the use of a single treatment. 2. No peripheral blood samples were taken from the animals to determine proof of target organ exposure.
Impact of the deviation:	1. A single administration of the test item was acceptable according to the test guideline available at the time of the study performed. The bone marrow samples from control and high dose group were

	taken twice (24h and 48h) and analysed for a single administration schedule as recommended by the current version of the guideline. 2. Even though the peripheral blood samples were not analysed, there is indirect evidence for the proof of target organ exposure. Reduction in the ratio of PCEs to erythrocytes in bone marrow in males and, mortality and severe clinical sign of toxicity at the top dose indicate target organ (bone marrow) exposure to inpyrfluxam. Therefore, the observed deviations do not impact the outcome of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The potential of inpyrfluxam to induce micronuclei was investigated in [redacted] mice using a GLP and OECD compliant study. A preliminary acute oral toxicity test in which doses of 500, 1000 and 2000 mg/kg bw were used, was performed to find the dose range for the main study (see findings below in the results section).

In the main study, groups of mice (5/sex/group) were administered with inpyrfluxam in corn oil at dose levels of 200, 400 and 800 mg/kg bw, negative control (corn oil at 10 mL/kg) or positive control (cyclophosphamide at 60 mg/kg bw) substances as a single treatment by oral gavage.

Due to the suspicion of mortality at top dose level of 800 mg/kg bw, an additional group of 5 animals/sex was also treated at 800 mg/kg bw. At 24 h (for all dose levels and controls), and 48 h after dosing (for negative control and 800 mg/kg bw only), the mice were sacrificed and bone marrow smears were prepared and micronuclei formed counted in 4000 PCEs per animal. Cytotoxicity to the bone marrow was also investigated by measuring the ratio of PCEs to erythrocytes.

Results

Preliminary acute toxicity test

At 1000 and 2000 mg/kg bw, the animals showed severe clinical signs of toxicity 4 h after dosing, and mortalities (5/10 and 6/10 deaths for 1000 mg/kg bw and 2000 mg/kg bw, respectively) within 2 days. There was no mortality in animals dosed at 500 mg/kg bw but ataxic gait and soft stools were observed after 4 h in both sexes. A statistically significant decrease in body weight was observed in males at 500 and 2000 mg/kg bw but not in females. Based on these results, 800 mg/kg bw was considered the maximum tolerable dose in each sex.

Micronucleus assay

One female animal was found dead one day after administration in the 48 h group dosed at 800 mg/kg bw, therefore one animal from the satellite group was used for the 48 h slide preparation. Clinical signs of toxicity such as ataxic gait and prone position were observed after inpyrfluxam treatment at 400 mg/kg bw and above in both sexes. Animals dosed at 200 mg/kg bw showed clinical signs of toxicity of soft stool, ataxic gait and hunched posture. Reduction in the ratio of PCEs to whole erythrocytes in males, and mortality and severe clinical signs of toxicity in females at 800 mg/kg bw after 48 h compared to concurrent controls confirmed that the animals were systemically exposed to inpyrfluxam and that it reached the bone marrow.

Inpyrfluxam did not induce an increase in the incidence of micronuclei in the bone marrow of male or female mice up to the top dose of 800 mg/kg bw. Positive and negative controls gave expected results which were within the laboratory HCD ranges. The results are presented in tables 6.4.2-1 and 6.4.2-2 and the HCD are summarised in table 6.4.2-3.

Table 6.4.2-1: Micronucleus test – male mice administered inpyrfluxam in a single dose via oral gavage

Test Substance	Dose level (mg/kg)	Time (h)	PCE/total E (%, mean ± SD)	Micronucleated PCE	
				(%, mean ± SD)	[range]
Vehicle control	0	24	53.0 ± 8.38	0.15 ± 0.089	[2-9] / 4000
S-2399 TG	200	24	56.9 ± 6.63	0.17 ± 0.065	[4-10] / 4000
	400	24	57.6 ± 4.61	0.16 ± 0.093	[2-12] / 4000
	800	24	55.5 ± 4.39	0.17 ± 0.041	[4-8] / 4000
Positive control (CP)	60	24	40.7 ± 6.88*	3.18 ± 1.210**	[80-180] / 4000
Vehicle control	0	48	55.5 ± 3.70	0.14 ± 0.045	[4-8] / 4000
S-2399 TG	800	48	45.7 ± 4.04**	0.12 ± 0.089	[1-8] / 4000

* p<0.05; ** p<0.01

Table 6.4.2-2: Micronucleus test – female mice administered inpyrfluxam in a single dose via oral gavage

Test Substance	Dose level (mg/kg)	Time (h)	PCE/total E (%, mean ± SD)	Micronucleated PCE	
				(%, mean ± SD)	[range]
Vehicle control	0	24	59.1 ± 4.14	0.16 ± 0.084	[3-12] / 4000
S-2399 TG	200	24	57.5 ± 5.62	0.14 ± 0.038	[3-7] / 4000
	400	24	54.1 ± 3.53	0.12 ± 0.037	[3-7] / 4000
	800	24	61.4 ± 4.94	0.15 ± 0.067	[3-9] / 4000
Positive control (CP)	60	24	50.9 ± 8.13	2.17 ± 1.067**	[24-136] / 4000
Vehicle control	0	48	51.6 ± 5.04	0.14 ± 0.076	[3-10] / 4000
S-2399 TG	800	48	50.4 ± 3.91	0.19 ± 0.086	[4-12] / 4000

* p<0.05; ** p<0.01

Table 6.4.2-3: Historical control data for the micronucleus tests conducted in CD-1 mice

Sex	Total no. of groups	PCEs with micronuclei
		Mean ± SD
Negative (vehicle)control		
Male	95	0.16 ± 0.06
Female	43	0.13 ± 0.04
Positive control (cyclophosphamide, 60 mg/kg, po, 24 h)		
Male	95	3.22 ± 0.71
Female	43	3.18 ± 0.68

The above values were calculated from the data obtained from 1986 to 2013 for negative control and 1996-2013 for positive control. Mean ± 2SD were set as the acceptable range.

Conclusion

Under the conditions of this GLP and OECD compliant study, inpyrfluxam did not induce an increase in the frequency of micronucleated immature erythrocytes of mice bone marrow up to a dose which caused bone marrow cytotoxicity and systemic toxicity. This provides indirect evidence of proof of target organ exposure.

[redacted] (2015)

B.6.4.3. In vivo studies in germ cells

No study is available. All the in vitro studies and the in vivo study in somatic cells performed with inpyrfluxam are negative, therefore it is not necessary to conduct any in vivo studies in germ cells.

B.6.4.4. Photomutagenicity

Tests for photomutagenicity are not necessary as there was no evidence of phototoxicity induction by inpyrfluxam.

B.6.4.5. Summary of genotoxicity

Inpyrfluxam was evaluated for its mutagenic potential in vitro using the bacterial reverse mutation test, mammalian gene mutation test, and the chromosomal aberration test. An in vivo mouse micronucleus test was also performed to investigate clastogenicity and aneugenicity. In these valid GLP and guideline studies, there was no evidence of genotoxicity and it is concluded that inpyrfluxam is not genotoxic. Therefore, inpyrfluxam does not meet the criteria for classification for germ cell mutagenicity according to Regulation 1272/2008 as it applies in GB.

Table 6.4.5-1: Summary of genotoxicity studies for inpyrfluxam

Data point/Type of study and *Acceptability*	Organism/ Cells	Dose range tested	Result	Reference
***In vitro* studies**				
KCA 5.4.1/01 Reverse mutation (OECD TG 471) *Acceptable*	*Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2*uvrA*)	1.5 to 5000 µg/plate	Negative	[redacted] (2014a/2017) TPT-0004
KCA 5.4.1/02 *In vitro* chromosomal aberration test (OECD TG 473) *Acceptable*	Chinese hamster lung cells (CHL/IU)	32.5-130 µg/mL (short-term treatment, -S9 Mix) 42.5-170 µg/mL (short-term treatment, +S9 Mix) 0.188-1.5 µg/mL (long-term treatment, -S9 Mix)	Negative	[redacted] (2014b) TPT-0005
KCA 5.4.1/03 Mammalian cell gene mutation (OECD TG 476) *Acceptable*	V79 cells – HPRT locus	6.5 to 39.0 µg/mL (4 h treatment -S9 Mix) 6.5 to 78.0 µg/mL (4 h treatment +S9 Mix) 13.0 to 78.0 µg/mL (24 h treatment -S9 Mix)	Negative	[redacted] (2014) TPT-0002
***In vivo* studies**				
KCA 5.4.2/01 Mouse micronucleus (OECD TG 474) *Acceptable*	CD-1 mice	200, 400 or 800 mg/kg bw (24 h sampling) 800 mg/kg bw (48 h sampling)	Negative	[redacted] (2015) TPT-0021

B.6.5. Long-term Toxicity and Carcinogenesis

The long-term toxicity and carcinogenicity of inpyrfluxam have been investigated via the oral route of exposure in rats and mice. Studies on mode of action for effects in the liver and thyroid in both species are also available but have been summarised in the ED section.

B.6.5.1. Long-term toxicity and carcinogenicity in rats

The long-term toxicity and carcinogenicity of inpyrfluxam have been investigated in rats via the oral (dietary) route. The study was GLP complaint and performed in accordance with OECD TG 453 (2009).

Reference:	KCA 5.5/01 and KCA 5.5/02

Report Title:	S-2399 Technical Grade: Combined Chronic Toxicity and Carcinogenicity Study in Rats
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. [redacted] 14-0046 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable methods of analysis available for dietary formulation and plasma concentration
Guideline(s):	OECD 453 (2009)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, Wistar Hannover female rats received inpyrfluxam by dietary administration at concentrations of 0, 150, 500 or 1500/1000 ppm over a period of 52 weeks for the chronic toxicity phase (21 animals/dose) or 104 weeks for the carcinogenicity phase (51 animals/dose). The top dose in females was changed from 1500 ppm to 1000 ppm at week 46 due to the considerable decrease in body weight (about 80%). Therefore, the high dose females in both the toxicity and carcinogenicity phases received inpyrfluxam at 1000 ppm for the remaining treatment period. Wistar Hannover male rats received inpyrfluxam by dietary administration at concentrations of 0, 150, 500 or 2000 ppm over a period of 52 weeks for the chronic toxicity phase (21 animals/dose) or 104 weeks for the carcinogenicity phase (51 animals/dose). The mean substance intakes at each dose level for males and females and the two phases are presented in the table 6.5.1-1.

Table 6.5.1-1: Mean test substance intake in the rat chronic/carcinogenicity study with inpyrfluxam

Dose level (ppm)	Males	Females

	150	500	2000	150	500	1500/1000
Chronic toxicity phase (52 weeks) mg/kg bw/day	6.77	22.8	95.9	8.84	30.1	86.4
Carcinogenicity phase (104 weeks) mg/kg bw/day	5.85	19.4	78.4	7.47	25.5	65.8

The dose levels were selected based on a previous repeated dose 90-day oral toxicity study in rats ([redacted] (2016) where adverse effects relevant to humans were observed from 2000 ppm (123 mg/kg bw/day) including significant decreases in body weights, body weight gains and food consumption, increased liver weight (with associated hypertrophy), adverse changes in coagulation and clinical-chemistry parameters indicative of liver damage, and histopathological findings of the thyroid, adrenal and ovary at 2000 ppm. In addition to these effects, it was also noted that the females were more sensitive than males.

In accordance with OECD test guideline 453, all animals were subjected to the required investigations. Analysis of the dietary formulation and plasma concentration of inpyrfluxam was in addition performed within the study. Plasma (n=4) was analysed at 14, 26 and 51 weeks of treatment.

Results

Toxicokinetics

Inpyrfluxam was detected in the plasma of females at all dose levels and in males at the high dose (at weeks 14, 26 and 51) whereas it was under the limit of quantification (LOQ) in the low and mid dose males. A dose dependent increase in plasma levels of inpyrfluxam was noted in females. At the high dose, the plasma concentrations in females were higher than in males. Furthermore, no accumulation after repeated oral administration was observed in both sexes.

Table 6.5.1-2: Summary of plasma levels of inpyrfluxam in rats administered inpyrfluxam in diet for 52 weeks.

Time point (week)	Sex and dose level (ppm)							
	Male (n=4)				Female (n=4)			
	0	150	500	2000	0	150	500	1500 / 1000
14	<LOQ	<LOQ	<LOQ [a]	0.06	<LOQ	0.02	0.15	0.24
26	<LOQ	<LOQ	<LOQ	0.07	<LOQ	0.03	0.17	0.31
51	<LOQ	<LOQ	<LOQ	0.04	<LOQ	0.02	0.11	0.22

<LOQ: below the limit of quantitation (<0.01 mg/L); [a]: computed as 0 for average calculation.

Mortality and general clinical signs of toxicity

There were no significant changes in the mortality of either sex compared to control. The survival rate was acceptable at all dose levels in lines with the requirement of OECD guideline.

Functional observational battery (FOBs)

There were no significant changes in any treated groups of either sex.

Body weight and body weight gain

Chronic toxicity phase

At the high dose, there were significant decreases in body weights (at weeks 7-11 in males and at weeks 1-52 in females) and body weight gain (at weeks 0-1 and 4-7 in males and throughout the treatment period in females). The decrease in total body weight gain in females (by 37%) was much more severe than in males (by 8%). The changes in body weights and bodyweight gains at the high dose are considered to be treatment related and adverse.

At 150 ppm, there was significant reduction in body weight gain at week 0-1 and increase at week 10-11. This is considered as incidental and not treatment related or adverse.

Carcinogenicity phase

At the high dose, there were significant decreases in body weights and body weight gains in both sexes throughout the treatment period (except at the week 64-68 in males). The decrease in total body weight gain in females (by 23%) was more severe than in males (18%). The changes in body weights and bodyweight gains at the high dose are considered as treatment related and adverse.

Overall, adverse decreases in body weights and body weight gains were noted at the high dose in both sexes and in both phases.

Table 6.5.1-3: Chronic toxicity phase - mean body weight (% of control) of rats administered with inpyrfluxam in the diet for 52 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	150	500	2000	150	500	1500/1000
1	99	98	97	98	101	95**
2	100	99	97	95	98	92**
3	100	98	96	97	99	91**
4	100	98	95	97	99	90**
5	101	98	94	97	100	89**
6	101	98	93	96	100	89**
7	101	98	92*	96	99	89**

8	101	98	92*	97	99	88**
9	102	98	92*	96	100	88**
10	102	98	92*	96	99	88**
11	102	99	92*	97	100	89**
12	103	99	93	97	100	88**
13	102	99	93	97	100	88**
16	103	98	94	98	98	87**
20	103	99	93	97	99	85**
24	103	100	94	98	99	85**
28	102	99	94	98	99	84**
32	103	100	95	99	97	82**
36	103	100	95	98	97	81**
40	103	100	94	99	98	80**
44	103	100	95	97	96	77**
48	103	100	95	97	95	77**
52	103	100	94	96	95	76**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.1-4: Chronic toxicity phase - mean body weight gain (% of control) of rats administered inpyrfluxam in the diet for 52 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	150	500	2000	150	500	1500/1000
0-1	98	93	89**	86*	105	68**
1-2	100	100	95	86	86	73**
4-5	108	100	79**	91	118	73**
5-6	111	94	78*	90	90	90
6-7	88	88	71**	100	88	100
7-8	117	108	83	88	88	50*
10-11	110	100	90	200*	133	167
16-20	100	104	87	91	118	45**
28-32	118	118	106	115	54	31**
32-36	110	110	90	89	78	44*
36-40	108	115	85	114	171	43**
40-44	85	85	100	58	42	8**
0-52	104	100	92	94	93	63**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.1-5: Carcinogenicity phase - mean body weight (% of control) of rats administered with inpyrfluxam in the diet for 104 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	150	500	2000	150	500	1500/1000

2	99	99	95**	101	99	96**
3	99	99	94**	100	99	94**
4	99	98	93**	99	99	93**
5	99	99	92**	100	99	92**
6	99	98	91**	100	99	92**
7	98	98	91**	100	98	92**
8	98	98	91**	100	98	91**
9	98	98	90**	100	98	92**
10	98	98	90**	99	98	91**
11	98	98	90**	99	98	90**
12	98	98	90**	99	97	90**
13	98	98	90**	99	98	90**
16	99	98	90**	100	98	89**
20	99	98	91**	99	97	88**
24	99	98	91**	99	97	87**
28	99	98	91**	100	97	86**
32	99	98	91**	100	97	85**
36	100	99	91**	99	97	83**
40	100	99	91**	99	97	81**
44	100	99	91**	99	96	80**
48	100	99	91**	99	95	79**
52	100	98	90**	99	95	80**
56	100	99	91**	100	95	80**
60	100	98	90**	100	95	79**
64	100	99	90**	100	95	79**
68	99	98	91**	100	95	79**
72	99	98	90**	100	95	78**
76	100	98	91**	100	95	78**
80	100	98	90**	99	93	78**
84	100	98	90**	100	93	78**
88	101	99	89**	100	93	78**
92	100	98	88**	100	94	79**
96	101	98	88**	102	96	81**
100	102	99	88**	101	96	81**
104	102	100	87**	101	98	81**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.1-6: Carcinogenicity phase - mean body weight gain (% of control) of rats administered with inpyrfluxam in the diet for 104 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	150	500	2000	150	500	1500/1000
0-1	96	100	89**	105	100	84**
1-2	100	96	87**	105	100	80**
2-3	100	97	86**	94	94	83**
3-4	97	97	83**	93	93	73**
4-5	96	96	76**	109	91	91
5-6	90	90	75**	100	100	89
6-7	100	100	87*	89	89	67**
7-8	100	92	83*	86	86	71
8-9	100	100	83*	100	100	100
13-16	105	89	89	111	100	56**
16-20	100	100	95	91	82	55**
20-24	100	100	90	100	78	56**
24-28	112	100	94	113	113	50**
28-32	94	106	100	110	90	40**
32-36	118	100	109	78	89	33**
36-40	108	117	92	90	90	10**
40-44	118	109	82	111	89	33**
56-60	110	100	70	122	100	44**
64-68	250**	150	250*	92	92	62*
68-72	92	58*	58**	100	86	43*
76-80	200	133	33*	100	0**	100
80-84	160	140	80	100	73	45*
92-96#	3	1	-5	4	5*	6*
0-104	103	99	82**	102	96	73**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); # actual body weight gain/loss (g): control values -2 g for males, and -3 g for females.

Food consumption

Chronic toxicity phase

At 2000 ppm, males showed relatively lower food consumption within the initial 10 weeks. There was significant reduction in food consumption in females at 1500/1000 ppm throughout the treatment period.

Carcinogenicity phase

At the high dose, there was a significant decrease in food consumption in both sexes throughout the treatment period.

Overall, there was adverse decrease in food consumption at high dose in both sexes in both phases.

Ophthalmology and urinalysis

No treatment related changes were observed in any of the treated groups in either sex.

Haematology

Chronic toxicity group

There were significant decreases in neutrophil (by 41%) and monocyte (by 40%) counts after 52 weeks in the high dose females. These changes were considered as treatment related and adverse.

Significant increases in haematocrit (by 4%) and haemoglobin concentration (by 4%) (after 14 weeks) and neutrophil (after 26 weeks) were observed in females at 500 ppm. These changes are considered as incidental due to the lack of a dose response.

Carcinogenicity group

At the high dose, there were significant decreases in neutrophil (by 20% in males and 35% in females) and monocytes (by 19% in males and 28% in females). Decreased white blood cell count (by 23%) was also noted in the high dose females.

Overall, there were adverse effects on differential leukocyte counts at the high dose in females of the chronic toxicity phase and in both sexes of the carcinogenicity phase.

Table 6.5.1-7: Chronic toxicity phase - summary of significant changes at haematology [mean ± SD (% of controls)] of rats administered inpyrfluxam in the diet for 52 weeks.

Para-meter	Time point (week)	Sex and dose level (ppm)							
		Male				Female			
		0 (N=10)	150 (N=10)	500 (N=10)	2000 (N=10)	0 (N=10)	150 (N=10)	500 (N=10)	1500 / 1000 (N=10)
Ht (%)	14	44.4 ± 1.3	44.9 ± 1.6 (101)	44.9 ± 1.4 (101)	45.2 ± 1.4 (102)	42.7 ± 1.4	43.3 ± 1.1 (101)	44.2 ± 1.2* **(104) ↑4%**	43.6 ± 1.1 (102)
Hb (g/dL)	14	15.5 ± 0.4	15.7 ± 0.5 (101)	15.7 ± 0.5 (101)	15.9 ± 0.6 (103)	14.9 ± 0.5	15.1 ± 0.4 (101)	15.5 ± 0.5* **(104) ↑4%**	15.2 ± 0.4 (102)
Differential leukocyte count									
	26	0.79 ± 0.27	0.76 ± 0.22	0.74 ± 0.25	0.77 ± 0.16	0.44 ± 0.17	0.56 ± 0.13	0.64 ± 0.19*	0.46 ± 0.23

Neut (10^3 /µL)			(96)	(94)	(97)		(127)	**(145) ↑45%**	(105)
	52	0.89 ± 0.29	1.09 ± 0.30 (122)	0.96 ± 0.34 (108)	1.13 ± 0.21 (127)	0.66 ± 0.28	0.67 ± 0.26 (102)	0.51 ± 0.13 (77)	0.39 ± 0.10** **(59) ↓41%**
Mono (10^3 / µL)	52	0.16 ± 0.05	0.21 ± 0.05 (131)	0.20 ± 0.07 (125)	0.18 ± 0.05 (113)	0.10 ± 0.04	0.11 ± 0.03 (110)	0.10 ± 0.02 (100)	0.06 ± 0.02* **(60) ↓40%**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test), N=number of animals used

Table 6.5.1-8: Carcinogenicity phase - summary of significant changes at haematology [mean ± SD (% of controls)] of rats administered with inpyrfluxam in the diet for 104 weeks.

Para-meter	Sex and dose level (ppm)							
	Male				Female			
	0 (N=42)	150 (N=46)	500 (N=41)	2000 (N=47)	0 (N=34)	150 (N=33)	500 (N=36)	1500 / 1000 (N=42)
WBC (10^3/µL)	6.20 ± 2.03	5.52 ± 1.60 (89)	5.53 ± 1.97 (89)	5.22 ± 2.19 (84)	3.61 ± 1.61	3.42 ± 1.28 (95)	3.44 ± 1.95 (95)	2.78 ± 1.15* ↓ **(77)**
Differential leukocyte count								
Neut (10^3/µL)	2.53 ± 1.24	2.21 ± 0.93 (87)	2.27 ± 1.39 (90)	2.02 ± 1.43* **(80) ↓20%**	1.59 ± 1.08	1.38 ± 0.69 (87)	1.40 ± 0.96 (88)	1.03 ± 0.56* **(65) ↓35%**
Mono (10^3/µL)	0.36 ± 0.14	0.32 ± 0.11 (89)	0.31 ± 0.13 (86)	0.29 ± 0.16* **(81) ↓19%**	0.18 ± 0.08	0.16 ± 0.07 (89)	0.16 ± 0.07 (89)	0.13 ± 0.04* **(72) ↓28%**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test), N=number of animals used

Clinical chemistry parameters

Chronic toxicity phase

At the high dose, significant increases/ increasing trend in γ-glutamyl transpeptidase (GGTP) and albumin/globulin ratio (A/G) were observed in males at week 14 and 26 which declined at week 52. There was also a significant decrease in globulin in top dose males.

These effects appear to be treatment-related and adverse. A dose dependent decrease in total bilirubin was observed in females at the top dose, whilst an increase was observed in top dose males. Given the conflicting results between sexes, the effects on bilirubin are considered to be incidental.

At 1500/1000 ppm, decreases in aspartate transaminase (AST) and alanine transaminase (AST) were noted at 14, 26 and/or 52 weeks in females. Given the direction of the change, these changes are not considered as treatment related or adverse. The changes in glucose, total protein, and blood urea nitrogen (BUN) in females are considered as incidental due to the lack of a dose response and/or the values being within the HCD (BUN). Minor changes were seen in chloride levels in males and females mainly at the top dose; however, these were inconsistent between sexes, timepoints and did not show a dose-response relationship (in females). Therefore, they are considered unrelated to treatment.

Overall, there were adverse effects on some clinical chemistry parameters (GGTP, A/G ratio and globulin) indicative of liver damage in top dose males.

Table 6.5.1-9: Chronic toxicity phase - summary of significant changes at clinical chemistry [mean ± SD (% of controls)] of rats administered inpyrfluxam in the diet for 52 weeks.

Para-meter	Time point (week)	Sex and dose level (ppm)							
		Male				Female			
		0 (N=10)	150 (N=10)	500 (N=10)	2000 (N=10)	0 (N=10)	150 (N=10)	500 (N=10)	1500 / 1000 (N=10)
ALP (U/L)	26	203 ± 37	209 ± 45 (103)	209 ± 25 (103)	218 ± 50 (107)	66 ± 4	77 ± 22 (117)	83 ± 15** **(126) ↑26%**	72 ± 20 (109)
AST (U/L)	26	71 ± 10	76 ± 12 (107)	76 ± 18 (107)	82 ± 15 (115)	150 ± 106	143 ± 70 (95)	91 ± 24 (61)	66 ± 11** **(44) ↓66%**
	52	75 ± 17	80 ± 23 (107)	64 ± 14 (85)	76 ± 16 (101)	137 ± 61	173 ± 101 (126)	100 ± 25 (73)	78 ± 30** **(57) ↓43%**
ALT (U/L)	14	27 ± 3	29 ± 5 (107)	30 ± 7 (111)	27 ± 4 (100)	26 ± 7	34 ± 24 (131)	27 ± 9 (104)	19 ± 2* **(73) ↓27%**
	26	30 ± 6	33 ± 8 (110)	31 ± 10 (103)	33 ± 5 (110)	58 ± 37	56 ± 29 (97)	46 ± 25 (79)	20 ± 4** **(34) ↓66%**
	52	42 ± 16	40 ± 15 (95)	31 ± 10 (74)	38 ± 11 (90)	63 ± 25	78 ± 50 (124)	62 ± 41 (98)	32 ± 12** **(51) ↓49%**

GGTP (U/L)	14	0.7 ± 0.1	0.7 ± 0.1 (100)	0.6 ± 0.1 (86)	1.8 ± 0.6** **(257)** **↑157%**	0.8 ± 0.2	0.8 ± 0.2 (100)	0.9 ± 0.2 (113)	1.8 ± 0.7** **(225)** **↑125%**
	26	0.8 ± 0.1	0.8 ± 0.1 (100)	0.6 ± 0.2 (75)	1.6 ± 0.5** **(200)** **↑100%**	0.8 ± 0.2	0.8 ± 0.2 (100)	0.9 ± 0.3 (113)	1.2 ± 0.5 (150)
	52	1.2 ± 0.2	1.2 ± 0.4 (100)	1.1 ± 0.3 (92)	2.1 ± 1.2 (175)	1.0 ± 0.3	1.0 ± 0.3 (100)	0.9 ± 0.2 (90)	0.9 ± 0.3 (90)
Creat (mg / dL)	14	0.34 ± 0.04	0.34 ± 0.05 (100)	0.35 ± 0.05 (103)	0.37 ± 0.05 (109)	0.41 ± 0.03	0.40 ± 0.03 (98)	0.36 ± 0.03* **(88)** **↓12%**	0.41 ± 0.05 (100)
BUN (mg / dL)	52	14.2 ± 2.0	14.4 ± 1.0 (101)	14.1 ± 1.1 (99)	14.9 ± 1.8 (105)	14.2 ± 1.3	16.1 ± 2.8 (113)	15.1 ± 1.9 (106)	17.1 ± 1.6** **(120)** **↑20%**
TP (g/dL)	26	6.42 ± 0.29	6.32 ± 0.19 (98)	6.29 ± 0.34 (98)	6.15 ± 0.27 (96)	7.07 ± 0.25	6.88 ± 0.31 (97)	6.83 ± 0.23 (97)	6.77 ± 0.31* **(96)** **↓4%**
Glob (g/dL)	14	2.04 ± 0.16	2.08 ± 0.14 (102)	1.90 ± 0.23 (93)	1.80 ± 0.22* **(88)** **↓12%**	1.72 ± 0.17	1.67 ± 0.21 (97)	1.71 ± 0.23 (99)	1.71 ± 0.14 (99)
	26	2.25 ± 0.22	2.17 ± 0.11 (96)	2.12 ± 0.19 (94)	1.96 ± 0.22** **(87)** **↓13%**	1.76 ± 0.14	1.74 ± 0.25 (99)	1.81 ± 0.19 (103)	1.79 ± 0.14 (102)
A/G	14	2.10 ± 0.23	2.08 ± 0.18 (99)	2.25 ± 0.23 (107)	2.44 ± 0.30* **(116)** **↑16%**	2.93 ± 0.46	3.07 ± 0.51 (105)	3.03 ± 0.49 (103)	2.76 ± 0.24 (94)
	26	1.87 ± 0.22	1.91 ± 0.14 (102)	1.98 ± 0.14 (106)	2.16 ± 0.25** **(116)** **↑16%**	3.05 ± 0.27	3.03 ± 0.59 (99)	2.81 ± 0.43 (92)	2.81 ± 0.24 (92)
Gluc (mg / dL)	52	150 ± 15	155 ± 16 (103)	152 ± 14 (101)	147 ± 8 (98)	152 ± 8	137 ± 10* **(90)** **↓10%**	154 ± 15 (101)	139 ± 16 (91)
T.Bil (mg / dL)	14	0.05 ± 0.01	0.06 ± 0.01 (120)	0.06 ± 0.02 (120)	0.07 ± 0.02* **(140)** **↑40%**	0.09 ± 0.03	0.08 ± 0.01 (89)	0.07 ± 0.01 (78)	0.06 ± 0.01** **(67)** **↓33%**
Cl (mEq / L)	14	107.1 ± 0.9	106.5 ± 1.1 (99)	105.9 ± 1.3 (99)	105.7 ± 1.3* **(99)** **↓1%**	108.3 ± 1.2	109.2 ± 1.6 (101)	108.2 ± 1.5 (100)	108.2 ± 0.7 (100)

	26	108.1 ± 1.1	107.3 ± 1.2 (99)	107.1 ± 1.4 (99)	106.6 ± 1.7* **(99)** **↓1%**	107.4 ± 1.3	109.1 ± 1.5** **(102)** **↑2%**	107.6 ± 1.2 (100)	107.5 ± 0.6 (100)
	52	109.5 ± 1.5	108.8 ± 1.9 (99)	108.0 ± 1.2 (99)	108.4 ± 1.7 (99)	108.0 ± 1.5	109.3 ± 1.5 (101)	108.8 ± 0.8 (101)	109.7 ± 2.0* **(102)** **↑2%**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test), N=number of animals used

Gross pathology

Overall, there were no treatment related gross pathological changes in the animals of either the chronic toxicity phase or carcinogenicity phase.

Organ weights

Chronic toxicity phase

At 2000 ppm, significant increases in relative weights of the liver (by 11%), heart (by 10%) and testes (by 9%) were noted in males. At 1500/1000, there were significant increases in relative brain (by 29%), thyroid (by 11%), kidney (by 18%), spleen (by 6%), adrenal gland (by 18%) and uterus (by 48%) weights of females. The effects on the liver in males is considered as adverse due to the associated altered clinical chemistry parameters. The 6% increase in spleen weight in top dose females, although associated with deposition of haemosiderin, it is not considered adverse given the low magnitude of the change and the lack of erythrocytic abnormalities at haematology. All the other changes are considered to be due to the decreased terminal body weight and not of toxicological significance.

From 500 ppm, dose dependent and/or significant increases in relative liver (by ≥ 5%) and ovary (by ≥ 25%) weights and decrease in absolute thyroid weight (by ≥ 9%) were observed in females. In the absence of histological findings or changes in clinical chemistry parameters, these findings are not considered as adverse.

Carcinogenicity phase

At 2000 ppm, significant decreases in absolute weights of thyroid (by 71%), kidney (by 21%), and adrenal gland (by 23%) were observed in males. At 1500/1000 ppm, females showed increased relative brain weight (by 21%) and decreased absolute kidney weight (by 15%). The significant decrease in relative thyroid weight recorded in top dose males was found to be due mainly to a single male (with thyroid mass at necropsy) in the control group. Therefore, the change in thyroid weight is considered as incidental and unrelated to treatment. All the other organ weight changes are considered to be secondary to the decreased terminal body weight.

Overall, there were adverse effects on relative liver weight in males at the high dose in the chronic toxicity phase.

Table 6.5.1-10: Summary of significant changes in absolute and relative organ weights in rats administered inpyrfluxam in the diet for 52/104 weeks.

Organ	Sex and dose level (ppm)							
	Male				Female			
	0	150	500	2000$^{\Phi}$	0	150	500	1500 / 1000
Toxicity group (N=21; $^{\Phi}$N=20)								
Terminal body weight (g)	552 ± 62	568 ± 65 (103)	556 ± 55 (101)	520 ± 51 (94)	338 ± 40	326 ± 44 (96)	322 ± 38 (95)	258 ± 19** **(76)** **↓24%**
Brain -Relative	0.39 ± 0.04	0.37 ± 0.04 (95)	0.38 ± 0.03 (97)	0.40 ± 0.04 (103)	0.58 ± 0.07	0.60 ± 0.09 (103)	0.61 ± 0.07 (105)	0.75 ± 0.07** **(129)** **↑29%**
Thyroid-Absolute (mg)	28.7 ± 4.3	29.0 ± 3.1 (101)	29.1 ± 4.4 (101)	27.9 ± 4.3 (97)	24.1 ± 4.5	25.2 ± 4.7 (105)	22.0 ± 4.6 (91)	20.7 ± 3.1* **(86)** **↓14%**
Thyroid - Relative	0.0052 ± 0.0006	0.0052 ± 0.0008 (100)	0.0053 ± 0.0006 (102)	0.0054 ± 0.0009 (104)	0.0072 ± 0.0013	0.0077 ± 0.0012 (107)	0.0068 ± 0.0010 (94)	0.0080 ± 0.0010* **(111)** **↑11%**
Heart -Relative	0.21 ± 0.01	0.21 ± 0.02 (100)	0.21 ± 0.01 (100)	0.23 ± 0.02** **(110)** **↑10%**	0.25 ± 0.02	0.25 ± 0.02 (100)	0.26 ± 0.02 (104)	0.31 ± 0.03** **(124)** **↑24%**
Liver - Absolute (g)	12.12 ± 1.83	12.77 ± 1.98 (105)	12.86 ± 1.78 (106)	12.62 ± 1.10 (104)	7.10 ± 0.88	6.86 ± 1.09 (97)	7.14 ± 0.95 (101)	6.34 ± 0.44** **(89)** **↓11%**
Liver -Relative	2.19 ± 0.15	2.24 ± 0.19 (102)	2.31 ± 0.21 (105)	2.43 ± 0.13** **(111)** **↑11%**	2.10 ± 0.15	2.10 ± 0.16 (100)	2.22 ± 0.12* **(106)** **↑6%**	2.46 ± 0.15** **(117)** **↑17%**
Kidneys – Absolute (mg)	2839 ± 324	2805 ± 378 (99)	2868 ± 299 (101)	2803 ± 327 (99)	2019 ± 175	1943 ± 294 (96)	1981 ± 161 (98)	1817 ± 311** **(90)** **↓10%**
Kidneys – Relative	0.51 ± 0.04	0.50 ± 0.05 (98)	0.52 ± 0.04 (102)	0.54 ± 0.04 (106)	0.60 ± 0.07	0.60 ± 0.06 (100)	0.62 ± 0.08 (103)	0.71 ± 0.13** **(118)** **↑18%**
Spleen – Absolute (mg)	822 ± 127	842 ± 163 (102)	835 ± 126 (102)	790 ± 83 (96)	590 ± 65	559 ± 82 (95)	566 ± 74 (96)	493 ± 53** **(84)** **↓16%**

Spleen – Relative	0.15 ± 0.02	0.15 ± 0.02 (100)	0.15 ± 0.02 (100)	0.15 ± 0.02 (100)	0.18 ± 0.03	0.17 ± 0.02 (94)	0.18 ± 0.02 (100)	0.19 ± 0.02* **(106)** **↑6%**
Adrenals – Relative	0.011 ± 0.001	0.010 ± 0.001 (91)	0.011 ± 0.001 (100)	0.011 ± 0.001 (100)	0.022 ± 0.004	0.021 ± 0.003 (95)	0.022 ± 0.004 (100)	0.026 ± 0.004** **(118)** **↑18%**
Testes – Relative	0.69 ± 0.06	0.66 ± 0.15 (96)	0.69 ± 0.05 (100)	0.75 ± 0.07* **(109)** **↑9%**				
Ovaries – Relative					0.024 ± 0.009	0.025 ± 0.008 (104)	0.032 ± 0.009* **(133)** **↑33%**	0.030 ± 0.011 (125)
Uterus – Relative					0.29 ± 0.11	0.28 ± 0.12 (97)	0.28 ± 0.10 (97)	0.43 ± 0.15** **(148)** **↑48%**
Carcinogenicity group (N=10; @N=9)								
Terminal body weight (g)	641 ± 74	651 ± 84 (102)	583 ± 75 (91)	562 ± 49 (88)	385 ± 52	388 ± 68 (101)	398 ± 38 (103)	316 ± 52* **(82)** **↓18%**
Brain – Relative	0.35 ± 0.05	0.34 ± 0.05 (97)	0.39 ± 0.04 (111)	0.39 ± 0.04 (111)	0.53 ± 0.06	0.52 ± 0.09 (98)	0.51 ± 0.04 (96)	0.64 ± 0.11* **(121)** **↑21%**
Thyroid – Absolute (mg)	109.5 ± 224.4	34.6 ± 12.3 (32)	36.6 ± 7.2 (33)	32.0 ± 9.5* **(29)** **↓71%**	26.0 ± 6.3	24.1 ± 4.2 (93)	29.0 ± 7.0 (112)	21.1 ± 3.4 (81)
Thyroid – Absolute (mg)	38.6 ± 7.3 #	34.6 ± 12.3 (90)	36.6 ± 7.2 (95)	32.0 ± 9.5 (83)	-			
Thyroid- Relative	0.0060 ± 0.0007	0.0053 ± 0.0014 (88)	0.0063 ± 0.0013 (105)	0.0056 ± 0.0012 (93)				
Kidneys – Absolute (mg)	4112 ± 1278	3613 ± 457 (88)	3945 ± 1238 (96)	3245 ± 773** **(79)** **↓21%**	2338 ± 316	2343 ± 321 (100)	2380 ± 220 (102)	1989 ± 114** **(85)** **↓15%**
Adrenals – Absolute (mg)	81.8 ± 16.7	69.7 ± 15.0 (85)	76.9 ± 24.7 (94)	62.6 ± 7.3** **(77)** **↓23%**	80.3 ± 11.9	74.4 ± 16.2 (93)	77.1 ± 10.0 (96)	70.6 ± 15.3 (88)

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test); # see text above for explanations

Histopathology

Non-neoplastic lesions - chronic toxicity phase

There was a significant increase in the incidence of deposition of brown pigment (identified as haemosiderin) in the spleen of top dose females. This is not considered as adverse as there were no associated erythrocytic abnormalities observed at haematology.

Non-neoplastic lesions - carcinogenicity phase

No treatment-related findings were noted.

Overall, there were no treatment-related or adverse non-neoplastic changes in either sex of either phase.

Table 6.5.1-11: Chronic toxicity phase - summary of significant non-neoplastic lesions (No. of affected animals) in rats administered inpyrfluxam in the diet for 52 weeks.

Organ & Lesion	Sex and dose level (ppm)							
	Male				Female			
	0	150	500	2000	0	150	500	1500 / 1000
Spleen – No. examined	21	0	0	20	21	21	21	21
Deposition, brown pigment, increased	0	-	-	0	0	0	0	5*

*: p≤0.05 (two-tailed Fisher's exact probability test)

Table 6.5.1-12: Carcinogenicity phase - summary of significant non-neoplastic lesions (No. of affected animals) in rats administered inpyrfluxam in the diet for 104 weeks.

Organ & Lesion	Sex and dose level (ppm)							
	Male				Female			
	0	150^	500^	2000	0	150^	500^	1500 / 1000
All animals								
Lung – No. examined	51	6	11	51	51	18	14	50
Accumulation, macrophage, alveolar	11	2	3	2*	5	5	3	7
Kidney – No. examined	51	11	16	51	51	21	17	51
Calculi	8	2	3	1*	17	9	5	12
Nephropathy, chronic	34	9	13	33	32	13	4	9**
Adrenal – No. examined	51	7	13	51	51	19	15	51
Peliosis	0	0	0	0	29	12	6	16*
Terminal kill								
Lung – No. examined	42	1	1	47	34	0	0	42
Accumulation, macrophage, alveolar	9	1	1	2*	3	-	-	5
Kidney – No. examined	42	6	6	47	34	3	2	42

Calculi	7	1	1	1*	11	0	0	8
Nephropathy, chronic	28	5	6	32	18	2	1	6**
Adrenal – No. examined	42	2	3	47	34	1	0	42
Peliosis	0	0	0	0	18	0	-	12*
Fatty change, cortical cell, diffuse	4	0	0	0*	0	0	-	0
Thymus – No. examined	42	1	1	47	34	0	3	42
Cyst(s)	4	0	0	0*	2	-	0	2
Killed *in extremis* or found dead (ke/fd)								
Thymus – No. examined	9	5	10	4	17	17	14	9
Hyperplasia, lymphocyte	0	0	0	0	0	2	4*	0
Pancreas – No. examined	9	5	10	4	17	18	15	8
Infiltration, fat	0	0	0	0	0	4	5*	0
Kidney – No. examined	9	5	10	4	17	18	15	9
Cast(s), urinary	0	0	0	0	0	1	4*	0
Nephropathy, chronic	6	4	7	1	14	11	3**	3*

^: organ examined only in animals that showed macroscopic lesions at terminal kill and/or on all animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.
*: p≤0.05; **: p≤0.01 (two-tailed Fisher's exact probability test)

Neoplastic lesions - carcinogenicity phase

There were no adverse neoplastic lesions observed in either sex.

Table 6.5.1-13: Carcinogenicity phase - summary of significant neoplastic lesions (No. of affected animals) in rats administered inpyrfluxam in the diet for 104 weeks.

Organ & Lesion	Sex and dose level (ppm)							
	Male				Female			
	0	150^	500^	2000	0	150^	500^	1500 / 1000
All animals								
Mammary gland – No. examined	51	5	11	51	51	30	22	51
Fibroadenoma (benign)	0	0	1	0	16	17	7	4**
Pituitary – No. examined	51	18	18	51	51	40	41	51
Adenocarcinoma, anterior (malignant)	1	0	0	0	5	1	1	0*
Terminal kill								
Mammary gland – No. examined	42	0	1	47	34	12	7	42
Fibroadenoma (benign)	0	-	1	0	13	8	4	2**
Killed *in extremis* or found dead (ke/fd)								
Mammary gland – No. examined	9	5	10	4	17	18	15	9
Fibroadenoma (benign)	0	0	0	0	3	9*	3	2

^: organ examined only in animals that showed macroscopic lesions at terminal kill and/or on all animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.
*: p≤0.05; **: p≤0.01 (two-tailed Fisher's exact probability test)

Conclusion

In conclusion, in a GLP and guideline study, dietary administration of inpyrfluxam to male and female Wistar Hannover rats at dietary concentrations of 0, 150, 500, 2000 (males only), or 1500/1000 ppm (females only) for 52 weeks (mean substance intakes: 0, 6.77, 22.8, 95.9 mg/kg bw/day for males and 0, 5.85, 19.4 and 78.4 mg/kg bw/day for females) or 104 weeks (mean substance intakes: 0, 8.84, 30.1, 86.4 mg/kg bw/day for males and 0, 7.47, 25.5, 65.8 mg/kg bw/day for females) caused adverse effects at the top dose only, including effects on body weights, body weight gains and food consumption, effects on differential leukocyte count, changes in some clinical-chemistry parameters and increased liver weights. The substance was not carcinogenic in the rat up to a dose causing significant toxicity.

Overall, a chronic NOAEL of 500 ppm (19.4 mg/kg bw/day) can be established from this study based on effects on body weight, body weight gain and food consumption, effects on differential leukocyte count, changes in some clinical-chemistry parameters and increased liver weight at the LOAEL of 1500/1000 ppm (65.8 mg/kg bw/day). The NOAEL for carcinogenicity is the top dose of 1500/1000 ppm (65.8 mg/kg bw/day).

[redacted] (2017)

B.6.5.2. Long-term toxicity and carcinogenicity in mice

The carcinogenicity of inpyrfluxam has been investigated in mice via the oral (dietary) route. The study was GLP complaint and performed in accordance with OECD TG 451 (2009).

Reference:	KCA 5.5/03
Report Title:	S-2399 Technical Grade: Carcinogenicity Study in Mice
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. [redacted] 14-0047 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable methods of analysis available for dietary formulation and plasma kinetics
Guideline(s):	OECD 451 (2009)
Deviations from current guideline:	No

Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, [redacted] [redacted] mice received inpyrfluxam by dietary administration at concentrations of 0, 700, 2000 or 7000/5000 ppm over a period of 78 weeks (52 animals/sex/dose). A satellite group containing 12 animals/sex/dose were treated at the same dose levels for 52 weeks. The mean substance intakes at each dose level for males and females in the satellite and carcinogenicity groups are presented in the table 6.5.2-1.

The top dose was changed from 7000 ppm to 5000 ppm at week 53 for males and week 52 for females due to the considerable decrease in body weight (about 80%) at 7000 ppm. Therefore, the high dose animals in both sexes received inpyrfluxam at 5000 ppm for the remaining treatment period.

Table 6.5.2-1: Mean test substance intake in the mouse chronic/carcinogenicity study with inpyrfluxam

Dose level (ppm)	Males			Females		
	700	2000	7000/5000	700	2000	7000/5000
Satellite group (52 weeks) mg/kg bw/day	77.1	240	826	72.9	222	790
Carcinogenicity group (78 weeks) mg/kg bw/day	77	224	775	69.3	210	701

The dose levels were selected based on a previous repeated dose 90-day oral toxicity study in mice ([redacted] (2016a) where adverse effects relevant to humans were observed from 3500 ppm (491 mg/kg bw/day), including effects on liver weight, clinical-chemistry parameters indicative of liver damage and histopathological changes in the liver. Furthermore, adverse histopathological effects were observed in the thyroid at 7000 ppm in both sexes.

In accordance with OECD test guideline 451, all animals were subjected to the required investigations. In addition, analysis of the dietary formulation and plasma concentrations of inpyrfluxam was performed within the study. Plasma was analysed at 52 weeks of treatment from 4 mice/sex/dose.

Results

Toxicokinetics

Inpyrfluxam was detected in the plasma of both sexes at the mid and high dose levels whereas it was under the limit of quantification (LOQ) at the low dose. A dose dependent increase in plasma levels of inpyrfluxam was noted in both sexes. At the high dose, the plasma concentrations in males were higher than in females.

Table 6.5.2-2: Summary of plasma levels of inpyrfluxam in mice (n=4) administered inpyrfluxam in diet for 52 weeks.

Time point (week)	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
52	<LOQ	<LOQ	0.006	0.092	<LOQ	<LOQ	0.004[a]	0.017

<LOQ: below the limit of quantitation (<0.004 mg/L); [a]: computed as 0 for average calculation.

Mortality

No treatment related mortality was observed in either sex compared to control. The survival rate was acceptable at all dose levels in lines with the requirement of OECD guideline.

Clinical signs of toxicity

At 7000/5000 ppm, increased incidences of emaciation, pale-coloured skin, and pale colour of the eye were noted in males of the carcinogenicity group. These changes were considered as treatment-related and adverse.

Body weight and body weight gain

Satellite group

At the high dose, there were decreases in body weights and body weight gains in both the sexes throughout the treatment period. The decreases in final body weights (by 6% in males and by 20% in females) and body weight gains (by 23% in males and by 35% in females) were more severe in females than in males. Furthermore, there was a decrease (19%) in the body weight gain in mid-dose females; however, this was not confirmed in the carcinogenicity phase and hence was not considered adverse.

Carcinogenicity group

At the high dose, there were decreases in body weight and body weight gain in both sexes throughout the treatment period. The decreases in final body weights (by 13% in males and by 11% in females) and body weight gains (by 37% in males and by 24% in females) were more severe in males than in females.

Overall, there were adverse decreases in body weight and body weight gain at the high dose in both sexes and in both groups.

Table 6.5.2-3: Satellite group - mean body weight (% of control) of mice administered with inpyrfluxam in the diet for 52 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
1	100	100	95	99	99	98
2	98	100	94*	96	99	96
3	98	99	96	96	99	95
4	97	99	95	94	95	94
5	99	100	96	98	99	95
6	99	98	95	95	93*	92*
7	100	98	95	96	97	90*
8	98	98	92*	92	94	90**
9	100	98	94	94	96	89*
10	99	97	94	93	91*	87**
11	99	98	93*	94	93	89
12	99	97	92*	92	90*	85**
13	100	98	93*	94	93	86**
16	100	97	93	93	90	84**
20	99	96	90*	95	89	83**
24	98	94	90*	96	93	83**
28	98	96	91	97	88*	82**
32	98	95	91	95	89	83**
36	98	95	91	97	90	80**
40	97	93	89	97	89	79**
44	99	96	89	97	89	78**
48	99	95	89*	98	90	78**
52	105	99	94	101	90	80** **(↓20%)**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.2-4: Satellite group - mean body weight gain (% of control) of mice administered inpyrfluxam in the diet for 52 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
0-1	97	97	41**	79	89	74
5-6	108	58	92	1.1#	-0.1*#	1.0#
7-8	0.4*#	0.8#	-0.2**#	33	43	100
8-9	0.3*#	-0.2#	0.6**#	0.3#	0.4#	-0.8#
11-12	67	44	33*	46	29	17
16-20	77	38	8*	115	70	67
24-28	200	900	400	1.9#	-1.3**#	0.9#
48-52	0.9#	1.1*#	0.3#	1.1#	-0.4#	1.2#
0-52	114	95	77	101	81	65** **(↓35%)**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test);
# actual body weight gain/loss (g): control values for males: 1.1 g @ wks 7-8, -0.4 g @ wks 8-9,.and -2.3 g @ wks 48-52; control values for females: 2.1 g @ wks 5-6, -0.3 g @ wks 8-9, 1.4 g @ wks 24-28, and -0.4 g @ wks 48-52

Table 6.5.2-5: Carcinogenicity group - mean body weight (% of control) of mice administered with inpyrfluxam in the diet for 78 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
1	101	100	97**	99	100	97**
2	101	99	95**	99	100	96**
3	99	97	94**	97	99	95**
4	99	99	94**	99	99	96**
5	100	99	94**	96*	100	93**
6	100	98	94**	99	100	94**
7	100	99	92**	99	98	92**
8	99	98	91**	99	98	92**
9	100	99	92**	98	98	90**
10	100	99	91**	99	98	89**
11	100	99	90**	98	98	88**
12	100	99	89**	98	98	87**
13	101	99	90**	98	98	87**
16	101	99	89**	98	98	85**
20	100	98	86**	100	97	85**
24	100	98	86**	99	98	84**
28	102	99	86**	100	97	84**
32	101	99	86**	100	97	84**
36	103	99	85**	100	98	85**

40	103	98	84**	102	99	85**
44	102	97	84**	102	100	84**
48	102	96	83**	102	99	86**
52	103	96	83**	103	101	86**
56	102	96	84**	102	99	86**
60	101	96	85**	101	98	87**
64	102	97	86**	100	98	86**
68	100	96	85**	98	94	86**
72	102	96	86**	101	96	89**
76	102	96	86**	97	94	89*
78	102	97	87** **(↓13%)**	98	95	89** **(↓11%)**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.2-6: Carcinogenicity group - mean body weight gain (% of control) of mice administered with inpyrfluxam in the diet for 78 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
0-1	112	96	58**	88	100	47**
1-2	95	84	63**	94	106	88
2-3	74**	68**	68**	72	67	72
3-4	113	150**	113	155	118	118
4-5	150	133	83	-0.2**#	0.9#	-0.1*#
6-7	111	122	22**	75	67	33
7-8	50**	60	20**	100	100	92
8-9	0.3#	0.3*#	0.5**#	0.1#	0.5#	-0.5#
9-10	100	78	56**	156	89	78
10-11	140	160	20*	92	108	58
11-12	75	50*	0**	79	79	43
12-13	300**	200	300*	400	200	200
13-16	108	92	62*	100	100	62*
16-20	92	85	8**	121	83	83
24-28	156*	144	78	136	57	79
32-36	267*	200	33	100	171	186
64-68	-1.0**#	-0.7#	-0.2#	-1.2#	-2.0**#	-0.7#
68-72	-0.3#	-1.1#	0.0*#	-1.0#	-1.0#	-0.2#
0-78	104	94	63** **(↓37%)**	95	90	76** **(↓24%)**

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test);
# actual body weight gain/loss (g): control values for males: 0.0 g @ wks 8-9, -0.4 g @ wks 64-68, and -1.1 g @ wks 68-72; control values for females: 0.7 g @ wks 4-5, 0.5 g @ wks 8-9, -0.1 g @ wks 64-68, and -1.3 g @ wks 68-72

Food consumption and food efficiency

Satellite group

At the high dose, there was a significant decrease in food consumption in both males (at weeks 1,44 and 48) and females (weeks 7 and 10). This change recovered in the later treatment periods.

Carcinogenicity group

At the high dose, there was a significant decrease in food consumption in both males (at weeks 1,7,28,40, 44 and 48) and females (weeks 1-12 and 36). This change recovered in the later treatment periods.

Overall, there were no adverse changes noted in food consumption at the end of the treatment period in both sexes and groups.

Table 6.5.2-7: Satellite group - Summary of food consumption at selected time points (% of control) of mice administered with inpyrfluxam in the diet for 52 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
1	98	96	84*	105	105	93
7	93	102	93	89	100	89*
8	92	105	88	94	88*	96
10	93	109	95	96	92	86*
44	100	104	92**	96	90	92
48	95	93	88**	102	96	89

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.5.2-8: Carcinogenicity group - Summary of food consumption at selected time points (% of control) of rats administered with inpyrfluxam in the diet for 78 weeks.

Week	Sex and dose level (ppm)					
	Male			Female		
	700	2000	7000 / 5000	700	2000	7000 / 5000
1	100	102	87**	98	98	87**
2	100	98	96	96	100	91**
3	100	100	96	98	98	91**
4	98	96	94	96	102	90**
6	100	100	98	100	100	90**
7	100	96	93*	100	102	90**
10	102	100	98	94	98	90**
12	104	98	98	100	102	92*
28	102	96	92**	110	114**	110
36	100	100	96	96	100	86*
40	98	98	93*	98	107	93
44	98	96	91**	100	104	94
48	98	96	91*	102	109	100

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Haematology

There were no treatment related changes in either sex.

Gross pathology
Satellite group

At the high dose, a significant increase in the incidence of dark coloured liver was observed in males. This is considered as treatment related and adverse.

Increased incidences of loss of tactile hair at 2000 ppm and ovary cysts at 700 ppm were noted. These changes are not considered adverse due to the lack of a dose response.

Carcinogenicity group

Significant increase or increasing tendency in the incidence of dark coloured liver (at the high dose in both sexes) and coarse surface of the kidney (in the high dose males - significant and from 2000 ppm in females) were observed. The changes in the liver and kidney are considered as treatment related and adverse.

Overall, there were adverse gross pathology effects on liver (in both groups) at the high dose and kidney (in the carcinogenicity group) from the mid dose in both sexes.

Table 6.5.2-9: Satellite group - summary of necropsy findings (No. of affected animals/No. of examined) in mice administered inpyrfluxam in the diet for 52 weeks.

Organ & lesion	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
Loss of tactile hair	0/9	0/11	0/10	0/11	0/12	0/11	5/12*	3/10
Liver								
Dark in colour	0/9	0/11	1/10	5/11*	0/12	0/11	0/12	0/10
Ovary								
Cyst(s)					1/12	6/11*	4/12	4/10

*: p≤0.05 (Fisher's exact probability test)

Table 6.5.2-10: Carcinogenicity group - summary of necropsy findings (No. of affected animals/No. of examined) in mice administered inpyrfluxam in the diet for 78 weeks.

Organ & lesion	Sex and dose level (ppm)	
	Male	Female

	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
All animals examined								
Loss of tactile hair	1/52	4/52	1/52	3/52	6/52	15/52*	8/52	11/52
Liver								
Dark in colour	0/52	0/52	0/52	8/52**	0/52	0/52	1/52	5/52
Lymph node								
Enlargement	0/52	4/52	2/52	3/52	8/52	4/52	1/52*	2/52
Seminal vesicle								
Hypertrophy	13/52	14/52	12/52	3/52*				
Ovary								
Cyst(s)					23/52	26/52	34/52*	26/52
Animals at terminal kill								
Loss of tactile hair	1/35	2/28	0/27	2/29	2/34	9/28*	7/31	8/30*
Loss of fur	10/35	6/28	5/27	8/29	8/34	13/28	16/31*	10/30
Liver								
Dark in colour	0/35	0/28	0/27	7/29**	0/34	0/28	0/31	2/30
Coagulating gland								
Hypertrophy	8/35	7/28	7/27	0/29**				
Ovary								
Cyst(s)					15/34	14/28	22/31*	16/30
Uterus								
Mass(es)					8/34	1/28*	5/31	3/30
Animals killed in extremis or found dead								
Thymus								
Enlargement	0/17	1/24	0/25	2/23	7/18	3/24	0/21**	6/22
Lymph node								
Enlargement	0/17	2/24	1/25	3/23	5/18	4/24	0/21*	2/22
Lung								
Mass(es)	3/17	7/24	7/25	3/23	7/18	6/24	1/21*	2/22
Liver								
Dark in colour	0/17	0/24	0/25	1/23	0/18	0/24	1/21	3/22
Kidney								
Coarse surface	0/17	2/24	1/25	6/23*	6/18	3/24	10/21	9/22

*: p≤0.05; **: p≤0.01 (Fisher's exact probability test)

Organ weights

Satellite group

At 7000/5000 ppm, increases in absolute (by 42% in males and 5% in females) and relative (by 53% in males and 28% in females) liver, and relative adrenal (by 40% in males and 35% in females) weights were observed. At the high dose, there were decreases in the absolute heart (by 15%) and kidney (by 12%) weights, and from 2000 ppm, dose dependent increase in the relative brain weight (significant only at the high dose) were noted in females. Given the presence of the associated histopathological findings, the changes in liver, and kidney

at the top dose are considered as treatment related and adverse. The changes in heart, adrenal and brain weights are considered the consequence of the reduced body weights.

Carcinogenicity group

At the high dose, significant increases in absolute (by 2%) and relative (by 14%) liver weights were noted in females. From the mid dose, there was a dose dependent increase (significant at the high dose) in relative adrenal weight in females. Significant increases in the relative brain (by 22%) and heart (16%) weights at the high dose, and absolute (not significant at the high dose) and relative testes weights from the mid dose were observed in males. Only the changes in liver weight at the top dose in females are considered adverse due to the associated histopathological changes. All other organ weight changes are not considered adverse, given the lack of associated histopathology and they are most likely the consequence of the reduced body weights reported at the top dose.

Overall, adverse effects were noted in liver and kidney weights at the top dose in the satellite group and in liver weight at the high dose in the carcinogenicity group.

Table 6.5.2-11: Summary of significant changes in absolute and relative organ weights in mice administered inpyrfluxam in the diet for 52/78 weeks.

Organ	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
Satellite group								
Terminal body weight (g)	49.1 ± 4.5	51.8 ± 5.8 (105)	48.5 ± 3.4 (99)	46.0 ± 5.6 (94)	56.2 ± 9.3	56.5 ± 9.7 (101)	50.4 ± 6.1 (90)	45.2 ± 5.6** **(80) ↓20%**
Brain – No. examined	9	11	10	11	12	11	12	10
Relative	1.03 ± 0.09	1.02 ± 0.14 (99)	1.06 ± 0.08 (103)	1.11 ± 0.11 (108)	0.92 ± 0.16	0.89 ± 0.13 (97)	1.03 ± 0.11 (112)	1.15 ± 0.17** **(125) ↑25%**
Heart – No. examined	9	11	10	11	12	11	12	10
Absolute (mg)	234 ± 32	234 ± 25 (100)	218 ± 16 (93)	228 ± 28 (97)	198 ± 49	176 ± 15 (89)	180 ± 14 (91)	168 ± 24* **(85) ↓15%**
Liver – No. examined	9	11	10	11	12	11	12	10
Absolute (g)	2.60 ± 0.51	3.03 ± 0.86 (117)	2.75 ± 0.59 (106)	3.69 ± 0.96** **(142) ↑42%**	2.37 ± 0.20	2.33 ± 0.25 (98)	2.35 ± 0.31 (99)	2.48 ± 0.30 (105)

Relative	5.26 ± 0.59	5.83 ± 1.33 (111)	5.65 ± 1.04 (107)	8.03 ± 1.95** **(153)** **↑53%**	4.31 ± 0.70	4.18 ± 0.52 (97)	4.69 ± 0.56 (109)	5.52 ± 0.67** **(128)** **↑28%**
Kidneys – No. examined	9	11	10	11	12	11	12	10
Absolute (mg)	891 ± 99	876 ± 82 (98)	868 ± 102 (97)	845 ± 97 (95)	538 ± 56	527 ± 56 (98)	529 ± 59 (98)	471 ± 48* **(88)** **↓12%**
Adrenals – No. examined	9	11	10	11	12	11	12	10
Relative	0.010 ± 0.003	0.010 ± 0.003 (100)	0.010 ± 0.002 (100)	0.014 ± 0.005 (140)	0.020 ± 0.005	0.018 ± 0.006 (90)	0.021 ± 0.008 (105)	0.027 ± 0.007* **(135)** **↑35%**
Main group								
Terminal body weight (g)	51.8 ± 6.7	50.2 ± 5.5 (97)	49.5 ± 7.6 (96)	42.4 ± 4.0** **(82)** **↓18%**	52.0 ± 8.9	53.5 ± 7.2 (103)	47.8 ± 7.2 (92)	45.8 ± 6.3 (88)
Brain – No. examined	10	10	10	10	10	10	10	10
Relative	0.98 ± 0.09	1.03 ± 0.12 (105)	1.04 ± 0.17 (106)	1.20 ± 0.14** **(122)** **↑22%**	1.03 ± 0.19	1.01 ± 0.14 (98)	1.14 ± 0.17 (111)	1.16 ± 0.17 (113)
Heart – No. examined	10	10	10	10	10	10	10	10
Relative	0.49 ± 0.09	0.51 ± 0.08 (104)	0.45 ± 0.05 (92)	0.57 ± 0.07* **(116)** **↑16%**	0.40 ± 0.12	0.40 ± 0.07 (100)	0.49 ± 0.10 (123)	0.45 ± 0.10 (113)
Liver – No. examined	10	10	10	10	10	10	10	10
Absolute (g)	3.26 ± 0.96	3.68 ± 1.84 (113)	3.21 ± 1.37 (98)	3.32 ± 1.22 (102)	2.87 ± 1.57	2.54 ± 0.27 (89)	2.78 ± 0.40 (97)	2.92 ± 0.38* **(102)** **↑2%**
Relative	6.49 ± 2.50	7.42 ± 3.87 (114)	6.30 ± 1.71 (97)	8.03 ± 3.93 (124)	5.63 ± 2.93	4.82 ± 0.80 (86)	5.85 ± 0.68 (104)	6.43 ± 0.91* **(114)** **↑14%**
Adrenals – No. examined	10	10	10	10	10	10	10	10
Relative	0.015 ± 0.008	0.013 ± 0.003 (87)	0.014 ± 0.003 (93)	0.015 ± 0.003 (100)	0.020 ± 0.008	0.023 ± 0.005 (115)	0.024 ± 0.005 (120)	0.028 ± 0.008* **(140)** **↑40%**
Testes – No. examined	10	10	10	10				
Absolute (mg)	171 ± 46	211 ± 66 (123)	249 ± 52** **(146)** **↑46%**	227 ± 40 (133)				

Relative	0.33 ± 0.09	0.42 ± 0.14 (127)	0.51 ± 0.11** **(155)** **↑55%**	0.54 ± 0.10** **(164)** **↑64%**	

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Histopathology

Non-neoplastic lesions – satellite group

At the high dose, significant increase in diffuse hepatocellular hypertrophy was observed in females. From 2000 ppm, there were increases in centrilobular hepatocellular hypertrophy and diffuse luminal dilatation of proximal renal tubules in males. These changes are considered as treatment related and adverse.

Non-neoplastic lesions – carcinogenicity group

At the high dose, significant increases in diffuse hepatocellular hypertrophy in females and diffuse luminal dilatation of renal proximal tubules were recorded in males. From 2000 ppm, increases in centrilobular hepatocellular hypertrophy in males (significant at the high dose) and amyloid nephropathy in both sexes (significant in high dose males) were noted. Reduction in the retention of secreted materials in the seminal vesicle and coagulating gland were noted in the high dose males. These changes are considered as treatment related and adverse.

In addition to the above changes, at the high dose, there were a significant increase in the incidence of amyloidosis in mesenteric lymph nodes, heart, forestomach, duodenum, liver, kidney, thyroid, adrenal and extra orbital lacrimal gland in males. From 2000 ppm, a significant increase in the incidence of amyloidosis in cervical lymph nodes and glandular stomach was observed in females. These changes are considered as treatment related and adverse.

Overall, there were adverse effects noted in liver and kidney from the mid dose in males and at the high dose in females in both satellite and carcinogenicity group. Adverse systemic amyloidosis was also noted at the high dose in males (various organs) and from the mid dose in females (cervical lymph node and glandular stomach) in the carcinogenicity group.

Table 6.5.2-12: Satellite group - summary of significant non-neoplastic lesions (No. of affected animals/ No of animals examined) in mice administered inpyrfluxam in the diet for 78 weeks.

Organ & Lesion	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000

Terminal kill								
Liver – No. examined	9	11	10	11	12	11	12	10
Hypertrophy, hepatocyte, centrilobular	0	0	3	6*	0	0	0	0
Hypertrophy, hepatocyte, diffuse	0	0	0	0	0	0	0	4*
Kidney - No. examined	9	11	10	11	12	0	2	10
Cyst, cortical	4	1	2	0*	1	-	2	1
Dilation, luminal, proximal tubule, diffuse	0	0	1	5*	0	-	0	0
Killed *in extremis* or found dead								
Kidney - No. examined	3	1	2	1	0	1	0	2
Dilation, luminal, proximal tubule, diffuse	1	0	0	0	-	1	-	0

*: p≤0.05 (two-tailed Fisher's exact probability test)

Table 6.5.2-13: Carcinogenicity group - summary of significant non-neoplastic lesions (No. of affected animals/ No of animals examined) in mice administered inpyrfluxam in the diet for 78 weeks.

Organ & Lesion	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
All animals								
Liver – No. examined	52	52	52	52	52	52	52	52
Hypertrophy, hepatocyte, centrilobular	0	0	3	7*	0	0	0	0
Hypertrophy, hepatocyte, diffuse	0	0	0	0	0	0	0	11**
Fatty change, hepatocyte, centrilobular	8	6	6	1*	0	0	0	0
Kidney – No. examined	52	52	52	52	52	29^	26^	52
Dilation, luminal, proximal tubule, diffuse	4	6	4	14*	4	1	2	2
Nephropathy, amyloid	5	3	9	16*	14	12	19	20
Seminal vesicle – No. examined	52	33^	33^	52	-			
Retention, secreted material	17	15	11	3**				
Coagulating gland - No. examined	52	31^	32^	52				

Retention, secreted material	10	9	11	1**				
Prostate – No. examined	52	24^	25^	52				
Prostatitis	6	3	2	0*				
Terminal kill								
Liver – No. examined	35	28	27	29	34	28	31	30
Hypertrophy, hepatocyte, centrilobular	0	0	3	7**	0	0	0	0
Hypertrophy, hepatocyte, diffuse	0	0	0	0	0	0	0	11**
Kidney – No. examined	35	28	27	29	34	5^	5^	30
Dilation, luminal, proximal tubule, diffuse	1	1	1	8**	1	0	0	0
Seminal vesicle – No. examined	35	9^	8^	29	-			
Retention, secreted material	16	9	8	3**				
Coagulating gland - No. examined	35	7^	7^	29				
Retention, secreted material	10	4	7	0**				
Killed *in extremis* or found dead								
Testis – No. examined	17	24	25	23	-			
Calcification, arterial wall	4	0*	1	1				

^: organ examined only in animals that showed macroscopic lesions at terminal kill and/or on all animals killed *in extremis* or found dead during the study. Not subjected to statistical analysis.
*: p≤0.05; **: p≤0.01 (two-tailed Fisher's exact probability test)

Table 6.5.2-14: Carcinogenicity group - Summary of amyloid lesions – All animals examined (No. of affected animals/No. of animals examined) in mice administered inpyrfluxam in the diet for 78 weeks.

Organ	Sex and dose level (ppm)							
	Male				Female			
	0	700	2000	7000 / 5000	0	700	2000	7000 / 5000
Lesion: Amyloidosis								
Lymph node (cervical)					5/52	12/52	15/52*	17/52**
Lymph node (mesenteric)	8/52	9/52	13/52	18/52*	-			
Heart	7/52	6/52	13/52	17/52*				
Forestomach	5/52	7/52	12/52	16/52 F* M**				
Glandular stomach					9/52	18/52	21/52*	19/52*
Duodenum	6/52	8/52	11/52	16/52*	-			
Liver	5/52	5/52	10/52	15/52*				

Kidney - Nephropathy	5/52	3/52	9/52	16/52 F* M**	
Thyroid	7/52	7/52	13/52	17/52*	
Adrenal	5/52	4/52	8/52	14/52*	
Extraorbital lacrimal gland	9/52	11/52	17/52	21/52*	

*: p≤0.05; **: p≤0.01 (F: Fisher's exact probability test & M: Mann-Whitney's U test); when not indicated, same level of significance at both tests

Neoplastic lesions - carcinogenicity group

There were no adverse neoplastic lesions observed in either sex.

Conclusion

In conclusion, in a GLP and guideline chronic/carcinogenicity study, dietary administration of inpyrfluxam to male and female [redacted] mice at dietary concentrations of 0, 700, 2000 and 7000/5000 ppm for 52 weeks (mean substance intakes: 0, 77.1, 240, 826 mg/kg bw/day for males and 0, 72.9, 222 and 790 mg/kg bw/day for females) or 78 weeks (mean substance intakes: 0, 77, 224 and 775 mg/kg bw/day for males and 0, 69.3, 210, 701 mg/kg bw/day for females) caused adverse effects from 2000 ppm, manifested as systemic amyloidosis in several organs and histopathology of liver and kidney. In addition, at the high dose there were adverse effects on body weights, body weight gains and gross pathological changes (in liver and kidney). The substance was not carcinogenic in the mice up to a dose causing significant toxicity.

Overall, a chronic NOAEL of 700 ppm (69.3 mg/kg bw/day) can be established from this study based on amyloidosis in several organs (cervical lymph node, glandular stomach, and kidney) and histopathological findings of liver and kidney at the LOAEL of 2000 ppm (210 mg/kg bw/day). The NOAEL for carcinogenicity is the top dose of 7000/5000 ppm (701 mg/kg bw/day).

[redacted] (2017)

B.6.5.3. Summary of long-term toxicity and carcinogenicity

The long-term toxicity and carcinogenicity of inpyrfluxam have been investigated via the oral route of exposure in rats and mice. Both studies were conducted in compliance with GLP and OECD test guidelines. The main findings are summarised in table below.

Table 6.5.3-1: Summary of long-term toxicity and carcinogenicity studies for inpyrfluxam

Data point/ Study *Acceptability*	Species / Strain/ sex	Doses	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects at LOAEL
KCA 5.5/01 *: Combined Chronic Toxicity and Carcinogenicity Study in Rats* OECD 453 (2009) GLP *Acceptable*	**Rat**/ Wistar Hannover rats/ M&F	Males: 0,150, 500, and 2000 ppm Females: 0, 150, 500, and 1000/1500 ppm <u>Mean substance intakes</u> *Males (chronic toxicity phase):* 0, 6.77,22.8 and 95.9 mg/kg bw/day *(carcinogenicity phase):* 0, 5.85,19.4 and 78.4 mg/kg bw/day *Females: (chronic toxicity phase):* 0, 8.84,30.1 and 86.4 mg/kg bw/day *(carcinogenicity phase):* 0, 7.47,25.5, and 65.8 mg/kg bw/day	<u>Chronic toxicity</u>: 500 ppm (19.4 mg/kg bw/day) <u>Carcinogenicity:</u> 1500/1000 ppm (65.8 mg/kg bw/day) - top dose	<u>Chronic toxicity:</u> 1500/1000 ppm (65.8 mg/kg bw/day) <u>Carcinogenicity:</u> N/A	**<u>Systemic chronic toxicity</u>** <u>Chronic toxicity phase:</u> ↓ bw (6% in M & 14%** in F) ↓ bw gain (8%* in M & 37%** in F) ↓ food consumption (M&F) ↓ neutrophil (41% in F), monocyte (40% in F) ↑ γ-glutamyl transpeptidase (157%* in M and 125%* in F at week 14), ↑albumin/globulin ratio (16%** in F at week 14 and 26) ↓ globulin (13% in F at week 26) ↑ relative liver weight (11%** in M) <u>Carcinogenicity phase:</u> ↓ bw (19%** in M & 13%** in F) ↓ bw gain (18%** in M & 27%** in F) ↓ food consumption (M&F) ↓ neutrophil (20% in M & 35% in F), monocytes (19% in M and 28% in F), white blood cell count (23% in F) **<u>Carcinogenicity</u>** Inpyrfluxam is not carcinogenic in rats
KCA 5.5/03 *Carcinogenicity Study in Mice*	**Mice/** [redacted] / M&F	0, 700, 2000 and 7000/5000 ppm	<u>Chronic toxicity</u>: 700 ppm (69.3	<u>Chronic toxicity:</u> 2000 ppm (210	**<u>Systemic chronic toxicity</u>** <u>Satellite group:</u>

OECD 451 (2009) GLP *Acceptable*		<u>Mean substance intakes</u> *Males (satellite group):* 0, 77.1, 240 and 826 mg/kg bw/day *(carcinogenicity group):* 0, 77, 224, and 775 mg/kg bw/day *Females: (chronic toxicity phase):* 0, 72.9, 222, and 790 mg/kg bw/day *(carcinogenicity phase):* 0, 69.3, 210, and 701 mg/kg bw/day	mg/kg bw/day) <u>Carcinogenicity:</u> 7000/5000 ppm (701 mg/kg bw/day) - top dose	mg/kg bw/day) <u>Carcinogenicity:</u> N/A	↓ bw (6% in M & 20%** in F) ↓ bw gain (23% in M & 35%** in F) coarse surface of the kidney in F centrilobular hepatocellular hypertrophy, diffuse luminal dilatation of proximal renal tubules in M <u>Carcinogenicity group:</u> ↓ bw (13%** in M & 11%** in F) ↓ bw gain (37%** in M & 24%** in F) centrilobular hepatocellular hypertrophy in M amyloid nephropathy in M&F amyloidosis in cervical lymph nodes, glandular stomach in F **<u>Carcinogenicity</u>** Inpyrfluxam is not carcinogenic in mice

Rat

In rats, inpyrfluxam was not carcinogenic up to the top dose of 1500/1000 ppm at which generalised and target organ toxicity occurred. The carcinogenicity NOAEL in the rat is therefore 1500/1000 ppm (65.8 mg/kg bw/day).

With regard to chronic toxicity, the main target organ was the liver. Increased liver weight with associated findings in clinical-chemistry (eg. increased γ-glutamyl transpeptidase, albumin/globulin ratio) and haematological (differential leukocyte count) parameters was seen at the top dose of 1500/1000 ppm (65.8 mg/ kg bw/day) in the chronic toxicity phase. Adverse effects on differential leukocyte count were also noted in the carcinogenicity phase. In addition to the toxic effects in the liver, decreases in body weight, body weight gain and food consumption were also observed at 65.8 mg/kg bw/day in both the chronic and carcinogenicity phase. The NOAEL for chronic toxicity in the rat is 500 ppm (19.4 mg/kg bw/day).

Mouse

In mice, inpyrfluxam was not carcinogenic up to the top dose of 7000/5000 ppm at which generalised and target organ toxicity occurred. The carcinogenicity NOAEL in the mice is therefore 7000/5000 ppm (701 mg/kg bw/day).

With regard to chronic toxicity, the main target organs were the liver and kidney. Increased liver and kidney weights were seen from the mid-dose of 2000 ppm (210 mg/kg bw/day) in the satellite group. These changes were associated with centrilobular hepatocellular hypertrophy, coarse surface of the kidney and diffuse luminal dilatation and hypertrophy of proximal renal tubules. Increases in centrilobular hepatocellular hypertrophy of the liver, amyloid nephropathy and amyloidosis in cervical lymph node and glandular stomach were also noted from 210 mg/kg bw/day in the carcinogenicity groups. In addition, decreases in body weight and body weight gain were observed from 210 mg/kg bw/day in both the satellite and carcinogenicity group. The NOAEL for chronic toxicity in the mouse is 700 ppm (69.3 mg/kg bw/day).

Overall conclusion

Overall, therefore, long term toxicity and carcinogenicity have been adequately investigated in studies in rats and mice. The substance is not carcinogenic in rats or mice. With regard to chronic toxicity, the critical target organ was the liver in both species with the kidney, cervix lymph nodes and stomach affected in mice. The most sensitive species to the long-term toxicity of inpyrfluxam is the rat, with a NOAEL of 19.4 mg/kg bw/day.
In addition, the following conclusions can be drawn:

- No classification for carcinogenicity is required (see aligned MCL dossier).
- The data requirements of assimilated Regulation 283/2013 have been met.

B.6.6. Reproductive Toxicity

The reproductive toxicity of inpyrfluxam has been investigated via the oral route of exposure in rats and rabbits. There were two generational toxicity studies (a preliminary one generation range finding study and a definitive two generation study in rats) and four developmental toxicity studies (two dose finding and two definitive developmental toxicity studies in rats; and two range finding and a definitive developmental toxicity study in rabbits).

B.6.6.1. Generational studies

1. Dose finding study in rats

The reproductive performance of inpyrfluxam has been investigated in the rat via the oral (dietary) route. A preliminary one-generation range-finding study was conducted prior to the definitive two-generation reproductive toxicity study. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.6.1/01

Report Title:	S-2399 Technical Grade: Dose Range-Finding Reproduction Toxicity Study in Rats
Author(s) & Year:	[redacted] (2015a)
Document No, Authority registration No	Study No. [redacted] 13-0080 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	No
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes, as a range finding study
Study relied upon:	Yes

Methods

Groups of 8 male and 8 female Wistar rats received inpyrfluxam by dietary administration at concentrations of 0, 300, 1000, 2000 or 4000 ppm over a period of 3 weeks before mating and until after weaning of F1 pups. At day 21 of lactation, F1 weanlings were given test substance-containing diets at the same concentrations as those given to their mothers until necropsy or observations for sexual development. The entire treatment period lasted approximately 15 weeks. The mean substance intakes at each dose level for males and females at various stages are presented in the table 6.6.1-1. The lowest values at each dose (in bold in the table) are taken forward to the identification of NOAELs and LOAELs.

The dose levels were selected based on a previous one-month oral toxicity study in rats described in Section B.6.3.1 ([redacted] 2014).

Table 6.6.1-1: Mean test substance intake in the rat range-finding reproductive toxicity study with inpyrfluxam

Dose level (ppm)	Males				Females			
	300	1000	2000	4000	300	1000	2000	4000

Pre-pairing period (mg/kg bw/day)	19.2	63.5	131	232	22.4	72	136	237
Breeding period (mg/kg bw/day)	**15.1**	**50.4**	**105**	**203**	-	-	-	-
Gestation period (mg/kg bw/day)	-	-	-	-	**20.4**	**68**	**132**	**254**
Lactation period ((mg/kg bw/day)	-	-	-	-	48.2	165	316	530

Results

Parental toxicity (F0)

At 4000 ppm, there were significant decreases in the body weights (throughout the treatment period) and body weight gains (throughout the treatment period in males; pre-mating and gestation periods, lactation days 0-14, and treatment weeks 0-11 in females) in both sexes. At 2000 ppm, significantly lower body weights (on gestation day 20 and lactation days 0, 4, 7, and 14) and body weight gains (during gestation days 0-20) were observed in females. These changes are considered treatment related and adverse.

Overall, adverse decreases were noted in body weights at 4000 ppm in both sexes, and in body weight gains from 2000 ppm in females and at 4000 ppm in males.

Table 6.6.1-2: Mean body weight (mean ± SD) of rats administered with inpyrfluxam in the diet for 15 weeks.

Study period	Week	Sex and dose level (ppm)				
		Males				
		0	300	1000	2000	4000
Pre-mating	0	263 ± 9	263 ± 9	263 ± 8	264 ± 9	264 ± 9
	1	293 ± 7	293 ± 11	291 ± 13	287 ± 13	264 ± 11** **(↓10%)**
	3	349 ± 16	346 ± 20	344 ± 18	340 ± 23	314 ± 11** **(↓10%)**
Breeding	4	368 ± 18	366 ± 16	358 ± 21	358 ± 28	327 ± 14** **(↓11%)**
	7	412 ± 24	409 ± 22	400 ± 24	402 ± 30	358 ± 12** **(↓13%)**
	9	434 ± 26	428 ± 25	417 ± 22	422 ± 32	371 ± 14** **(↓15%)**
After 10 weeks of treatment		442 ± 25	442 ± 28	430 ± 22	435 ± 35	380 ± 13** **(↓14%)**
Study period	**Week or day (d)**	**Females**				
		0	**300**	**1000**	**2000**	**4000**
Pre-mating	0	180 ± 5	180 ± 5	180 ± 5	180 ± 5	181 ± 5
	1	187 ± 5	192 ± 8	185 ± 6	181 ± 6	162 ± 10** **(↓13%)**
	3	208 ± 7	213 ± 13	206 ± 6	205 ± 7	195 ± 9* **(↓6%)**
Gestation	d 0	210 ± 7	218 ± 13	207 ± 5	206 ± 7	197 ± 6* **(↓6%)**

	d 7	241 ± 11	250 ± 13	241 ± 6	234 ± 8	220 ± 7** **(↓6%)**
	d 14	268 ± 13	277 ± 17	267 ± 7	257 ± 10	235 ± 10** **(↓9%)**
	d 21	333 ± 21	344 ± 21	327 ± 8	312 ± 12* **(↓6.3)**	274 ± 17** **(↓18)**
Lactation	d 0	252 ± 8	256 ± 21	239 ± 16	225 ± 12** **(↓10.7)**	202 ± 19** **(20%)**
	d 4	271 ± 12	282 ± 17	258 ± 11	238 ± 18** **(↓12.2)**	211 ± 12** **(↓22%)**
	d 7	280 ± 14	291 ± 19	273 ± 10	253 ± 14** **(↓9.6)**	220 ± 13** **(↓21%)**
	d 14	295 ± 15	300 ± 16	287 ± 8	266 ± 15** **(↓9.8)***	227 ± 13** **(↓23%)**
	d 21	276 ± 15	285 ± 15	272 ± 5	268 ± 15	224 ± 16** **(↓19%)**
After 11 weeks of treatment		252 ± 15	263 ± 10	246 ± 6	241 ± 13	214 ± 9** **(↓15%)**

*: p ≤ 0.05; **: p ≤ 0.01

Table 6.6.1-3: Mean body weight gain (g, mean ± SD) of rats administered with inpyrfluxam in the diet for 15 weeks.

Study period	Week	Sex and dose level (ppm)				
		Males				
		0	300	1000	2000	4000
Pre-mating	0-1	30 ± 5	30 ± 3	28 ± 8	24 ± 8	0 ± 13** **(↓100%)**
	0-3	85 ± 10	82 ± 14	81 ± 12	76 ± 19	50 ± 13** **(↓41%)**
Breeding	0-6	136 ± 15	134 ± 14	126 ± 19	126 ± 27	87 ± 14** **(↓36%)**
	0-9	171 ± 22	165 ± 19	154 ± 17	159 ± 27	107 ± 19** **(↓37%)**
Overall: weeks 0-10		179 ± 22	178 ± 21	167 ± 17	171 ± 30	116 ± 17**(↓35%)**
Study period	**Week or day (d)**	**Females**				
		0	**300**	**1000**	**2000**	**4000**
Pre-mating	0-1	7 ± 4	12 ± 6	5 ± 4	1 ± 7	-19 ± 8** **(↓371%)**
	0-3	28 ± 6	33 ± 10	26 ± 6	25 ± 5	14 ± 5** **(↓50%)**
Gestation	d 0-7	31 ± 6	33 ± 5	35 ± 7	28 ± 4	23 ± 4*(↓26%)**
	d 0-20	123 ± 15	126 ± 12	120 ± 11	106 ± 7* **(↓14%)**	78 ± 17** **(↓37%)**
Lactation	d 0-4	19 ± 6	26 ± 15	19 ± 9	14 ± 12	9 ± 12
	d 0-7	27 ± 8	35 ± 14	34 ± 9	28 ± 11	18 ± 11
	d 0-14	43 ± 12	44 ± 20	48 ± 13	42 ± 10	24 ± 13* **(↓44%)**
	d 0-21	23 ± 12	29 ± 16	33 ± 14	44 ± 10* **(↑91%)**	22 ± 18

Overall: weeks 0-11	72 ± 11	82 ± 8	65 ± 5	61 ± 12	32 ± 9** **(↓56%)**

*: p ≤ 0.05; **: p ≤ 0.01

At the high dose, there was a significant decrease in food consumption in males (at weeks 1, 2, 7,8,9 and 10) and females (throughout the treatment period). At 2000 ppm, a significant decrease in food consumption was noted in females at week 1 and during lactation days 7-14.

Overall, there were adverse effects on food consumption in males at the high dose and in females from 2000 ppm.

At 4000 ppm, dark coloured liver was noted in 3 males.

From 2000 ppm, a significant and adverse increase in relative liver weight (by ≥ 15%) was observed in males and at the top dose in females. From 2000 ppm, there was a significant decrease in absolute ovary weight (by ≥18%) in females. These changes in liver and ovary weight are considered treatment related and adverse.

At 4000 ppm, significant increases in relative testes and epididymis weights were recorded in males. These are considered secondary to decreased body weights and not specific.

Overall, there were adverse effects on liver weight from 2000 ppm in males and at 4000 ppm in females, and ovary weight from 2000 ppm in females.

Table 6.6.1-4: Summary of organ weights (mean ± SD) of parental rats (F0) administered inpyrfluxam in the diet for 15 weeks.

Organ		Sex and dose level (ppm)				
		Males				
		0 (n=8)	300 (n=8)	1000 (n=8)	2000 (n=8)	4000 (n=8)
Terminal body weight (g)		442 ± 25	442 ± 28	430 ± 22	435 ± 35	380 ± 13** **(↓14%)**
Liver	Absolute (mg)	12764 ± 1563	13581 ± 1138	13680 ± 1414	14621 ± 1520* **(↑15%)**	13618 ± 1062
	Relative %	2.89 ± 0.30	3.08 ± 0.16	3.18 ± 0.22* **(↑10%)**	3.36 ± 0.21** **(↑16%)**	3.58 ± 0.17** **(↑24%)**
Testes	Absolute (mg)	3479 ± 211	3613 ± 337	3402 ± 218	3401 ± 207	3550 ± 138
	Relative %	0.789 ± 0.060	0.820 ± 0.088	0.793 ± 0.056	0.786 ± 0.067	0.937 ± 0.049** **(↑19%)**
Epidid.	Absolute (mg)	1281 ± 63	1294 ± 95	1215 ± 65	1244 ± 82	1219 ± 86

	Relative %	0.290 ± 0.012	0.294 ± 0.030	0.283 ± 0.022	0.287 ± 0.019	0.322 ± 0.025* **(↑11%)**
Organ		**Females**				
		0 (n=8)	**300 (n=7)**	**1000 (n=8)**	**2000 (n=8)**	**4000 (n=7)**
Terminal body weight (g)		252 ± 15	263 ± 10	246 ± 6	241 ± 13	214 ± 9** **(↓15%)**
Liver	Absolute (mg)	9929 ± 1256	11070 ± 1216	10454 ± 1192	11326 ± 2644	10425 ± 2591
	Relative %	3.94 ± 0.31	4.21 ± 0.33	4.26 ± 0.43	4.67 ± 0.87	4.84 ± 1.08* **(↑23%)**
Ovary	Absolute (mg)	115.3 ± 14.2	109.7 ± 8.6	110.7 ± 12.9	94.2 ± 18.6* **(↓18%)**	81.8 ± 11.0** **(↓29%)**
	Relative %	0.0459 ± 0.0059	0.0418 ± 0.0037	0.0452 ± 0.0061	0.0391 ± 0.0075	0.0383 ± 0.0064
Uterus	Absolute (mg)	928 ± 104	935 ± 126	972 ± 192	884 ± 196[a]	713 ± 195[b]
	Relative %	0.370 ± 0.051	0.357 ± 0.054	0.395 ± 0.076	0.371 ± 0.078[a]	0.335 ± 0.100[b]

*: p ≤ 0.05; **: p ≤ 0.01; a: n=7. b: n=5

Reproductive toxicity

At 2000 ppm and 4000 ppm, a significant delay (by 4 days and 12 days respectively) in vaginal opening was observed in F1 weanlings. This is considered the consequence of the low body weights during the lactation period and the following growth period observed in these animals. This is supported by the fact that when vaginal opening was attained by these pups, there were no changes in body weights compared to controls and the lower dose F1 females.

Overall, there were no adverse changes in sexual maturation of the F1 females.

There were no other reproductive effects noted.

Table 6.6.1-7: Summary of sexual development in offspring (F1) females (mean ± SD) of rats administered inpyrfluxam in the diet for 15 weeks.

Parameter	Dose level (ppm)				
	0	300	1000	2000	4000
No. of females examined	24	20	22	24	15
Days of age at vaginal opening	31.2 ± 1.4	31.4 ± 2.2	32.6 ± 2.4	35.6 ± 3.6**	43.4 ± 8.3**
Body weight (g) at vaginal opening	101 ± 8	102 ± 11	101 ± 11	97 ± 15	90 ± 16
Body weight (g) at 26 days of age	74 ± 4	76 ± 4	70 ± 3	57 ± 6**	36 ± 8**

Body weight gain (g) – day 26 to day of vaginal opening	27 ± 8	26 ± 12	31 ± 12	40 ± 14**	54 ± 21**

**: p ≤ 0.01

Offspring toxicity (F1)

There were no clinical signs of toxicity during lactation days 0-14, although several pups were found dead or lost by cannibalism of a dam at the high dose. At 4000 (2 pups) and 2000 ppm (1 pup) enlargement of the eye was observed in pups during lactation days 15-21. The enlargement of the eye is considered treatment related and adverse due to occurrence in different litters.

At 2000 and 4000 ppm, there were significant decreases in the mean body weights of F1 male and female pups from lactation day 4. These effects are considered treatment-related and adverse.

Table 6.6.1-5: Summary of clinical findings during lactation days (0-21) in offspring (F1) of rats administered inpyrfluxam in the diet for 15 weeks.

Parameter		Dose level (ppm)				
		0	300	1000	2000	4000
No. of litters		8	7	8	8	7
Found dead (%,mean ± SD)	Day 0	0.8 ± 2.4	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 2.5	0.0 ± 0.0
Lost (%,mean ± SD)	Days 1-4	0.0 ± 0.0	1.3 ± 3.4	0.0 ± 0.0	0.0 ± 0.0	11.5 ± 27.1
Found dead (%,mean ± SD)	Days 5-7	0.0 ± 0.0	3.6 ± 9.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Found dead (%,mean ± SD)	Days 8-14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 4.7
Lost (%,mean ± SD)		0.0 ± 0.0	0.0 ± 0.0	1.6 ± 4.4	0.0 ± 0.0	6.5 ± 12.7
Lost (%,mean ± SD)	Days 15-21	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 4.4	0.0 ± 0.0	0.0 ± 0.0
Male pup weight (mean ± SD)	Day 0	5.7 ± 0.2	6.0 ± 0.3	5.6 ± 0.5	5.3 ± 0.5	5.4 ± 0.6
	Day 4	9.8 ± 0.7	10.3 ± 0.7	9.7 ± 1.4	7.2 ± 1.6**	7.8 ± 1.6*
	Day 7	16.1 ± 1.4	16.7 ± 0.9	15.6 ± 1.9	11.6 ± 2.5**	10.1 ± 2.7**
	Day 14	34.2 ± 1.9	35.7 ± 1.8	33.3 ± 2.7	25.7 ± 3.4**	18.6 ± 3.7**
	Day 21	55.8 ± 2.8	56.6 ± 2.8	52.7 ± 3.2	42.2 ± 4.7**	28.3 ± 4.0**
Female pup weight (mean ± SD)	Day 0	5.5 ± 0.2	5.7 ± 0.3	5.4 ± 0.5	5.1 ± 0.5	5.2 ± 0.6
	Day 4	9.3 ± 0.8	10.2 ± 1.0	9.2 ± 1.3	7.0 ± 1.6**	6.9 ± 1.7**
	Day 7	15.3 ± 1.3	16.1 ± 1.8	14.8 ± 1.7	11.2 ± 2.4**	8.6 ± 3.2**
	Day 14	33.1 ± 2.0	34.6 ± 2.6	31.7 ± 2.0	25.1 ± 3.5**	16.2 ± 4.7**

	Day 21	53.2 ± 2.6	54.0 ± 3.4	49.9 ± 2.6	40.8 ± 4.2**	25.6 ± 5.2**

*: p ≤ 0.05; **: p ≤ 0.01

Consistent with the findings at the clinical observations, at 4000 ppm, enlargement of the eye (2 pups) along with opacity of the eye (in one of them) was noted in males. At 2000 ppm, enlargement of the eye was noted in one female pup. Histopathological examination of the abnormal eyes revealed synechia and cataract in the female pup at 2000 ppm, and synechia, haemorrhage, cataract and/or retinal atrophy in the male pups at 4000 ppm.

Overall, histopathology was noted in the eyes of male pups at 4000 ppm and one female pup at 2000 ppm.

At the high dose, significant decreases in the absolute brain (by >10%) and relative spleen weights (by >25%) were noted in both sexes. From 2000 ppm, there were significant increases in relative brain weight (by >25%) in both sexes. Absolute thymus weight was significantly decreased from 2000 ppm in males (by >30%) and from 1000 ppm in females (by >15%). From 1000 ppm, significant reduction in absolute uterus weight was recorded in females. Given the magnitude and the dose response relationship, these changes were considered treatment related and adverse.

Overall, adverse effects were observed in spleen weight at 4000 ppm, brain weight from 2000 ppm in the pups of both sexes, thymus weight from 2000 ppm in male pups and from 1000 ppm in female pups, and uterus weight in female pups from 1000 ppm.

Table 6.6.1-6: Summary of organ weights (mean ± SD) in offspring (F1) of rats administered inpyrfluxam in the diet for 15 weeks.

Organ		Sex and dose level (ppm)				
		Males				
		0 (n=8)	300 (n=7)	1000 (n=8)	2000 (n=8)	4000 (n=6)
Terminal body weight (g)		81 ± 6	85 ± 3	76 ± 4	62 ± 7** **(↓23%)**	42 ± 6** **(↓48%)**
Brain	Absolute (mg)	1519 ± 55	1563 ± 51	1552 ± 42	1469 ± 46	1344 ± 43** **(↓12%)**
	Relative %	1.87 ± 0.09	1.84 ± 0.10	2.04 ± 0.08	2.40 ± 0.28** **(↑28%)**	3.28 ± 0.39** **(↑75%)**
Spleen	Absolute (mg)	340 ± 53	316 ± 22	282 ± 23*	225 ± 52** **(↓34%)**	122 ± 31** **(↓64%)**
	Relative %	0.417 ± 0.051	0.373 ± 0.026	0.371 ± 0.037	0.361 ± 0.059	0.290 ± 0.038** **(↓30%)**
Thymus	Absolute (mg)	298 ± 33	324 ± 36	279 ± 31	207 ± 31** **(↓31%)**	115 ± 20** **(↓61%)**

	Relative %	0.366 ± 0.023	0.382 ± 0.040	0.367 ± 0.045	0.333 ± 0.024	0.276 ± 0.022** **(↓25%)**
Organ		**Females**				
		0 (n=8)	**300 (n=7)**	**1000 (n=8)**	**2000 (n=8)**	**4000 (n=7)**
Terminal body weight (g)		75 ± 5	73 ± 4	69 ± 4	55 ± 5** **(↓27%)**	33 ± 8** **(↓56%)**
Brain	Absolute (mg)	1469 ± 37	1469 ± 46	1466 ± 42	1394 ± 55	1228 ± 122** **(↓16%)**
	Relative %	1.97 ± 0.12	2.02 ± 0.12	2.12 ± 0.10	2.55 ± 0.20** **(↑29%)**	3.84 ± 0.56** **(↑95%)**
Spleen	Absolute (mg)	282 ± 37	259 ± 29	257 ± 37	185 ± 33** **(↓34%)**	88 ± 20** **(↓69%)**
	Relative %	0.376 ± 0.038	0.353 ± 0.025	0.370 ± 0.052	0.335 ± 0.041	0.268 ± 0.019** **(↓29%)**
Thymus	Absolute (mg)	309 ± 40	280 ± 40	261 ± 40* **(↓16%)**	197 ± 30** **(↓36%)**	87 ± 35** **(↓72%)**
	Relative %	0.411 ± 0.033	0.382 ± 0.040	0.377 ± 0.054	0.357 ± 0.041	0.254 ± 0.048** **(↓38%)**
Uterus	Absolute (mg)	71.2 ± 10	74.7 ± 11.0	56.0 ± 7.6** **(↓21%)**	46.9 ± 10.7** **(↓34%)**	31.4 ± 5.9** **(↓56%)**
	Relative %	0.0958 ± 0.0182	0.1023 ± 0.0159	0.0807 ± 0.0102	0.0847 ± 0.0145	0.0982 ± 0.0221

*: $p \leq 0.05$; **: $p \leq 0.01$

Conclusion

In conclusion, in one-generation range-finding study in rats, dietary administration of inpyrfluxam over a period of 3 weeks before mating and until necropsy of F1 weanlings (at 26 days of age) at dietary concentrations of 0, 300, 1000, 2000 or 4000 ppm (15.1, 50.4, 105 or 203 mg/kg bw/day in males and 20.4, 68, 132 or 254 mg/kg bw/day in females) caused adverse effects mainly from 2000 ppm (105 mg/kg bw/ day) in parents (F0), including effects on body weight gain, food consumption, liver and ovary weights. From 2000 ppm, there were adverse effects in offspring (F1) including effects on body weight, eyes, and brain weights. Moreover, the thymus and uterus weights were affected in offspring (F1) from 1000 ppm (50.4 mg/kg bw/day).

As this was a range finding non-GLP study, no robust points of departure can be set. Based on the observed results, a dose level of less than 2000 ppm (105/132 mg/kg bw/day) was considered a suitable top dose for the subsequent definitive reproductive toxicity study in rats.

[redacted] (2015a)

2. Two generation reproductive toxicity study

The reproductive performance of inpyrfluxam has been investigated in a two-generation reproductive toxicity study in the rat via the oral (dietary) route. The study was performed according to GLP and OECD test guidelines.

Reference:	KCA 5.6.1/02
Report Title:	S-2399 Technical Grade: Reproduction Toxicity Study in Rats
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. [redacted] 15-0018 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Acceptable method of analysis available for dietary formulation
Guideline(s):	OECD Test guideline 416 (2001)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 24 male and 24 female Wistar Hannover rats received inpyrfluxam by dietary administration at concentrations of 0, 150, 500 or 1250 (females) / 2000 (males) ppm for two successive generations. The mean substance intakes at each dose level for males and females at various stages are presented in the table 6.6.2-1. The lowest values at each dose (in bold in the table) are taken forward to the identification of NOAELs and LOAELs.

Table 6.6.1-8: Mean test substance intake in the rat reproductive toxicity study with inpyrfluxam

Dose level (ppm)	Males			Females		
	150	500	2000	150	500	1250
F0-Pre-pairing period (mg/kg bw/day)	9.38	31.3	124	**10.9**	**35.5**	**86**
F0-overall (weeks 1-17) (mg/kg bw/day)	**8.34**	**27.8**	**113**	12.9	42.9	106
F1-Pre-pairing period (mg/kg bw/day)	11.6	38.7	156	12.2	41.4	103
F1-overall (weeks 1-17) (mg/kg bw/day)	10.1	33.1	136	13.7	46.3	116

The dose levels were selected based on a previous dose finding reproductive toxicity study ([redacted] 2015a) described above.

In accordance with OECD test guideline 416, all animals were subjected to the required investigations. Analysis of the dietary formulation was performed within the study.

Results

Parental toxicity

F0

At the high dose, there were decreases in mean body weights and body weight gains of both F0 parental male and female (statistically significant from week 2 for body weight and from week 1 for body weight gain) animals throughout the treatment period. These decreases were more severe in females than in males and are considered treatment related and adverse.

Table 6.6.1-9: Summary of body weight (g) in F0 parental animals (mean ± SD) (n=24 unless otherwise specified) administered with inpyrfluxam in the diet for 18 weeks.

Study period	Week	Sex and dose level (ppm)			
		Males			
		0	150	500	2000
Pre-mating	0	162 ± 6	162 ± 6	162 ± 6	162 ± 6
	1	209 ± 9	210 ± 8	209 ± 9	205 ± 10
	2	254 ± 13	256 ± 10	255 ± 12	247 ± 13
	3	288 ± 18	295 ± 11	292 ± 15	280 ± 17
	4	313 ± 20	321 ± 12	320 ± 17	303 ± 22
	5	336 ± 23	346 ± 13	340 ± 21	322 ± 26
	6	355 ± 25	365 ± 13	359 ± 24	338 ± 29
	7	366 ± 27	378 ± 14	372 ± 27	349 ± 30
	8	381 ± 28	394 ± 16	388 ± 29	363 ± 32
	9	389 ± 30	404 ± 17	399 ± 31	372 ± 32
	10	398 ± 31	414 ± 18	409 ± 33	381 ± 33
Breeding	11	406 ± 32	421 ± 19	414 ± 32	391 ± 33
	12	413 ± 33	429 ± 19	423 ± 34	401 ± 34

	13	420 ± 34	436 ± 20	428 ± 34	407 ± 34
	14	426 ± 36	440 ± 19	432 ± 35	410 ± 35
	15	437 ± 38	447 ± 19	442 ± 36	420 ± 36
	16	445 ± 39	453 ± 20	449 ± 37	426 ± 37
	17	452 ± 39	459 ± 20	455 ± 37	433 ± 37
After 18 weeks of treatment		450 ± 39	459 ± 21	458 ± 37	434 ± 38
Study period	**Week or day (d)**	**Females**			
		0	**150**	**500**	**1250**
Pre-mating	0	133 ± 5	133 ± 5	133 ± 5	133 ± 5
	1	152 ± 6	154 ± 6	155 ± 7	148 ± 7
	2	173 ± 10	172 ± 7	173 ± 9	164 ± 8**
	3	188 ± 10	187 ± 8	189 ± 11	178 ± 9**
	4	199 ± 10	199 ± 8	201 ± 12	188 ± 11**
	5	209 ± 11	207 ± 9	210 ± 14	196 ± 12**
	6	220 ± 11	217 ± 9	218 ± 13	205 ± 13**
	7	225 ± 13	222 ± 9	224 ± 14	210 ± 14**
	8	233 ± 13	231 ± 9	232 ± 14	216 ± 15**
	9	238 ± 14	238 ± 10	237 ± 15	222 ± 14**
	10	244 ± 14	243 ± 10	243 ± 15	226 ± 14**
Gestation	d 0	244 ± 14	244 ± 10	244 ± 16	223 ± 14**
	d 7	266 ± 12	265 ± 9	263 ± 14	250 ± 15**
	d 14	291 ± 12	288 ± 11	289 ± 14	277 ± 18**
	d 20	355 ± 15	348 ± 17	351 ± 18	337 ± 25**
Lactation	d 0	268 ± 13	270 ± 13	269 ± 16	252 ± 16**
	d 4	292 ± 13	291 ± 12	288 ± 14	273 ± 19**
	d 7	297 ± 14	296 ± 13	296 ± 13	281 ± 17**
	d 14	314 ± 13	311 ± 12	306 ± 15	292 ± 17** (n=23)
	d 21	293 ± 10	293 ± 10	291 ± 13	287 ± 16
After 18 weeks of treatment		279 ± 14	278 ± 12	271 ± 12	263 ± 21**

*: p ≤ 0.05; **: p ≤ 0.01

Table 6.6.1-10: Summary of body weight gain (g) in F0 parental animals (mean ± SD) administered with inpyrfluxam in the diet for 18 weeks.

Study period	**Week**	**Sex and dose level (ppm)**			
		Males			
		0	**150**	**500**	**2000**
Pre-mating	0-1	47 ± 4	48 ± 5	48 ± 4	43 ± 5*
	0-2	92 ± 9	94 ± 7	93 ± 8	85 ± 9*
	0-5	174 ± 20	184 ± 13	179 ± 18	160 ± 23
	0-10	237 ± 28	252 ± 18	247 ± 31	220 ± 31
Breeding	0-11	245 ± 28	260 ± 19	252 ± 29	229 ± 31
	0-14	264 ± 32	278 ± 20	270 ± 32	248 ± 33
	0-17	290 ± 35	298 ± 21	293 ± 34	271 ± 35
Weeks 0-18		288 ± 35	298 ± 22	296 ± 34	272 ± 35
Study period	**Week or day (d)**	**Females**			
		0	**150**	**500**	**1250**
Pre-mating	0-1	19 ± 5	21 ± 4	21 ± 4	15 ± 5**
	0-2	40 ± 7	39 ± 5	40 ± 6	31 ± 6**

	0-5	76 ± 8	75 ± 7	76 ± 12	63 ± 10**
	0-10	111 ± 12	110 ± 8	110 ± 13	93 ± 11**
Gestation	d 0-7	23 ± 5	21 ± 6	20 ± 6	27 ± 5*
	d 0-14	48 ± 7	44 ± 9	46 ± 10	54 ± 8*
	d 0-20	112 ± 10	105 ± 17	107 ± 16	114 ± 16
Lactation	d 0-4	24 ± 10	21 ± 14	19 ± 12	21 ± 10
	d 0-7	30 ± 11	26 ± 16	27 ± 12	29 ± 10
	d 0-14	46 ± 12	41 ± 15	37 ± 12*	41 ± 13
	d 0-21	25 ± 14	22 ± 11	22 ± 11	35 ± 14*
Weeks 0-18		146 ± 13	145 ± 11	138 ± 11	130 ± 19**

*: p ≤ 0.05; **: p ≤ 0.01

At the high dose, there were significant decreases in the food consumption in weeks 1 and 5 in males, and weeks 1, 2, 4, 5, 6 and 8 and on lactation days 7-14 in females. These decreases are considered treatment related and adverse.

At the high dose, there were adverse increases in and relative liver weights (by 16.8 % in females). At the high dose, there were adverse and statistically significant increases in both absolute (by 26.1%) and relative (by 33.3%) thyroid weights in females.

Statistically significant increases in relative kidney weight from mid dose and relative brain weight at high dose were noted in females. The increases in kidney weights were small (5%) and showed no dose-response. Therefore, they are considered to be incidental. In the absence of histopathological findings, the change in brain weight is considered as the consequence of the decreased terminal body weight and not adverse.

Overall, there were adverse effects noted in liver and thyroid weight in females at the top dose of 1250 ppm.

Table 6.6.1-11: Summary of selected organ weights in F0 parental animals (mean ± SD; n=24) administered with inpyrfluxam in the diet for 18 weeks.

Organ		Sex and dose level (ppm)			
		Males			
		0	150	500	2000
Terminal body weight (g)		450 ± 39	459 ± 21	458 ± 37	434 ± 38
Liver	Absolute (mg)	12676 ± 1185	13259 ± 1338	13559 ± 1421	13639 ± 1322* **(↑7.6%)**
	Relative %	2.82 ± 0.11	2.88 ± 0.21	2.96 ± 0.14** **(↑5.0%)**	3.15 ± 0.21** **(↑11.7%)**
Thyroid	Absolute (mg)	25.2 ± 3.9	25.4 ± 3.5	24.5 ± 3.5	26.3 ± 3.7
	Relative %	0.00561 ± 0.00081	0.00553 ± 0.00069	0.00535 ± 0.00074	0.00607 ± 0.00082
Kidneys	Absolute (mg)	2761 ± 297	2779 ± 185	2836 ± 294	2775 ± 269
	Relative %	0.614 ± 0.051	0.605 ± 0.032	0.619 ± 0.037	0.640 ± 0.044
Brain	Absolute (mg)	1993 ± 78	1984 ± 59	2031 ± 57	2000 ± 79
	Relative %	0.445 ± 0.034	0.433 ± 0.021	0.446 ± 0.040	0.464 ± 0.040
Organ		Females			
		0	150	500	1250

Terminal body weight (g)		279 ± 14	278 ± 12	271 ± 12	263 ± 21** **(↓5.7%)**
Liver	Absolute (mg)	11316 ± 1540	11472 ± 1365	11358 ± 1378	12501 ± 2107* **(↑10.5%)**
	Relative %	4.05 ± 0.42	4.12 ± 0.39	4.19 ± 0.46	4.73 ± 0.54** **(↑16.8%)**
Thyroid	Absolute (mg)	18.8 ± 3.5	19.5 ± 2.9	19.2 ± 2.8	23.7 ± 3.9** **(↑26.1%)**
	Relative %	0.00675 ± 0.00113	0.00702 ± 0.00105	0.00709 ± 0.00088	0.00900 ± 0.00137** **(↑33.3%)**
Kidneys	Absolute (mg)	2117 ± 139	2169 ± 161	2170 ± 132	2104 ± 180
	Relative %	0.760 ± 0.042	0.780 ± 0.046	0.802 ± 0.047** **(↑5.5%)**	0.801 ± 0.047** **(↑5.4%)**
Brain	Absolute (mg)	1842 ± 54	1873 ± 66	1854 ± 56	1833 ± 70
	Relative %	0.663 ± 0.038	0.674 ± 0.036	0.685 ± 0.032	0.700 ± 0.053** **(↑5.6%)**

*: p ≤ 0.05; **: p ≤ 0.01

At the high dose, there was an increase in diffuse hepatocyte hypertrophy in males, but this was not considered adverse as it did not result in increased liver weight > 15%. Thyroid follicular hypertrophy was noted in the high dose females.

Hyaline droplets in the proximal tubules of the kidney were seen in males at 2000 ppm. Based on the results of the 90-day toxicity study, the change in kidney is considered to be related to renal deposition of male rat specific α2μ-globulin (section B.6.3.2 - [redacted] 2016). These findings are male rat specific, and hence not relevant to humans and are not considered further.

Overall, adverse histopathological findings of relevance to humans were observed in the thyroid in females at the high dose.

Table 6.6.1-12: Summary of histopathological changes in F0 parental animals successfully yielding litters (No. affected animals / No. of animals examined) administered with inpyrfluxam in the diet for 18 weeks.

Organ & Lesion	Sex and dose level (ppm)			
	Males			
	0	150	500	2000
Liver				
Hypertrophy, hepatocyte, diffuse	0/24	0/23	0/24	**7/24****
Kidney				
Deposition, hyaline droplet, proximal tubular cell, increased	0/24	0/23	0/24	**2/24**
Thyroid				
Hypertrophy, follicular cell	0/24	0/0	0/0	0/24
Organ & Lesion	**Females**			
	0	**150**	**500**	**1250**

Liver				
Hypertrophy, hepatocyte, diffuse	0/24	0/0	0/0	0/24
Kidney				
Deposition, hyaline droplet, proximal tubular cell, increased	0/24	0/0	0/0	0/24
Thyroid				
Hypertrophy, follicular cell	3/24	1/23	0/24	**16/24****

**: p ≤ 0.01

F1

At the high dose, there were decreases in body weight and bodyweight gains throughout the treatment period in both sexes. These are considered treatment related and adverse.

Table 6.6.1-13: Summary of body weight (g) in F1 parental animals (mean ± SD) (n=24 unless otherwise specified) administered with inpyrfluxam in the diet for 18 weeks.

Study period	Week	Sex and dose level (ppm)			
		Males			
		0	150	500	2000
Pre-mating	0	68 ± 8	70 ± 8	67 ± 7	66 ± 8
	1	111 ± 12	113 ± 12	110 ± 11	103 ± 11*
	2	162 ± 13	164 ± 15	161 ± 15	149 ± 14**
	3	211 ± 17	211 ± 18	209 ± 17	193 ± 17**
	4	261 ± 20	260 ± 21	258 ± 20	237 ± 20**
	5	303 ± 21	301 ± 20	298 ± 21	274 ± 21**
	6	337 ± 23	334 ± 20	331 ± 25	304 ± 24**
	7	362 ± 25	359 ± 20	357 ± 28	327 ± 27**
	8	386 ± 27	383 ± 21	380 ± 31	350 ± 31**
	9	403 ± 29	399 ± 23	397 ± 34	365 ± 34**
	10	417 ± 31	412 ± 24 (n=23 up to termination)	411 ± 35	378 ± 36**
Breeding	11	426 ± 31	422 ± 24	422 ± 38	387 ± 35**
	12	435 ± 33	432 ± 25	431 ± 37	399 ± 35**
	13	448 ± 35	443 ± 27	443 ± 37	413 ± 35**
	14	459 ± 37	453 ± 26	454 ± 39	421 ± 36**
	15	467 ± 39	460 ± 28	463 ± 40	429 ± 36**
	16	475 ± 40	468 ± 29	471 ± 41	436 ± 35**
	17	482 ± 41	474 ± 30	480 ± 42	443 ± 37**
After 18 weeks of treatment		486 ± 42	477 ± 30	482 ± 43	444 ± 37**
Study period	**Week or day (d)**	**Females**			
		0	**150**	**500**	**1250**
Pre-mating	0	65 ± 7	67 ± 7	63 ± 6	62 ± 7
	1	100 ± 10	101 ± 8	97 ± 9	94 ± 9
	2	135 ± 10	133 ± 10	130 ± 10	126 ± 10**
	3	158 ± 13	154 ± 10	154 ± 11	148 ± 11**
	4	179 ± 15	176 ± 11	173 ± 13	166 ± 13**
	5	197 ± 16	193 ± 12	193 ± 15	181 ± 13**

	6	211 ± 17	206 ± 12	205 ± 16	192 ± 13**
	7	220 ± 18	215 ± 12	214 ± 17	200 ± 15**
	8	231 ± 19	225 ± 12	225 ± 18	208 ± 15**
	9	238 ± 19	234 ± 14	232 ± 20	216 ± 15**
	10	244 ± 19	239 ± 14	240 ± 20	220 ± 15**
Gestation	d 0	249 ± 18 (n=21 up to termination)	241 ± 15 (n=23 up to termination)	239 ± 19 (n=22 up to termination)	219 ± 14** (n=22 up to termination)
	d 7	264 ± 15	258 ± 15	257 ± 20	243 ± 16**
	d 14	290 ± 16	283 ± 17	280 ± 21	268 ± 19**
	d 20	349 ± 21	344 ± 20	339 ± 26	323 ± 25**
Lactation	d 0	273 ± 19	264 ± 19	267 ± 19	248 ± 18**
	d 4	294 ± 19	289 ± 20	285 ± 23	264 ± 20**
	d 7	297 ± 17	293 ± 19	289 ± 20	272 ± 19**
	d 14	312 ± 16	308 ± 17	302 ± 21	283 ± 19**
	d 21	290 ± 18	288 ± 16	287 ± 20	281 ± 17
After 18 weeks of treatment		277 ± 20	277 ± 15	273 ± 20	260 ± 21*

*: p ≤ 0.05; **: p ≤ 0.01

Table 6.6.2-14: Summary of body weight gain (g) in F1 parental animals (mean ± SD) administered with inpyrfluxam in the diet for 18 weeks.

Study period	Week	Sex and dose level (ppm)			
		Males			
		0	150	500	2000
3Pre-mating	0-1	43 ± 5	43 ± 5	43 ± 5	37 ± 4**
	0-2	94 ± 8	93 ± 8	94 ± 8	83 ± 8**
	0-5	235 ± 18	230 ± 15	231 ± 16	208 ± 16**
	0-10	349 ± 28	342 ± 19	345 ± 30	312 ± 32**
Breeding	0-11	358 ± 28	352 ± 20	356 ± 33	321 ± 32**
	0-14	391 ± 34	383 ± 22	387 ± 34	355 ± 33**
	0-17	414 ± 38	404 ± 27	413 ± 37	377 ± 34**
Weeks 0-18		418 ± 39	407 ± 27	416 ± 37	378 ± 34**
Study period	**Week or day (d)**	**Females**			
		0	**150**	**500**	**1250**
Pre-mating	0-1	35 ± 4	34 ± 3	34 ± 5	33 ± 4*
	0-2	70 ± 7	66 ± 6*	67 ± 7	64 ± 6**
	0-5	132 ± 14	126 ± 9	129 ± 13	119 ± 10**
	0-10	180 ± 18	172 ± 12	177 ± 18	159 ± 14**
Gestation	d 0-7	15 ± 7	17 ± 4	17 ± 7	24 ± 6**
	d 0-14	41 ± 10	42 ± 7	41 ± 10	49 ± 7*
	d 0-20	100 ± 17	103 ± 11	100 ± 13	104 ± 13
Lactation	d 0-4	21 ± 10	25 ± 9	18 ± 7	15 ± 9
	d 0-7	24 ± 9	30 ± 10	23 ± 7	24 ± 11
	d 0-14	39 ± 13	44 ± 12	35 ± 8	35 ± 10
	d 0-21	17 ± 14	24 ± 12	21 ± 9	32 ± 12**
Weeks 0-18		211 ± 20	210 ± 14	211 ± 19	199 ± 20

*: p ≤ 0.05; **: p ≤ 0.01

At the high dose, there were significant decreases in the food consumption at weeks 1-7 in males, and at weeks 6-8, and on lactation days 7-14 in females. These decreases are considered treatment related and adverse.

From the mid dose, there were statistically significant increases in both absolute (by 9.3% at the mid dose and 17.7% at the high dose) and relative (by 11.1% at the mid dose and 25.1% at the high dose) liver weights in females. Males at the high dose showed a statistically significant increase in relative liver weight (by 7.8%). Only the increase at the top dose in females is considered adverse as it is >15% [3].

[3] TOX-TAB template refined with Annex (europa.eu)

At the high dose, there were statistically significant increases in the absolute (by 26.7%) and relative (by 35.2%) thyroid weights in females. Given the magnitude of the changes and the presence of histopathological findings, the changes in thyroid weights are considered treatment related and adverse. There was a statistically significant increase in relative brain weights at the high dose in females. However, given the small magnitude (7%) and the lack of associated histopathology, it is not considered adverse.

Overall, there were adverse increases in liver and thyroid weight at the top dose in females.

Table 6.6.1-15: Summary of selected organ weights in F1 parental animals (mean ± S; n=24) administered with inpyrfluxam in the diet for 18 weeks.

Organ		Sex and dose level (ppm)			
		Males			
		0 (n=24)	150 (n=23)	500 (n=24)	2000 (n=24)
Terminal body weight (g)		486 ± 42	477 ± 30	482 ± 43	444 ± 37** **↓8.6%**
Liver	Absolute (mg)	14999 ± 1831	14991 ± 1218	15443 ± 1627	14789 ± 1265
	Relative %	3.09 ± 0.25	3.15 ± 0.19	3.20 ± 0.21	3.33 ± 0.17** **↑7.8%**
Thyroid	Absolute (mg)	26.0 ± 4.2	24.7 ± 2.4	25.5 ± 3.2	25.9 ± 4.3
	Relative %	0.00536 ± 0.00074	0.00520 ± 0.00061	0.00532 ± 0.00076	0.00583 ± 0.00091
Kidneys	Absolute (mg)	2773 ± 276	2853 ± 185	2818 ± 194	2812 ± 271
	Relative %	0.572 ± 0.040	0.601 ± 0.055	0.586 ± 0.031	0.634 ± 0.041** **↑10.8%**
Brain	Absolute (mg)	2015 ± 74	2044 ± 71	2020 ± 86	2007 ± 75

	Relative %	0.418 ± 0.036	0.430 ± 0.030	0.422 ± 0.037	0.454 ± 0.035** **↑8.6%**
Adrenals	Absolute (mg)	59.8 ± 7.5	62.1 ± 6.6	62.6 ± 10.2	59.8 ± 7.4
	Relative %	0.01235 ± 0.00149	0.01304 ± 0.00138	0.01299 ± 0.00191	0.01350 ± 0.00152* **↑9.3%**
Testes	Absolute (mg)	3655 ± 281	3729 ± 305	3697 ± 268	3794 ± 229
	Relative %	0.751 ± 0.060	0.785 ± 0.072	0.764 ± 0.072	0.861 ± 0.087** **↑14.6%**
Epididymis	Absolute (mg)	1221 ± 103	1251 ± 100	1224 ± 86	1276 ± 79
	Relative %	0.251 ± 0.025	0.263 ± 0.025	0.253 ± 0.025	0.290 ± 0.030** **↑16%**
Seminal vesicles	Absolute (mg)	1790 ± 221	1885 ± 187	1829 ± 170	1882 ± 163
	Relative %	0.371 ± 0.054	0.397 ± 0.044	0.382 ± 0.052	0.425 ± 0.036** **↑14.6%**
Organ		**Females**			
		0 (n=21)	**150 (n=23)**	**500 (n=22)**	**1250 (n=22)**
Terminal body weight (g)		277 ± 20	277 ± 15	273 ± 20	260 ± 21* **↓6.1%**
Liver	Absolute (mg)	11055 ± 1603	11575 ± 1016	12088 ± 1740* **↑9.3%**	13015 ± 2329** **↑17.7%**
	Relative %	3.98 ± 0.36	4.19 ± 0.35	4.42 ± 0.49** **↑11.1%**	4.98 ± 0.55** **↑25.1%**
Thyroid	Absolute (mg)	19.5 ± 2.4	20.4 ± 2.9	20.7 ± 1.8	24.7 ± 3.7** **↑26.7%**
	Relative %	0.00705 ± 0.00069	0.00739 ± 0.00103	0.00760 ± 0.00065	0.00953 ± 0.00125** **↑35.2%**
Kidneys	Absolute (mg)	2162 ± 199	2148 ± 125	2196 ± 195	2125 ± 241
	Relative %	0.781 ± 0.044	0.778 ± 0.049	0.805 ± 0.054	0.816 ± 0.049
Brain	Absolute (mg)	1843 ± 76	1850 ± 54	1860 ± 56	1850 ± 80
	Relative %	0.668 ± 0.043	0.670 ± 0.039	0.684 ± 0.045	0.715 ± 0.049** **↑7.0%**
Adrenals	Absolute (mg)	83.1 ± 9.5	82.9 ± 9.3	85.0 ± 10.1	79.9 ± 9.7
	Relative %	0.0301 ± 0.0033	0.0301 ± 0.0042	0.0312 ± 0.0030	0.0308 ± 0.0031
Spleen	Absolute (mg)	539 ± 42	560 ± 80	594 ± 54* **↑10.2%**	522 ± 63
	Relative %	0.195 ± 0.019	0.203 ± 0.031	0.218 ± 0.018** **↑11.8%**	0.201 ± 0.019

*: $p \leq 0.05$; **: $p \leq 0.01$

At the high dose, there were increases in diffuse hepatocyte hypertrophy of the liver and hyaline droplet deposition in the proximal tubular cell of the kidney in males. The kidney findings are not relevant to humans. The liver hypertrophy in males was not considered adverse as it did not result in increased liver weight of >15%. Thyroid follicular hypertrophy was noted in the high dose females.

Overall, there were adverse histopathological findings in the thyroid at the high dose in females.

Table 6.6.1-16: Summary of histopathological changes in F1 parental animals successfully yielding litters (No. affected animals / No. of animals examined) administered with inpyrfluxam in the diet for 18 weeks.

Organ & Lesion	Sex and dose level (ppm)			
	Males			
	0	150	500	2000
Liver				
Hypertrophy, hepatocyte, diffuse	0/21	0/21	0/22	**11/22****
Kidney				
Deposition, hyaline droplet, proximal tubular cell, increased	1/21	0/21	0/22	**7/22***
Thyroid				
Hypertrophy, follicular cell	0/21	0/0	0/0	0/22
Organ & Lesion	Females			
	0	150	500	1250
Liver				
Hypertrophy, hepatocyte, diffuse	0/21	0/0	0/0	0/22
Kidney				
Deposition, hyaline droplet, proximal tubular cell, increased	0/21	0/0	0/0	0/22
Thyroid				
Hypertrophy, follicular cell	0/21	0/22	0/22	**10/22****

*: $p \leq 0.05$; **: $p \leq 0.01$

Reproductive toxicity

No adverse changes were noted in the reproductive performance of F0 or F1 parental animals.

Table 6.6.1-17: Summary of reproductive parameters in F1 parental animals successfully yielding litters administered with inpyrfluxam in the diet for 18 weeks.

Parameter	Dose level (ppm)

		0	150	500	1250/2000
Oestrus cycle					
Length (days, mean ± SD)		4.1 ± 0.3	4.1 ± 0.2	4.3 ± 0.4	4.2 ± 0.4
Regularity of oestrus cycles – fraction and %		22/24 91.7	24/24 100.0	24/24 100.0	23/24 95.8
Mating index					
Male – fraction and %		24/24 100.0	23/24 95.8	24/24 100.0	24/24 100.0
Female – fraction and %		24/24 100.0	24/24 100.0	24/24 100.0	24/24 100.0
Number of days until mating (mean ± SD)		1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.6	1.1 ± 0.3
Fertility index – fraction and %		21/24 87.5	23/24 95.8	22/24 91.7	22/24 91.7
Gestation index – fraction and %		21/21 100.0	23/23 100.0	22/22 100.0	22/22 100.0
Duration of gestation (days; mean ± SD)		22.0 ± 0.2	22.1 ± 0.3	22.0 ± 0.2	22.0 ± 0.0
No. of implantation sites (mean ± SD)		13.1 ± 2.3	13.3 ± 1.3	12.8 ± 2.0	12.0 ± 1.8*
No. of pups delivered (mean ± SD)		11.8 ± 2.4	12.5 ± 1.6	11.6 ± 2.0	11.3 ± 1.7
Viability index (%, mean ± SD)	Day 0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Day 4	100.0 ± 0.0	99.7 ± 1.4	99.3 ± 3.3	99.6 ± 1.8
	Day 7	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Day 14	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
	Day 21	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Sex ratio		0.474	0.465	0.514	0.435

*: p ≤ 0.05

At the high dose, there were statistically significant decreases in body weights (by 5%) and a delay (by 1.6 days) in completion of preputial separation in F1 weanlings. The delay in completion of preputial separation is considered the consequence of the low body weights and hence not a specific reproductive effect.

Table 6.6.1-18: Summary of sexual maturation of F1 parental animals successfully yielding litters administered with inpyrfluxam in the diet for 18 weeks.

Parameter	Dose level (ppm)			
	0	150	500	1250/2000
Completion of preputial separation				
Days of age	42.1 ± 2.2	42.2 ± 2.1	43.3 ± 1.8	43.7 ± 2.1*
Body weight (g) at completion	196 ± 12	197 ± 18	203 ± 13	186 ± 16*
Completion of vaginal opening				
Days of age	32.5 ± 2.0	31.6 ± 2.1	32.3 ± 1.5	33.9 ± 2.5

Body weight (g) at completion	112 ± 14	107 ± 9	109 ± 7	111 ± 12

*: p ≤ 0.05

Offspring toxicity (F1 and F2)

At the high dose, there was a statistically significant decrease in body weight during lactation days 14 and 21 (by 5.8% and 6.2% in F1 male pups and by 6.6 % and 6.9% in F1 female pups; by 7.2% and 8.2% in F1 male pups and by 6.3% and 7.7% in F1 female pups). This is considered treatment related and adverse.

At the high dose, there was an appearance of thread like tail (external malformation) in one F1 pup in one litter. This change was not present in F2 pups, and a similar finding was noted in the historical controls at the test facility, therefore it is not considered treatment related.

Overall, there were no gross pathological observations in any group.

Table 6.6.1-19: Summary of percent litter incidences of macroscopic findings in F1 and F2 pups (mean ± SD) of rats administered with inpyrfluxam in the diet for 18 weeks.

Time point (lactation days)	Findings	Dose level (ppm)			
		0	150	500	1250
F1					
0-4	No. of litters examined	24	21	23	23
	No abnormality detected	80.8 ± 20.5	80.2 ± 18.1	77.3 ± 21.7	71.0 ± 25.0
	Malpositioned subclavian branch (right)	0.0 ± 0.0	0.0 ± 0.0	4.3 ± 20.9	0.0 ± 0.0
	Hepatodiaphragmatic nodule	0.8 ± 4.1	1.2 ± 5.5	0.0 ± 0.0	0.0 ± 0.0
	Liver: white spots	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 7.2	0.0 ± 0.0
	Persistent left umbilical artery	18.4 ± 20.9	19.8 ± 18.1	18.4 ± 16.1	27.4 ± 24.5
	Thread-like tail	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 5.2
	Partly cannibalised	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 2.6
	Autolysis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 2.6
	No. of litters examined	24	24	24	24
	No abnormality detected	93.2 ± 10.8	94.3 ± 11.8	97.9 ± 5.6	96.4 ± 10.4
	Sebum on eyelid	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 3.4
	Bend tail	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 3.4
	Dilation of renal pelvis	6.8 ± 10.8	4.9 ± 11.5	2.1 ± 5.6	2.3 ± 8.2
F2					
0-4	No. of litters examined	19	23	21	21

	No abnormality detected	80.4 ± 19.1	79.2 ± 24.8	87.9 ± 24.3	79.4 ± 25.8
	Persistent left umbilical artery	19.6 ± 19.1	20.8 ± 24.8	12.1 ± 24.3	20.6 ± 25.8
5-21	No. of litters examined	21	23	22	22
	No abnormality detected	93.5 ± 8.5	96.7 ± 7.7	97.7 ± 6.3	97.7 ± 4.9
	Dilation of renal pelvis	6.5 ± 8.5	3.3 ± 7.7	2.3 ± 6.3	2.3 ± 4.9

At the high dose, a number of organs (brain and spleen in F1 and F2 weanlings and thymus and uterus in F2 weanlings only) weights were affected. However, given the small magnitude of the change and the absence of associated histopathology, these are not considered adverse.

Table 6.6.1-20: Summary of organ weights in F1 weanlings (mean ± SD) of rats administered with inpyrfluxam in the diet for 18 weeks.

Organ		Sex and dose level (ppm)			
		Males			
		0	150	500	1250
Terminal body weight (g)		83 ± 7	86 ± 4	82 ± 5	79 ± 4* **(↓4.8%)**
Brain	Absolute (mg)	1524 ± 52	1535 ± 45	1525 ± 45	1522 ± 45
	Relative %	1.84 ± 0.14	1.78 ± 0.08	1.86 ± 0.11	1.93 ± 0.11* **(↑4.95%)**
Spleen	Absolute (mg)	317 ± 61	343 ± 59	333 ± 45	287 ± 26* **(↓9.5%)**
	Relative %	0.378 ± 0.059	0.397 ± 0.066	0.404 ± 0.047	0.363 ± 0.033
Thymus	Absolute (mg)	300 ± 55	299 ± 44	287 ± 46	295 ± 40
	Relative %	0.360 ± 0.056	0.347 ± 0.050	0.349 ± 0.049	0.373 ± 0.047
Organ		Females			
		0	150	500	1250
Terminal body weight (g)		78 ± 4	80 ± 5	77 ± 5	73 ± 5** **(↓6.4%)**
Brain	Absolute (mg)	1459 ± 53	1486 ± 62	1474 ± 44	1455 ± 54
	Relative %	1.88 ± 0.11	1.87 ± 0.11	1.91 ± 0.12	1.99 ± 0.11** **(↑5.9%)**
Spleen	Absolute (mg)	283 ± 40	299 ± 40	297 ± 42	260 ± 41
	Relative %	0.363 ± 0.047	0.376 ± 0.050	0.384 ± 0.045	0.356 ± 0.049
Thymus	Absolute (mg)	294 ± 63	283 ± 39	283 ± 43	275 ± 49
	Relative %	0.377 ± 0.077	0.356 ± 0.044	0.366 ± 0.049	0.375 ± 0.051
Uterus	Absolute (mg)	69.6 ± 16.1	71.0 ± 16.0	68.9 ± 11.9	61.0 ± 12.1
	Relative %	0.0892 ± 0.0193	0.0890 ± 0.0172	0.0888 ± 0.0134	0.0833 ± 0.0155

*: $p \leq 0.05$; **: $p \leq 0.01$

Table 6.6.1-21: Summary of organ weights in F2 weanlings (mean ± SD) of rats administered with inpyrfluxam in the diet for 18 weeks.

Organ	Sex and dose level (ppm)

		Males			
		0	150	500	1250
Terminal body weight (g)		84 ± 6	83 ± 5	83 ± 6	77 ± 5** **(↓8.3%)**
Brain	Absolute (mg)	1514 ± 59	1504 ± 61	1515 ± 48	1524 ± 53
	Relative %	1.81 ± 0.12	1.80 ± 0.08	1.84 ± 0.14	1.98 ± 0.10** **(↑9.4%)**
Spleen	Absolute (mg)	313 ± 45	311 ± 34	322 ± 41	276 ± 39** **(↓11.8%)**
	Relative %	0.372 ± 0.043	0.373 ± 0.036	0.391 ± 0.048	0.358 ± 0.043
Thymus	Absolute (mg)	307 ± 43	297 ± 37	293 ± 32	271 ± 43** **(↓11.7%)**
	Relative %	0.365 ± 0.038	0.356 ± 0.043	0.356 ± 0.037	0.350 ± 0.042
Organ		**Females**			
		0	**150**	**500**	**1250**
Terminal body weight (g)		78 ± 5	75 ± 6	77 ± 5	72 ± 4** **(↓7.7%)**
Brain	Absolute (mg)	1462 ± 43	1450 ± 46	1475 ± 42	1457 ± 38
	Relative %	1.87 ± 0.12	1.93 ± 0.15	1.92 ± 0.12	2.03 ± 0.09** **(↑8.6%)**
Spleen	Absolute (mg)	278 ± 40	271 ± 32	295 ± 40	246 ± 36* **(↓11.5%)**
	Relative %	0.354 ± 0.045	0.360 ± 0.035	0.383 ± 0.041	0.342 ± 0.048
Thymus	Absolute (mg)	296 ± 52	284 ± 37	287 ± 43	257 ± 41* **(↓13.2%)**
	Relative %	0.377 ± 0.056	0.377 ± 0.047	0.372 ± 0.043	0.355 ± 0.044
Uterus	Absolute (mg)	73.9 ± 12.3	67.8 ± 25.8* **(↓8.3%)**	68.5 ± 13.9	60.6 ± 8.4** **(↓18%)**
	Relative %	0.0943 ± 0.0149	0.0905 ± 0.0372	0.0893 ± 0.0181	0.0842 ± 0.0119

*: $p \leq 0.05$; **: $p \leq 0.01$

Conclusion

In conclusion, in a guideline compliant two-generation study in rats, dietary administration of inpyrfluxam at concentrations of 0, 150, 500 or 1250/2000 ppm (females/males) (0, 8.34, 27.8 or 113 mg/kg bw/day in males and 0, 10.9, 35.5 or 86 mg/kg bw/day in females) for two successive generations caused no effects on fertility and reproductive performance. Therefore, a NOAEL for reproductive toxicity of 1250/2000 ppm (86 mg/kg bw/day) can be identified. Parental toxicity was noted at the top dose in F0 and F1 animals and consisted of reductions in body weights, body weight gains and food consumption, increased liver weight with associated hypertrophy and increased thyroid weight with associated follicular hypertrophy mainly in females. Offspring toxicity was also noted at the top dose with decreased body weights in both F1 and F2 weanlings. A NOAEL of 500 ppm (27.8 mg/kg bw/day) is therefore identified for both parental and offspring toxicity.

[redacted] (2017)

B.6.6.2. Developmental toxicity studies

The developmental toxicity of inpyrfluxam has been investigated via the oral (gavage) route of exposure in rats and rabbits. Four studies (two dose finding and two definitive developmental toxicity studies) in rats and two studies (one dose finding and a definitive developmental toxicity study) in rabbits were available.

Rats

1. Preliminary dose finding study in rats

A relatively old preliminary dose range-finding developmental toxicity study was conducted prior to the definitive study. This study was submitted as supporting information for the recent dose finding and subsequent definitive studies. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.6.2/07
Report Title:	Preliminary prenatal developmental toxicity study in rats with S-2399
Author(s) & Year:	[redacted] (2012)
Document No, Authority registration No	Study No. TPT-0103 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	No
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes, as a supporting dose finding study
Study relied upon:	Yes

Methods

Groups of 7 pregnant female Wistar Hannover rats received inpyrfluxam via oral gavage at doses of 0, 12.5, 25, 50 or 100 mg/kg bw/day from gestation day 6 to 19. The dams were examined for general signs of clinical toxicity, mortality, body weight, body weight gain, and food consumption before sacrificing on gestation day 20. After sacrifice, gross pathology and uterine contents were examined.

Results

Maternal toxicity

At the high dose, 3 out of 7 females were found dead on gestation days 8 and 9. Clonic convulsion and/or lateral position were noted in all the three animals before death.

At 100 mg/kg bw/day, irregular respiration (6 of 7) was noted after 3-4 h of administration, even though the animals recovered by the next day. At the top dose, stains in the periocular region, and on the body surface (ventral neck, perinasal region, and perioral region) and wet and/or stain in the genital region were observed. From 25 mg/kg bw/day, prone position (1 of 7, 6 of 7, and 7 of 7 animals at 25, 50 and 100 mg/kg bw/day respectively) and from 50 mg/kg bw/day, ataxic gait (3 of 7, and 6 of 7 animals at 50 and 100 mg/kg bw/day respectively) were observed 1 h after administration, all of which disappeared after 3-4 h from dosing. These changes occurring from 25 mg/kg bw/day are not considered adverse as they were transient, disappearing a few hours after dosing.

Overall, mortality and clinical signs of toxicity (stains all over the body, clonic convulsion and irregular respiration) were noted at the high dose.

At the high dose, significant decrease in mean body weights and body weight gains were noted throughout the treatment period. This is considered treatment related and adverse.

Table 6.6.2-1: Summary of mean body weight and body weight gains (g) in dams (mean ± SD) administered with inpyrfluxam via oral gavage from gestation day 6 to 19.

Day of gestation	Inpyrfluxam (mg/kg bw/day)				
	0 (control)	12.5	25	50	100
6 to 9	9 (±4.6)	13 (±2.8)	11 (±1.9)	5 (±5.2) (↓44.4%)	-10 (±11.0)**(↓**211%)**
6 to 12	26 (±4.0)	30 (±4.1)	25 (±4.1)	18 (±5.8) (↓30.8%)	-4 (±16.4)*(↓**115%)**
6 to 15	40 (±4.3)	47 (±3.6)	39 (±2.9)	32 (±6.5) (↓20%)	8 (±24.3)*(↓**80%)**
6 to 17	58 (±4.9)	62 (±1.9)	57 (±3.1)	48 (±6.8) (↓17.2%)	16 (±31.8)*(↓**72%)**
6 to 20	98 (±9.9)	103 (±7.5)	95 (±6.4)	86 (±8.7) (↓12.2%)	41 (±36.7)*(↓**58.2%)**

*(p<0.05); ** (p<0.01)

At 100 mg/kg bw/day, retention of foamy fluid in trachea (3 of 3), staining of the perinasal region (1 of 3) and small cecum (1 of 3) were noted in dead animals. These changes are considered treatment related and adverse.

Developmental toxicity

At the high dose, decreases in the mean number of live foetuses per litter (by 28.6%) and in foetal weight were noted. These changes are considered treatment related and adverse. No other changes were noted, and no malformations were observed.

Conclusion

In conclusion, in a dose range-finding developmental toxicity study, administration of inpyrfluxam via oral gavage to Wistar Hannover rats at doses of 0, 12.5, 25, 50 or 100 mg/kg bw/day from gestation day 6 to 19 caused adverse effects mainly at 100 mg/kg bw/day including effects on body weight, body weight gain, clinical signs of toxicity and gross pathology in dams. In addition, there were decreases in the mean number of live foetuses per litter and foetal weight at the high dose.

As this was a range finding non-GLP study, no robust points of departure can be set. Based on the results, a dose level less than 100 mg/kg bw/day was considered as a suitable top dose for the subsequent developmental toxicity study in rats.

[redacted] (2012)

2. Second dose finding study in rats

A second dose range-finding developmental toxicity study was conducted prior to the definitive study. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.6.2/01
Report Title:	S-2399 Technical Grade: Dose Range-Finding Teratogenicity Study in Rats
Author(s) & Year:	[redacted] (2015b)
Document No, Authority registration No	Study No. [redacted] 13-0087 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	No
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes, as a range finding study
Study relied upon:	Yes

Methods

Groups of 8 or 7 pregnant female Wistar Hannover rats received inpyrfluxam via oral gavage at doses of 0, 20, 40 or 80 mg/kg bw/day from gestation day 6 to 19. The dams were examined for general signs of clinical toxicity, mortality, body weight, body weight gain, and food consumption before sacrificing on day 20 of gestation. After sacrifice, gross pathology and uterine contents were examined.

The dose levels were selected based on a previous range-finding study described above ([redacted] 2012).

Results

Maternal toxicity

At the high dose, decreased mean body weights and body weight gains (statistically significant) were noted throughout the treatment period. These decreases are considered treatment related and adverse.

Table 6.6.2-2: Summary of mean body weight and body weight gains (g) in dams (mean ± SD) administered with inpyrfluxam via oral gavage from gestation day 6 to 19.

Body weight (g) at Gestation day	0 mg/kg/day (n=7)	20 mg/kg/day (n=8)	40 mg/kg/day (n=7)	80 mg/kg/day (n=8)
0	238 ± 13	238 ± 13	237 ± 9	240 ± 9
6	262 ± 14	260 ± 15	261 ± 8	262 ± 13
9	274 ± 15	273 ± 17	271 ± 9	262 ± 19
12	287 ± 15	285 ± 13	281 ± 8	272 ± 21
15	303 ± 16	298 ± 17	297 ± 9	285 ± 20
18	340 ± 22	327 ± 20	327 ± 10	317 ± 20
20	369 ± 26	355 ± 21	353 ± 12	341 ± 25
Gravid uterine weight (g)	75 ± 13	68 ± 7	70 ± 9	73 ± 9
Adjusted body weight (g)	294 ± 16	288 ± 14	284 ± 10	268 ± 19**
Body weight gain during Gestation days				
6-9	12 ± 5	12 ± 4	9 ± 5	-1 ± 10**
6-12	25 ± 6	24 ± 5	20 ± 4	10 ± 10**
6-15	41 ± 5	38 ± 6	35 ± 5	23 ± 11**
6-18	78 ± 10	66 ± 8	66 ± 3	55 ± 11**
6-20	108 ± 14	95 ± 11	92 ± 7	79 ± 15**

**: p ≤ 0.01

At the high dose, there were significant decreases in food consumption on gestation days 6-9, 9-12, 15-18, and 18-20. These decreases are considered treatment related and adverse.

Table 6.6.2-3: Summary of food consumption in dams (mean ± SD) with inpyrfluxam via oral gavage from gestation day 6 to 19.

Food consumption (g/rat/day) during Gestation days	0 mg/kg/day (n=7)	20 mg/kg/day (n=8)	40 mg/kg/day (n=7)	80 mg/kg/day (n=8)
0-6	18.1 ± 4.5	17.2 ± 2.0	17.5 ± 3.0	17.4 ± 2.8
6-9	21.7 ± 6.3	20.2 ± 4.5	18.3 ± 5.2	14.2 ± 4.7*
9-12	23.3 ± 2.0	22.0 ± 3.2	20.2 ± 3.3	16.5 ± 3.5**
12-15	21.4 ± 6.0	19.7 ± 3.3	19.6 ± 5.6	15.9 ± 2.2
15-18	23.8 ± 2.2	22.0 ± 0.8	20.3 ± 3.0	19.1 ± 3.1**
18-20	24.9 ± 5.7	23.1 ± 3.7	21.4 ± 6.4	18.1 ± 3.7*

*: p ≤ 0.05; **: p ≤ 0.01

Developmental toxicity

At the high dose, there was a decrease in mean foetal weight. This is considered treatment related and adverse. No other changes were noted.

Conclusion

In conclusion, in a second dose range-finding developmental toxicity study, administration of inpyrfluxam via oral gavage to Wistar Hannover rats at doses of 0, 20, 40 or 80 mg/kg bw/day from gestation day 6 to 19 caused adverse effects mainly at the top dose of 80 mg/kg bw/day, including effects in body weight, body weight gain and food consumption in dams. In addition, there was a decrease in foetal weight at the high dose.

As this was a range finding non-GLP study, no robust points of departure can be set. Based on the results, a dose level of 80 mg/kg bw/day was considered as a suitable top dose for the subsequent definitive developmental toxicity study in rats.

[redacted] (2015b)

3. Definitive developmental toxicity study in rats

The developmental toxicity of inpyrfluxam has been investigated in the rat via the oral (gavage) route. The study was GLP complaint and performed in accordance with OECD TG 414 (2001).

Reference:	KCA 5.6.2/02
Report Title:	S-2399 Technical Grade: Teratogenicity Study in Rats
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. [redacted] 14-0071 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	OECD 414 (2001)
Deviations from current guideline:	No measurement of plasma thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) in dams at termination. No AGD in foetuses was measured.
Impact of the deviation:	The observed deviations do not impact the validity of the results.

	The test guideline was updated later to include endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals. These deviations do not affect the validity of the study as the thyroid hormone investigations have been addressed in mode of action studies in rats and pathological evaluations of the thyroid gland were conducted in the 90-day study in rats and two generation study in rats. AGD was measured in the two-generation study in rats.
GLP or GEP:	Yes-GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant study, groups of 22-24 pregnant female Wistar Hannover rats received inpyrfluxam via oral gavage at doses of 0, 10, 25 or 80 mg/kg bw/day from gestation day 6 to 19 to evaluate potential toxic effects on maternal rats and their foetuses.

In accordance with OECD test guideline 414, all animals were subjected to the required investigations except for the deviations recorded above.

The dose levels were selected based on the second dose finding study described above ([redacted] 2015b).

Results

Maternal toxicity

At the high dose, decreased mean body weights (statistically significant on gestation days 12, 18 and 20) and body weight gains (statistically significant from gestation days 6-9) were noted throughout the treatment period. These are considered treatment related and adverse.

At the high dose, there was a significant decrease in food consumption from gestation days 6-9 to termination. This is considered treatment related and adverse.

There were no treatment related changes in the mean gravid uterine weight, mean numbers of corpora lutea and implantations in any group.

Developmental toxicity

At the high dose, statistically significant decreases in mean foetal weight were observed. This is considered treatment related and adverse.

There were numerous malformations noted in the treated groups, but the incidences were similar to those in controls. Among the malformations, the unusual occurrence of cyclopia was also noted in one foetus at the high dose. Although cyclopia is observed spontaneously, the incidence of cyclopia is quite low and this malformation had not appeared in the HCD of the testing facility. Therefore, relation to treatment could not be excluded and an additional developmental toxicity study in rats was conducted to determine whether the malformation was likely to be treatment related.

Conclusion

In conclusion, in a guideline compliant developmental toxicity study, administration of inpyrfluxam via oral gavage to Wistar Hannover rats at doses of 0, 10, 25 or 80 mg/kg bw/day from gestation day 6 to 19 caused adverse effects mainly at 80 mg/kg bw/day including effects on body weight, body weight gain and food consumption in dams. In addition, there was a decrease in foetal weight and one incidence of cyclopia at the high dose.

Overall, a NOAEL of 25 mg/kg bw/day for maternal toxicity can be established from this study based on the effects on body weight, body weight gain and food consumption in dams at the LOAEL of 80 mg/kg bw/day.

A NOAEL of 25 mg/kg bw/day for developmental toxicity can be established from this study based on the effects on foetal weight and one occurrence of cyclopia at the LOAEL of 80 mg/kg bw/day.

([redacted] 2017a)

4. Additional developmental toxicity study in rats

To confirm whether the occurrence of cyclopia was related to inpyrfluxam treatment, an additional study was conducted using a slightly higher dose and larger group size. The study was GLP complaint and performed in accordance with OECD TG 414 (2001).

Reference:	KCA 5.6.2/03
Report Title:	S-2399 Technical Grade: Additional teratogenicity Study in Rats
Author(s) & Year:	[redacted] (2017b)
Document No, Authority registration No	Study No. [redacted] 16-0018 [redacted]

Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	OECD 414 (2001)
Deviations from current guideline:	No measurement of plasma thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH) in dams at termination. No AGD in foetuses was measured. Only one dose level was used.
Impact of the deviation:	The observed deviations do not impact the validity of the results. The test guideline was updated later to include endocrine-sensitive endpoints intended to improve detection of potential endocrine activity of test chemicals. These deviations do not affect the validity of the study as the thyroid hormone investigations have been addressed in mode of action studies in rats and pathological evaluations of the thyroid gland were conducted in the 90-day study in rats and two generation study. AGD was measured in the two-generation study. Although only one dose level was used, the study was conducted mainly to investigate the potential relation to treatment of cyclopia.
GLP or GEP:	Yes-GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and OECD compliant developmental toxicity study, groups of 39 or 40 pregnant female Wistar Hannover rats received inpyrfluxam via oral gavage at doses of 0 or 90 mg/kg bw/day from gestation day 6 to 19.

In accordance with OECD test guideline 414, all animals were subjected to the required investigations except for the deviations recorded above.

Results

Maternal toxicity

At 90 mg/kg bw/day, staggering gait was noted in one dam on gestational day 12. Additionally, mass in the axillary region was noted in another dam from gestational day 18 until after the dosing period (day 20). Given the low frequency in occurrence, these changes are considered to be incidental.

In the treated dams, significant decreases in mean body weights and body weight gains were noted from gestation day 9. These are considered treatment related and adverse.

At 90 mg/kg bw/day, there were significant decreases in food consumption from gestation days 6-9 to termination. These are considered treatment related and adverse.

In the treated dams, dilatation of the kidney in 3 animals were noted. This is considered incidental due to the low frequency in occurrence.

Also, a significant decrease in mean gravid uterine weight was noted in the treated dams. This is considered treatment related and adverse.

There were no treatment related changes in the mean numbers of corpora lutea and implantations.

Developmental toxicity

At 90 mg/kg bw/day, a significant decrease in mean foetal weight was observed. This is considered treatment related and adverse.

No treatment related adverse changes in the mean number of live foetuses, percentage incidence of resorptions, foetal deaths and sex ratio were recorded in any group.

Microphthalmia was noted in one treated foetus. Based on the observations from a previous study where microphthalmia was noted in one foetus of the control group (section B.6.6.3-2, [redacted] 2017a), the case of microphthalmia is not considered to be treatment related.

Cyclopia was not observed in any foetuses. In this second study, the number of examined foetuses had increased to 533 compared to 309 in the previous study. The tested dose was increased from 80 to 90 mg/kg bw/day to confirm that a maximal tolerated dose had been achieved. It is also noted that cyclopia was not observed neither in F1 or F2 pups in the rat multigeneration study up to a dose of 86-113 mg/kg

bw/day (See section, B.6.6.1; [redacted] (2017). It can be concluded that cyclopia ~~was not~~ is unlikely to be related to treatment with inpyrfluxam.

Conclusion

In conclusion, in an investigative developmental toxicity study, administration of inpyrfluxam via oral gavage to Wistar Hannover rats at doses of 0 or 90 mg/kg bw/day from gestation day 6 to 19 caused adverse effects on body weight, body weight gain and food consumption in dams and reductions in mean foetal weight. It is also concluded that cyclopia, observed in the previous study is ~~not related~~ unlikely to be related to inpyrfluxam treatment.

[redacted] (2015b)

Rabbits

1. Preliminary dose finding study in rabbits

A preliminary dose range-finding developmental toxicity study was conducted in rabbits prior to the definitive developmental toxicity study. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.6.2/04
Report Title:	S-2399 Technical Grade: Dose Range-Finding Teratogenicity Study in Rabbits
Author(s) & Year:	[redacted] (2015c)
Document No, Authority registration No	Study No. [redacted] 13-0088 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	No
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No

Acceptability:	Yes, as a range finding study
Study relied upon:	Yes

Methods

Groups of 8 pregnant female Japanese White rabbits received inpyrfluxam via oral gavage at doses of 0, 15, 50 or 150 mg/kg bw/day from gestation day 6 to 27 to evaluate potential toxic effects on maternal animals and their foetuses. The does were examined for general signs of clinical toxicity, mortality, body weight, body weight gain and food consumption before sacrificing on gestation day 28. After sacrifice, gross pathology and uterine contents were examined.

Results

Maternal toxicity

No effects were observed.

Developmental toxicity

No effects were noted.

Conclusion

In conclusion, in a preliminary dose range-finding developmental toxicity study in rabbits, administration of inpyrfluxam via oral gavage to Japanese White rabbits at doses of 0, 15, 50 or 150 mg/kg bw/day from gestation day 6 to 27 caused no adverse effects in maternal animals or foetuses.

Based on the observed results, it was concluded that none of the tested dose levels reached the maximum tolerated dose (MTD) and a dose level greater than 150 mg/kg bw/day was considered a more suitable top dose for the subsequent dose finding study.

[redacted] (2015c)

2. Second dose finding study in rabbits

A second dose range-finding developmental toxicity study was conducted in rabbits to find the maximum tolerated dose prior to the definitive study. The study was not conducted according to GLP or OECD test guidelines.

Reference:	KCA 5.6.2/05
Report Title:	S-2399 Technical Grade: Additional Dose Range-Finding Teratogenicity Study in Rabbits

Author(s) & Year:	[redacted] (2015d)
Document No, Authority registration No	Study No. [redacted] 14-0031 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	No
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes, as a range finding study
Study relied upon:	Yes

Methods

Groups of 8 pregnant female Japanese White rabbits received inpyrfluxam via oral gavage at doses of 0, 300, 500 or 1000 mg/kg bw/day from gestation day 6 to 27 to evaluate potential toxic effects on maternal animals and their foetuses. The does were examined for general signs of clinical toxicity, mortality, body weight, body weight gain and food consumption before sacrificing on gestation day 28. After sacrifice, gross pathology and uterine contents were examined.

Results

Maternal toxicity

From 500 mg/kg bw/day, mortality occurred (1/8 at 500 mg/kg bw/day and 4/8 at 1000 mg/kg bw/day). The remaining animals were killed in extremis on gestation day 6 or 7 at the high dose and on gestation day 17 or 18 at the mid dose due to the severe toxicity noted.

From 300 mg/kg bw/day, lateral position, prone position, decreased spontaneous motor activity, abnormal behaviour (convulsion), and bradypnoea were observed. At

300 mg/kg bw/day, abortion was recorded in 3 animals. These changes are considered treatment related and adverse.

Overall, clinical signs of toxicity and abortions were noted from 300 mg/kg bw/day.

Table 6.6.2-4: Summary clinical signs of toxicity [No. of affected animals (No. of non-pregnant)] in does administered inpyrfluxam via oral gavage from gestation day 6-27.

Parameter	0 mg/kg/day	300 mg/kg/day	500 mg/kg/day	1000 mg/kg/day
No. of females	8 (0)	8 (0)	8 (0)	6 (2)
Lateral position	0 (0)	1 (0)	4 (0)*	2 (1)
Prone position	0 (0)	3 (0)	6 (0)**	5 (0)**
Decreased spontaneous activity	0 (0)	0 (0)	0 (0)	2 (0)
Abnormal behaviour (convulsion)	0 (0)	1 (0)	2 (0)	1 (0)
Bradypnoea	0 (0)	0 (0)	0 (0)	2 (0)
Loose stool	0 (0)	2 (0)	2 (0)	0 (0)
Prematurely terminated	0 (0)	0 (0)	7 (0)**	4 (0)*
Abortion	0 (0)	3 (0)	0 (0)	0 (0)
Found dead	0 (0)	0 (0)	1 (0)	2 (2)

*: $p \leq 0.05$; **: $p \leq 0.01$

At 300 and 500 mg/kg bw/day, decreases in mean body weight and body weight gains were noted in surviving animals throughout the treatment period. These changes are considered treatment related and adverse.

At 300 and 500 mg/kg bw/day, there was a significant decrease in food consumption. This is considered treatment related and adverse.

There was no treatment related gross pathology changes observed in the animals that survived until terminal kill. However, gross pathological changes were noted in the stomach and large intestine from 300 mg/kg bw/day in the animals that died or were killed in extremis.

Developmental toxicity

At 300 mg/kg bw/day, there were no treatment related changes in mean gravid uterine weight or mean numbers of corpora lutea and implantations in the surviving animals.

At 300 mg/kg bw/day, there were no effects on mean number of live foetuses and percent incidences of resorptions and deaths, sex ratio and mean foetal weights.

At 300 mg/kg bw/day, there were no malformations noted in the foetuses.

Conclusion

In conclusion, in a second dose range-finding developmental toxicity study, administration of inpyrfluxam via oral gavage at doses of 0, 300, 500 or 1000 mg/kg bw/day to Japanese White rabbits from gestation day 6 to 27 caused adverse effects in maternal animals from 300 mg/kg bw/day including clinical signs of toxicity, abortions, decreases in body weight, body weight gains and food consumption, and pathological changes in stomach and large intestine. In addition, mortality/early sacrifice occurred from 500 mg/kg bw/day. There were no adverse effects on foetuses up to a dose of 300 mg/kg bw/day (foetuses at higher doses could not examined).

As this was a range finding non-GLP study, no robust points of departure can be set. Based on the observed results, a dose level between 150 and 300 mg/kg bw/day was considered a suitable top dose for the definitive developmental toxicity study in rabbits.

[redacted] (2015d)

3. Definitive developmental toxicity study in rabbits

The developmental toxicity of inpyrfluxam has been investigated in rabbits via the oral (gavage) route. The study was GLP complaint and performed in accordance with OECD TG 414 (2001).

Reference:	KCA 5.6.2/06
Report Title:	S-2399 Technical Grade: Teratogenicity Study in Rabbits
Author(s) & Year:	[redacted] (2017c)
Document No, Authority registration No	Study No. [redacted] 15-0017 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	OECD 414 (2001)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes-GLP
Acceptability:	Yes

Study relied upon:	Yes

Methods

In a GLP and OECD compliant developmental toxicity study, groups of 23-25 pregnant Japanese White rabbits received inpyrfluxam via oral gavage at doses of 0, 20, 60, or 200 mg/kg bw/day from gestation day 6 to 27.

In accordance with OECD test guideline 414, all animals were subjected to the required investigations.

The dose levels were selected based on a previous dose range finding study described above ([redacted] 2015d).

Results

Maternal toxicity

At 200 mg/kg bw/day, red discharge in the tray and abortions were noted in 2 animals on gestation day 21 and 23. These findings are considered treatment related and adverse.

There were no adverse changes in mean body weights in any group. At the high dose, decreased body weight gains (statistically significant on gestation days 6-9, 6-12 and 6-21) were noted throughout the treatment period. Animals which aborted showed marked body weight losses. These decreases are considered treatment related and adverse.

Table 6.6.2-5: Summary of mean body weight and body weight gains (g) in does (mean ± SD) administered with inpyrfluxam via oral gavage from gestation day 6 to 27.

Body weight (g) at Gestation Day	0 mg/kg/day (n=21 to day 27, then 20)	20 mg/kg/day (n=24)	60 mg/kg/day (n=24)	200 mg/kg/day (n=25 to day 18, 24 at day 21, then 23)
0	3416 ± 279	3386 ± 296	3382 ± 267	3382 ± 295
6	3552 ± 309	3513 ± 311	3527 ± 279	3504 ± 301
9	3567 ± 291	3534 ± 314	3541 ± 296	3489 ± 290
12	3603 ± 283	3562 ± 317	3573 ± 290	3477 ± 264
15	3666 ± 271	3635 ± 305	3656 ± 310	3540 ± 262
18	3662 ± 281	3631 ± 313	3653 ± 311	3545 ± 263
21	3699 ± 286	3677 ± 318	3697 ± 305	3544 ± 257
24	3744 ± 290	3721 ± 329	3745 ± 304	3596 ± 250

27	3788 ± 307	3772 ± 306	3805 ± 300	3660 ± 253
28	3824 ± 309	3790 ± 308	3825 ± 298	3681 ± 248
Gravid uterine weight (g)	484 ± 131	472 ± 106	464 ± 88	446 ± 124
Adjusted body weight (g)	3340 ± 246	3318 ± 312	3361 ± 283	3235 ± 218
Body weight gain during Gestation days				
6-9	15 ± 33	21 ± 27	14 ± 39	-15 ± 38*
6-12	51 ± 53	49 ± 44	46 ± 48	-27 ± 89**
6-15	115 ± 93	122 ± 71	130 ± 82	36 ± 168
6-18	110 ± 123	118 ± 70	127 ± 101	41 ± 181
6-21	148 ± 147	164 ± 91	170 ± 93	65 ± 174*
6-24	192 ± 171	208 ± 125	219 ± 103	138 ± 105
6-27	236 ± 234	259 ± 116	278 ± 123	202 ± 119
6-28	286 ± 212	277 ± 134	298 ± 123	223 ± 114

*: p ≤ 0.05; **: p ≤ 0.01

At the high dose, there was significant decrease in food consumption during gestation days 6-9, 9-12, 12-15 and 15-18. Two animals which aborted showed obvious decreases in food consumption and/or stopped feeding. These changes are considered treatment related and adverse.

Table 6.6.2-6: Summary of food consumption (mean ± SD) in does administered with inpyrfluxam via oral gavage from gestation day 6 to 27.

Food consumption (g/rabbit/day) during Gestation days	0 mg/kg/day	20 mg/kg/day	60 mg/kg/day	200 mg/kg/day
0-3	188 ± 22 (21)	185 ± 21 (23)	189 ± 26 (24)	187 ± 23 (25)
3-6	190 ± 24 (20)	191 ± 22 (24)	193 ± 24 (24)	186 ± 24 (22)
6-9	182 ± 21 (21)	179 ± 19 (23)	178 ± 25 (24)	149 ± 32** (24)
9-12	172 ± 26 (20)	167 ± 24 (24)	171 ± 32 (22)	109 ± 47** (23)
12-15	150 ± 43 (21)	151 ± 40 (24)	150 ± 39 (23)	100 ± 52** (22)
15-18	154 ± 47 (21)	162 ± 30 (24)	152 ± 39 (19)	126 ± 59* (21)
18-21	146 ± 49 (20)	157 ± 29 (24)	161 ± 28 (22)	131 ± 49 (18)
21-24	122 ± 43 (20)	131 ± 36 (21)	140 ± 30 (21)	123 ± 31 (17)
24-27	111 ± 43 (20)	117 ± 33 (23)	130 ± 31 (23)	123 ± 15 (19)
27-28	126 ± 37 (19)	127 ± 29 (24)	137 ± 25 (24)	131 ± 18 (18)

*: p ≤ 0.05; **: p ≤ 0.01

Developmental toxicity

There were no treatment related changes in the mean gravid uterine weight, mean numbers of corpora lutea and implantations in any group.

Table 6.6.2-7: Summary of caesarean section (mean ± SD) in does administered with inpyrfluxam via oral gavage from gestation day 6 to 27.

Parameter	0 mg/kg/day (n= 20)	20 mg/kg/day (n=24)	60 mg/kg/day (n=24)	200 mg/kg/day (n=23)
No. of corpora lutea	11.5 ± 2.5	11.3 ± 2.3	12.8 ± 1.9	11.9 ± 2.9
No. of implantations	10.0 ± 3.1	9.8 ± 2.5	9.5 ± 2.4	9.5 ± 3.0
No. of live foetuses	9.1 ± 2.8	9.0 ± 2.9	8.7 ± 2.3	8.5 ± 2.8
% Resorption and foetal deaths	8.8 ± 9.3	9.5 ± 13.0	8.5 ± 10.6	8.8 ± 14.1

*: $p \leq 0.05$

No treatment related adverse changes in the mean number of live foetuses, percentage incidence of resorptions, foetal deaths, sex ratio and foetal weight were observed in any group.

Table 6.6.2-8: Summary of foetal observations (mean ± SD) in does administered with inpyrfluxam via oral gavage from gestation day 6 to 27.

Parameter	0 mg/kg/day (n= 20)	20 mg/kg/day (n=24)	60 mg/kg/day (n=24)	200 mg/kg/day (n=23)
Foetal weight – male (g)	37.7 ± 7.2	36.9 ± 6.5	38.2 ± 4.7	36.7 ± 5.2
Foetal weight – female (g)	36.6 ± 6.0	36.8 ± 5.2	36.7 ± 5.0	35.9 ± 4.0
Sex ratio	0.580	0.475*	0.483	0.480
No. of females with malformed foetuses (%)	3 (15.0)	7 (29.2)	9 (37.5)	4 (17.4)
No. of females with foetuses with variations (%)	18 (90.0)	22 (91.7)	21 (87.5)	20 (87.0)

*: $p \leq 0.05$

There were no external, skeletal or visceral malformations in the foetuses of any group. There were no treatment related variations noted in any group.

Conclusion

In conclusion, in a guideline compliant developmental toxicity study, administration of inpyrfluxam via oral gavage to Japanese White rabbits at doses of 0, 20, 60 or 200 mg/kg bw/day from gestation day 6 to 27 caused adverse effects in the maternal animals at 200 mg/kg bw/day including effects on body weight gain and food consumption, clinical signs of toxicity (red discharge in the tray and abortions). There were no adverse effects noted in the foetuses.
Overall, a NOAEL of 60 mg/kg bw/day for maternal toxicity can be established from this study based on clinical signs of toxicity and effects on body weight gain and food consumption at the LOAEL of 200 mg/kg bw/day.

A NOAEL of 200 mg/kg bw/day (highest dose tested) can be established for developmental toxicity as there were no adverse effects at any dose.

([redacted] 2017a)

B.6.6.3. Summary of reproductive toxicity

The reproductive toxicity of inpyrfluxam has been investigated via the oral route of exposure. There were two generational toxicity studies (a preliminary one generation range finding study and a definitive two generation study in rats) and four developmental toxicity studies (two dose finding and two definitive developmental toxicity studies in rats; and two range finding and a definitive developmental toxicity study in rabbits). All the definitive studies were conducted in compliance with GLP and OECD test guidelines. The main findings are summarised in table below.

Additional information on the potential effects of inpyrfluxam on reproductive organs from the repeated dose toxicity studies in rats, mice and dogs has been taken into account. In the 28-day oral (dietary) toxicity study in rats, at 3000 ppm (246.4 mg/kg bw/day), there were decreases in ovary and uterus weight along with associated histopathological findings (section B.6.3.1, [redacted] 2014). In the 90-day studies in rats, histopathological findings were noted in the ovary at 2000 ppm (123 mg/kg bw/day) and 4000 ppm (255 mg/kg bw/day) (section B.6.3.2, [redacted] 2016). Similar effects were not seen in the multigeneration study (although lower doses were employed). Therefore, it is most likely these were high dose effects occurring in the excess of the MTD (significant effects on body weights, body weight gains, liver toxicity, adrenal toxicity and histopathology of other organs) and hence of limited relevance at realistic exposure levels.

Table 6.6.3-1: Summary of reproductive toxicity studies for inpyrfluxam

Data point/ Study *Acceptability*	Species / Strain/ sex	Doses	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects at LOAEL
Reproduction toxicity					
KCA 5.6.1/01 *Dose Range-Finding One-generation Reproduction Toxicity Study in Rats* - not GLP or OECD compliant	**Rat**/ Wistar Hannover rats/M &F	Males and Females: 0, 300,1000,2000, and 4000 ppm Mean substance intakes	Not set as range-finding study	Not set as range-finding study	**2000 ppm**: *Reproductive toxicity* No adverse effects up to top dose. *Parental toxicity (F0)* ↓ bw in F (on gestation day 20 and lactation days 0, 4, 7, and 14)

- Range finding study *Acceptable as a range-finding study*		*Males:* 15.1, 50.4, 105 or 203 mg/kg bw/day *Females:* 20.4, 68, 132 or 254 mg/kg bw/day			↓ bw gain in F (during gestation days 0-20) ↓ food consumption (F) ↑ relative liver weight (16%) in M) ↓ absolute ovary weight (18% in F) <u>*Offspring toxicity (F1)*</u> ↓ bw in M & F (from lactation day 4) enlargement of the eye associated with synechia and cataract ↓ absolute thymus and brain weight (in M and F) ↓ absolute uterus weight in F
KCA 5.6.1/02 *Definitive Two-generation Reproduction Toxicity Study in Rats* - GLP -OECD 416 (2001) *Acceptable*	**Rat**/ Wistar Hannover rats/M&F	Males: 0, 150,500, and 2000 ppm Females: 0, 150,500, and 1250 ppm <u>Mean substance intakes</u> *Males:* 0, 8.34, 27.8 or 113 mg/kg bw/day *Females:* 0, 10.9, 35.5 or 86 mg/kg bw/day	<u>*Reproductive toxicity*</u> 1250/2000 ppm (86 mg/kg bw/day) <u>*Parental and offspring toxicity*</u> 500 ppm (27.8 mg/kg bw/day)	<u>*Reproductive toxicity*</u> N/A <u>*Parental and offspring toxicity*</u> 1250/2000 ppm (86 mg/kg bw/day)	<u>*Reproductive toxicity*</u> <u>No adverse effects up to the top dose</u> <u>*Parental toxicity*</u> <u>*F0*</u> ↓ bw in M & F ↓ bw gain in M & F ↓ food consumption in M & F ↑absolute and relative liver weights in F ↓ absolute and relative thyroid weight in F ↑ Thyroid follicular hypertrophy in F <u>*F1*</u> ↓ bw in M & F

					↓ bw gain in M & F ↓ food consumption in F ↑absolute and relative liver weights in F ↓ absolute and relative thyroid weight in F ↑ Thyroid follicular hypertrophy in F *Offspring toxicity* ↓ bw in M & F
Developmental toxicity					
KCA 5.6.2/07 *Preliminary prenatal developmental toxicity study in rats* - not GLP or OECD compliant - Range finding study *Acceptable as a range-finding study*	**Rat**/ Wistar Hannover rats/F	0, 12.5, 25, 50 or 100 mg/kg bw/day	Not set as range-finding study	Not set as range-finding study	**100 mg/kg bw/day** *Maternal toxicity* Mortality Clinical signs of toxicity (stains all over the body, clonic convulsion and irregular respiration) ↓ bw and bw gain retention of foamy fluid in trachea *Developmental toxicity* ↓ mean number of live foetuses per ↓ foetal weight
KCA 5.6.2/01 *Dose Range-Finding Teratogenicity Study in Rats* - not GLP or OECD compliant	**Rat**/ Wistar Hannover rats/F	0, 20, 40 or 80 mg/kg bw/day	Not set as range-finding study	Not set as range-finding study	**80 mg/kg bw/day** *Maternal toxicity* ↓ bw and bw gain ↓ food consumption *Developmental toxicity* ↓ foetal weight

- Range finding study *Acceptable as a range-finding study*					
KCA 5.6.2/02 *Definitive Teratogenicity Study in Rats* - GLP -OECD 414 (2001) *Acceptable*	**Rat**/ Wistar Hannover rats/F	0, 10, 25 or 80 mg/kg bw/day	*Maternal toxicity* 25 mg/kg bw/day *Developmental toxicity* 25 mg/kg bw/day	*Maternal toxicity* 80 mg/kg bw/day *Developmental toxicity* 80 mg/kg bw/day	*Maternal toxicity* ↓ bw and bw gain ↓ food consumption *Developmental toxicity* ↓ foetal weight Cyclopia (n=1)
KCA 5.6.2/03 *Investigative Teratogenicity Study in Rats* - GLP -OECD 414 *Acceptable as additional study*	**Rat**/ Wistar Hannover rats/F	0 or 90 mg/kg bw/day	*Maternal toxicity* N/A *Developmental toxicity* N/A	*Maternal toxicity* N/A *Developmental toxicity* N/A	**90 mg/kg bw/day** *Maternal toxicity* ↓ bw and bw gain ↓ food consumption ↓ mean gravid uterine weight *Developmental toxicity* ↓ foetal weight
KCA 5.6.2/04 *Dose Range-Finding Teratogenicity Study in Rabbits* - not GLP or OECD compliant - Range finding study	**Rabbit**/ Japanese White /F	0, 15, 50 or 150 mg/kg bw/day	Not set as range-finding study	Not set as range-finding study	**150 mg/kg bw/day** *Maternal toxicity* None *Developmental toxicity* None

Acceptable as a range-finding study					
KCA 5.6.2/05 *Additional Dose Range-Finding Teratogenicity Study in Rabbits* - not GLP or OECD compliant - Range finding study *Acceptable as a range-finding study*	**Rabbit**/ Japanese White /F	0, 300, 500, or 1000 mg/kg bw/day	Not set as range-finding study	Not set as range-finding study	**300 mg/kg bw/day** <u>*Maternal toxicity*</u> Clinical signs of toxicity (lateral and prone position, decreased spontaneous motor activity, abnormal behaviour (convulsion), bradypnoea, abortion) ↓ bw and bw gain ↓ food consumption Gross pathological changes in stomach and large intestine <u>*Developmental toxicity*</u> None
KCA 5.6.2/06 *Definitive Teratogenicity Study in Rabbits* - GLP -OECD 414 (2001) *Acceptable*	**Rabbit**/ Japanese White /F	0, 20, 60, or 200 mg/kg bw/day	<u>*Maternal toxicity*</u> 60 mg/kg bw/day <u>*Developmental toxicity*</u> 200 mg/kg bw/day	<u>*Maternal toxicity*</u> 200 mg/kg bw/day <u>*Developmental toxicity*</u> N/A	<u>*Maternal toxicity*</u> Clinical signs of toxicity (red discharge in the tray and abortions) ↓ bw gain ↓ food consumption <u>*Developmental toxicity*</u> None

<u>*Effects on fertility and reproductive performance*</u>

The potential effects of inpyrfluxam on fertility and reproductive performance has been investigated in the rat via the oral (dietary) route of exposure in a guideline-compliant 2-generation study.

Administration of inpyrfluxam at concentrations of 0, 150, 500 or 1250/2000 ppm (females/males) (0, 8.34, 27.8 or 113 mg/kg bw/day in males and 0, 10.9, 35.5 or 86 mg/kg bw/day in females) for two successive generations caused no effects on fertility and reproductive performance. Therefore, a NOAEL of 1250/2000 ppm (86 mg/kg bw/day) can be established for reproductive toxicity. Parental toxicity was noted at the top dose in F0 and F1 animals and consisted of reductions in body weights, body weight gains and food consumption, increased liver weight with associated hypertrophy and increased thyroid weight with associated follicular hypertrophy, mainly in females. Offspring toxicity was also noted at the top dose with decreased body weights in both F1 and F2 weanlings. A NOAEL of 500 ppm (27.8 mg/kg bw/day) is therefore established for both parental and offspring toxicity.

Developmental toxicity

The developmental toxicity of inpyrfluxam has been investigated via the oral (gavage) route of exposure in rats and rabbits in guideline-compliant studies.

Rats

Administration of inpyrfluxam via oral gavage to rats at doses of 0, 10, 25 or 80 mg/kg bw/day caused developmental toxicity (decreased foetal weight) and maternal toxicity (reduced body weights, body weight gains and food consumption) at the top dose of 80 mg/kg bw/day. On this basis, a NOAEL of 25 mg/kg bw/day was identified for both maternal and developmental toxicity. It is most likely that the foetal effects are the secondary unspecific consequence of maternal toxicity. An additional developmental toxicity study was performed in rats (using 40 dams) at the high dose of 90 mg/kg bw/day to investigate whether a single occurrence of cyclopia observed in the main study at 80 mg/kg bw/day was related to treatment with inpyrfluxam. There was no evidence of cyclopia, confirming that inpyrfluxam ~~does not~~ is unlikely to cause cyclopia.

It should be noted that although cyclopia in rats and optic nerve fibre degeneration in dogs are effects on the eye, they are completely unrelated.

Rabbits

Administration of inpyrfluxam via oral gavage to rabbits at doses of 0, 20, 60 or 200 mg/kg bw/day did not cause developmental toxicity. However, maternal toxicity (clinical signs of toxicity and reduced body weight gain and food consumption) was observed at the top dose of 200 mg/kg bw/day. On this basis the NOAEL for developmental toxicity is 200 mg/kg bw/day, the highest dose tested and the NOAEL for maternal toxicity is 60 mg/kg bw/day.

Overall conclusion

Overall, therefore, reproduction and developmental toxicity have been adequately investigated in studies in rats and rabbits. The substance does not affect fertility in rats and is not a specific developmental toxicant in rats or rabbits. The reproductive toxicity NOAEL from the two-generation study is 86 mg/kg bw/day. The lowest developmental toxicity NOAEL is 25 mg/kg bw/day from the rat developmental toxicity study.

In addition, the following conclusions can be drawn:

- No classification for reproductive toxicity is required (see aligned MCL dossier).
- The data requirements of assimilated Regulation 283/2013 have been met.

B.6.7. Neurotoxicity

The neurotoxicity of inpyrfluxam was investigated via the oral (gavage or dietary) route of exposure. There were two acute neurotoxicity studies (a dose range finding and a definitive study) and a sub-chronic neurotoxicity study in Wistar rats. All the studies were conducted in compliance with GLP and OECD test guidelines.

B.6.7.1. Neurotoxicity studies in rodents

1. Acute Neurotoxicity Study of Inpyrfluxam in Rats – range finding

Reference:	KCA 5.7.1/01
Report Title:	S-2399 Technical Grade: Dose Range-Finding Study for Acute Neurotoxicity Study in Rats
Author(s) & Year:	[redacted] (2015)
Document No, Authority registration No	Study No. [redacted] 14-0107 [redacted].
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 424 (1997)
Deviations from current guideline :	Yes, Only 3 animals per sex per dose were used whereas the guideline recommends the use of 10 animals per sex per dose.

Impact of the deviation:	Since it is only a range finding study to finalise the doses for the definitive study, the observed deviation does not affect the validity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and guideline study, groups of three male and female Wistar rats received inpyrfluxam at doses of 0, 10, 30, 100, 200 and 400 mg/kg bw once by oral gavage. On the day of administration, animals were subjected to hourly detailed clinical observations for 8 h after dosing. Further, the animals were observed daily for mortality and clinical signs of toxicity for 7 days after treatment. After the 7-day observation period, all animals were euthanized and subjected to necropsy.

All the investigations required by the test guideline were performed.

Results

At 400 and 200 mg/kg bw, mortality was noted among females (one animal per dose) after 3h and 1h of administration respectively.

At 400 mg/kg bw, the following signs of clinical toxicity were noted in females: prone position (at 1–2 h), tremors (at 1 h and 4–5 h), ataxia (at 1–2 h), red nose (at 2 h), pale skin (at 2 h), hypothermia (at 1–2 h); ananastasia (at 2 h); bradypnoea/laboured respiration (at 1–6 h), staggering gait (at 2–5 h), decreases in spontaneous motor activity (at 3 h), muscle tone (at 1–2 h), alertness (at 1–3 h) and exploration (at 1–8 h). At 400 mg/kg bw, bradypnoea and/or laboured respiration (at 1 h), decreases in muscle tone (at 1–3 h) and alertness (at 1 h) were observed in males. At 200 mg/kg bw, abnormal gait and, decreases in muscle tone, alertness and exploration were observed in females. All these changes are considered treatment related and adverse.

No treatment related changes were noted in body weight or at necropsy (including the animals that were found dead) at any dose levels of either sex.

Overall, there were clinical signs of toxicity and/or changes in neurofunction in males at 400 mg/kg bw and in females from 200 mg/kg bw.

Conclusion

In a guideline dose range finding acute neurotoxicity study, administration of inpyrfluxam to rats at doses of 0, 10, 30, 100, 200 and 400 mg/kg bw once by oral gavage caused adverse effects, including mortality and clinical signs of toxicity and/or

neurofunction (eg. abnormal gait and, decreases in muscle tone, alertness, and exploration) from 200 mg/kg bw. Most of these changes were noted in the 1 – 5 h time window after administration, and therefore, the time to peak effect was estimated as 1 – 5 h after administration.

Based on the results of this dose finding study, a dose level around 200 mg/kg bw was considered as the recommended top dose level for the definitive acute neurotoxicity study.

([redacted] Y, 2015)

2. Acute Neurotoxicity Study of Inpyrfluxam in Rats – definitive

Reference:	KCA 5.7.1/02
Report Title:	S-2399 Technical Grade: Acute Oral Neurotoxicity Study in Rats
Author(s) & Year:	[redacted] (2016b)
Document No, Authority registration No	Study No. [redacted] 14-0108 [redacted].
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Not required
Guideline(s):	OECD 424 (1997)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and guideline study, groups of ten male and female Wistar rats received inpyrfluxam once via oral gavage at doses of 0, 30, 100 and 200 mg/kg bw.

All the investigations required by the test guideline were performed.

Results

Mortality and general clinical signs

No mortality was observed in any of the groups in either sex.

At the high dose, a decrease in muscle tone was noted in two females on the first day after administration which disappeared on day 2. This is considered adverse.

Functional observation battery

At 200 and 100 mg/kg bw, there were significant decreases in body temperature and motor activity (at 0 - 10 minutes, 10 - 20 minutes, 20 - 30 minutes after week 2, and total (60 minutes) of measurement) in females. Given the lack of any associated histopathological/neurostructural findings, these changes are considered to be related to systemic toxicity but not associated with neurotoxicity.

Overall, adverse decreases in body temperature and motor activity associated with systemic toxicity were noted in females from 100 mg/kg bw.

Body weight, necropsy, and histopathology

No treatment related changes were noted in body weight, at necropsy, or histopathology in either sex.

Conclusion

In conclusion, in a guideline acute neurotoxicity study, administration of inpyrfluxam once via oral gavage to Wistar rats at doses of 0, 30, 100 and 200 mg/kg bw caused adverse effects including decreased body temperature and motor activity from 100 mg/kg bw and decrease muscle tone at the top dose. These effects were considered an expression of generalised toxicity rather than neurotoxicity as there was no consistent pattern of neurotoxic effects, including neuropathology. No neurostructural changes were noted at any tested doses.

Overall, a **NOAEL of 30 mg/kg bw** can be established for **systemic toxicity** from this study based on reduced body temperature and motor activity at 100 mg/kg bw.

A **NOAEL of 200 mg/kg bw** (highest tested dose) can be established for **neurotoxicity** as there were no adverse effects up to this dose**.**

([redacted] 2016b).

3. Neurotoxicity Study of Inpyrfluxam in Rats – repeated dose

Reference:	KCA 5.7.1/03
Report Title:	S-2399 Technical Grade: Repeated Dose 90-Day Oral Neurotoxicity Study in Rats
Author(s) & Year:	[redacted] (2016)
Document No, Authority registration No	Study No. [redacted] 15-0037 [redacted].
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch:13CG0617G Purity: 95.0%
Method of analysis:	Acceptable method of analysis available for dietary formulation
Guideline(s):	OECD 424 (1997)
Deviations from current guideline :	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a GLP and guideline study, groups of 10 Wistar Hannover rats received inpyrfluxam by dietary administration over 91 consecutive days. Dietary concentrations of inpyrfluxam were 0, 500, 2000 and 4000 ppm (mean substance intakes: 0, 30, 118.9 and 240 mg/kg bw/day) for males and 0, 500, 1000 and 2000 ppm (mean substance intakes:0, 35.2, 68 and 133 mg/kg bw/day) for females. The dose levels were selected based on a previous repeated dose 90-day oral toxicity study in rats ([redacted] 2016).

In accordance with OECD test guideline 424, all animals were subjected to the required investigations. Analysis of the dietary formulation was performed within the study.

Results

No treatment related mortality or clinical signs of toxicity were observed in any of the groups in either sex.

Body weight

At the top dose of 4000 ppm, a statistically significant decrease in body weight was observed in males throughout the treatment period. At the top dose of 2000 ppm (weeks 6-13) and mid dose of 1000 ppm (weeks 8 and 13), a statistically significant decrease in body weight was noted in females. This change in body weight is considered treatment related and adverse due its magnitude (around 10% or greater).

Table 6.7.1-1: Mean body weight (% of control) of rats administered inpyrfluxam in the diet for 90 days.

Week	Sex and dose level (ppm)					
	Male			Female		
	500	2000	4000	500	1000	2000
0	99	100	98	98	100	99
1	99	100	98	96	98	94
2	100	98	88**	98	97	93
3	99	97	87**	100	97	94
4	98	97	87**	99	94	93
5	98	95	87**	99	95	92
6	97	94	85**	100	94	92*
7	96	93	85**	100	94	91*
8	97	93	84**	99	91*	91*
9	97	92	84**	100	92	90*
10	97	92	85**	100	92	91*
11	97	93	85**	100	93	90*
12	97	93	85**	101	92	91*
13	97	92	84**	100	92*	90*
Week 0 = initiation of treatment; * and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)						

Food consumption

At the high dose, there was a statistically significant decrease in food consumption throughout the treatment period in both males (except at weeks 9 and 10) and females (except at weeks 3,6,7,9,10 and 12). In addition, food consumption was approximately 10% lower in the mid-dose females up to week 8. This is considered treatment related and adverse.

Table 6.7.1-2: Summary of food consumption (% of control) of rats administered inpyrfluxam in the diet for 90 days.

Week	Sex and dose level (ppm)					
	Male			Female		
	500	2000	4000	500	1000	2000

1	100	95	76**	96	91*	80**
2	104	101	85**	99	93	89**
3	97	95	93*	101	91	91
4	98	96	91**	100	90	87*
5	97	93	90**	101	94	88*
6	100	93	86**	103	94	91
7	100	93	88**	103	94	90
8	100	95	88**	101	89	88*
9	101	96	91	106	96	91
10	100	96	90	104	95	93
11	100	99	91*	105	97	89*
12	100	94	89*	103	95	91
13	99	96	91*	104	94	87**
* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)						

Overall, there were adverse decreases in body weight and food consumption in males at 4000 ppm and in females from 1000 ppm.

Functional tests

At 4000 ppm, a statistically significant decrease in forelimb grip strength was recorded in males (at week 13). This is considered incidental due to the lack of associated clinical signs of toxicity, other central nervous system related functional changes or neuro-histopathological findings.

From 1000 ppm, a significant increase in motor activity in females was noted. This is also considered incidental as this was noted only at one interval (40-50 min) within the 60 min observation period.

Overall, three were no adverse functional changes in either sex in any of the treated groups.

Necropsy and histopathology

No treatment related changes were noted at necropsy or histopathology in either sex.

Conclusion

In conclusion, in a guideline sub chronic neurotoxicity study, dietary administration of inpyrfluxam over 91 consecutive days to male Wistar rats at concentrations of 0, 500, 2000 and 4000 ppm (mean substance intakes: 30, 118.9 and 240 mg/kg bw/day) and female Wistar at concentrations of 0, 500, 1000, 2000 ppm (mean substance intakes: 35.2, 68 and 133 mg/kg bw/day) caused adverse effects in body weight and food consumption from 1000 ppm (68 mg/kg bw/day). No neurotoxicity was observed at any tested dose.

Overall, a **NOAEL of 500 ppm (35.2 mg/kg bw/day)** can be established for **systemic toxicity** based on the decrease in body weight and food consumption at 1000 ppm (68 mg/kg bw/day).

A **NOAEL of 2000 ppm (133 mg/kg bw/day-**highest tested dose) can be established for **neurotoxicity** as there were no adverse effects at any dose**.**

(2016).

B.6.7.2. Delayed polyneuropathy studies

According to assimilated Reg. (EU) 283/2013, these studies shall be performed for active substances of similar or related structures to those capable of inducing delayed polyneuropathy such as organophosphorus compounds. Inpyrfluxam does not belong to the chemical classes suspected to cause delayed neurotoxicity. Therefore, no delayed neurotoxicity study is required.

B.6.7.3. Summary of neurotoxicity

The neurotoxicity of inpyrfluxam was investigated via the oral (gavage or dietary) route of exposure. There were two acute neurotoxicity studies (a preliminary dose range finding and a definitive study) and a sub-chronic neurotoxicity study in rats. All the studies were conducted in compliance with GLP and OECD test guidelines. The main findings of these studies are summarised in table 6.1.6-1 below. Additional information on the potential effects of inpyrfluxam on the nervous system from the short and long-term toxicity studies in rats that included neuro behavioural evaluation (FOB and motor activity) has been taken into account. There were no adverse effects on neurobehavioral parameters, brain weight or neuropathology in these studies.

In the 90-day repeat dose study in dogs, optic nerve fibre degeneration was noted at the top dose (700/500 mg/kg bw/day) in males and from the mid dose (160 mg/kg bw/day) in females (2016; section B.6.3.2). However, significant systemic toxicity was noted at and above 160 mg/kg bw/day including vomiting, signs of anaemia, changes in clinical-chemistry parameters indicative of liver ~~damage~~ changes, increased liver weight and histopathological findings in the liver, gall bladder, kidney, thyroid and adrenal. The top dose of 700/500 mg/kg bw/day was highly toxic with additional signs of toxicity, such as mortality, clinical signs of toxicity, effects on body weight and food consumption, changes in urinalysis parameters and additional effects on other organs.

In the one-year repeat dose study in dogs, degeneration of the optic nerve was noted at 160 mg/kg bw/day in females (2017; section B.6.3.3). However, significant systemic toxicity was noted at and above 30 mg/kg bw/day (vomiting, clinical chemistry parameters indicative of liver ~~damage~~ changes, increased liver weights, and histopathological findings of the liver) with the MTD being reached at 30 mg/kg bw/day already.

Overall, although significant systemic toxicity occurred at the same doses at which the optic nerve fibre degeneration was observed, it is unclear whether the degeneration was specific or secondary. Therefore, in the absence of any further information, it was concluded that inpyrfluxam was neurotoxic in dogs at highly toxic doses (at and above 160 mg/kg bw/day).

~~the increased incidence in optic nerve degeneration in dogs occurred above the MTD and therefore this does not represent specific neurotoxicity.~~

Table 6.7.3-1 Summary of available neurotoxicity studies

Data point/ Study *Acceptability*	Species / Strain / sex	Doses	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Adverse effects at LOAEL
KCA 5.7.1/02 *Acute Oral Neuro toxicity* - OECD TG 424 (1997) *Acceptable*	**Rat**/ Wistar Hannover rats/ M&F	0, 30, 100, and 200 mg/kg bw	**Systemic toxicity** 30 mg/kg bw **Neurotoxicity** 200 mg/kg bw	**Systemic toxicity** 200 mg/kg bw (highest tested dose) **Neurotoxicity** -	**Systemic toxicity** ↓ motor activity ↓ body temperature **Neurotoxicity** None
KCA 5.7.1/03 *Oral Neuro toxicity- Repeat dose* - OECD TG 424 (1997) *Acceptable*	**Rat**/ Wistar Hannover rats/ M&F	*Males* 0, 500, 2000 and 4000 ppm (mean substance intakes: 30, 118.9 and 240 mg/kg bw/day) *Females* 0, 500, 1000, 2000 ppm (mean substance intakes: 0, 35.2, 68 and 133 mg/kg bw/day)	**Systemic toxicity** 35.2 mg/kg bw/day **Neurotoxicity** 133 mg/kg bw	**Systemic toxicity** 133 mg/kg bw/day (highest tested dose) **Neurotoxicity** -	**Systemic toxicity** ↓ Body weight ↓ Food consumption **Neurotoxicity** None

In the acute neurotoxicity study in rats, systemic toxicity related decreases in body temperature and motor activity were noted from 100 mg/kg bw in both sexes. Therefore, a **NOAEL of 30 mg/kg bw** was established for **systemic toxicity**. Inpyrfluxam did not induce any neurotoxicity up to the highest tested dose of 200 mg/kg bw. Therefore, a **NOAEL of 200 mg/kg bw** was established for **neurotoxicity.**

In the 90-day repeat dose neurotoxicity study in rats, decreases in body weight and food consumption were observed from around 1000 ppm (68 mg/kg bw/day). Therefore, a **NOAEL of 500 ppm (35.2 mg/kg bw/day)** was established for **systemic toxicity.** Inpyrfluxam did not induce any neurotoxicity up to the highest tested dose of 2000 ppm (133 mg/kg bw/day). Therefore, A **NOAEL of 2000 ppm (133 mg/kg bw/day)** was established for **neurotoxicity.** Overall, inpyrfluxam is not neurotoxic.

Inpyrfluxam has neither a structural relationship to any chemical class that causes delayed neurotoxicity, nor did repeat-dose studies show any indications of delayed neurotoxicity. Therefore, a specific study for delayed neurotoxicity of inpyrfluxam is not required.

B.6.8. Other Toxicological Studies

B.6.8.1. Toxicity studies on metabolites and relevant impurities

Metabolites

ADME data in rats show that the plant metabolites 1'-COOH-S-2840, N-des-Me-1'-CH_2OH-S-2840 and 1'-CH_2OH-S-2840 glucuronide are major rat metabolites, representing more than 10% of the administered dose (AD). Therefore, the toxicological evaluation of these metabolites is considered to be "covered" by the parent compound.

The plant metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 have been individually tested for their acute (oral) toxicity and genotoxicity potential (covering gene mutations, clastogenicity and aneugenicity potential) in Ames, in vitro mammalian cell gene mutation, in vitro chromosomal aberration and in vitro micronucleus tests. In addition to these studies, a 90-day oral toxicity study in rats was also submitted for metabolite 3'-OH-S-2840.

Other plant and livestock metabolites (i.e. DFPA, N-des-Me-DFPA and DFPA-CONH2) are common to other succinate dehydrogenase inhibitor (SDHI) fungicides and toxicological data (genotoxicity and general toxicity studies) are already available and been evaluated at EU level before the UK left the EU. The applicant has access to these data.

For additional plant and livestock metabolites (Gly-1'-CH_2OH-S-2840, 1',1'-bis-(CH_2OH)-S-2840, N-des-Me-S-2840, Glc-NDM-S-2399A and Glc-NDM-S-2399B), only a QSAR analysis has been submitted.

Experimental data on some metabolites of inpyrfluxam

3'-OH-S-2840

1.Acute oral toxicity study of 3'-OH-S-2840 in rats

Reference:	KCA 5.8.1.1/01
Report Title:	Acute Oral Toxicity Study of 3'-OH-S-2840 in Rats
Author(s) & Year:	[redacted] (2017b)
Document No, Authority registration No	Study No. 4361 [redacted]
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160520-1 Purity: 99.6%
Method of analysis:	Not required
Guideline(s):	OECD 423 (2001)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes
Study relied upon:	Yes

The acute oral toxicity of 3'-OH-S-2840 was assessed in a GLP and guideline study using the toxic class method. Two groups (first and second) of three female RccHan Wistar rats were administered 2000 mg/kg bw of 3'-OH-S-2840 via oral gavage. All the investigations required by the guideline were performed. The dose level was

selected based on a previous oral toxicity study where there were no mortalities or clinical signs of toxicity at 50 mg/kg bw or 300 mg/kg bw (2016)[3].

No mortality, clinical signs of toxicity, macroscopic pathological findings or changes in bodyweight or body weight gain were noted in any of the groups. Under the conditions of this GLP and guideline study, the oral LD50 of 3'-OH-S-2840 was >2000 mg/kg bw in female RccHan:WIST rats. 3'-OH-S-2840 is not acutely toxic via the oral route and does not meet the criteria for classification for acute oral toxicity according to Regulation 1272/2008 as it applies in GB.

(2017b)

2. 90-day oral toxicity study in rats

Reference:	KCA 5.8.1.1/02
Report Title:	3'-OH-S-2840: Repeated Dose 90-Day Oral Toxicity Study in Rats
Author(s) & Year:	(2018)
Document No, Authority registration No	Study No. 17-0024
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160914-1 Purity: 99.7%
Method of analysis:	Acceptable method of analysis available for dietary formulation
Guideline(s):	OECD 408 (1998)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes
Study relied upon:	Yes

[3] T., Acute Oral toxicity study of 3'-COOH-S-2840 in rats, , Technical report, (Study No.: SI924), (2016)

Methods

In a GLP and guideline study, groups of 10 male and 10 female Wistar Hannover rats received 3'-OH-S-2840 by dietary administration at concentrations of 0, 500, 2000 or 4000 ppm (mean substance intakes: 0, 32.2, 128 and 258 mg/kg bw/day for males and 0, 37.9, 157 and 291 mg/kg bw/day for females) over 91 consecutive days. The dose levels were selected based on a previous 28-day oral toxicity study in rats (not submitted).

In accordance with OECD test guideline 408, all animals were subjected to the required investigations. Analysis of the dietary formulation was performed within the study.

Results

There was no treatment related effect on mortality, clinical signs of toxicity, FOB, ophthalmology or urinalysis.

Body weight

At the high dose, there were slight decreases in body weight (up to 8% reduction compared to control) and body weight gains (up to 12% reduction compared to control) in males. This is considered adverse. The results are summarised in table 6.8.1-1 and -2.

Table 6.8.1-1: Mean body weight (% of control) of rats administered 3'-OH-S-2840 in the diet for 90 days.

Week	Sex and dose level (ppm)					
	Male			Female		
	500	2000	4000	500	2000	4000
1	100	99	95**	102	101	99
2	100	100	96	102	101	98
3	100	101	96	101	100	98
4	100	101	95	101	101	97
5	99	100	95	101	101	98
6	99	100	94	100	101	97
7	99	100	94	99	100	97
8	99	101	93	99	100	97
9	99	101	93	98	101	97
10	99	101	93	100	101	97
11	98	101	93	100	101	100
12	98	101	93	101	103	100
13	98	101	92	101	103	99

**: p≤0.01 (Dunnett's test or Dunnett-type test)

Table 6.8.1-2: Summary of body weight gain (absolute value (g)/ % of control taken as 100%) of rats administered 3'-OH-S-2840 in the diet for 90 days.

Week	Sex and dose level (ppm)							
	Male				Female			
	0	500	2000	4000	0	500	2000	4000
0-1	49 / 100	48 / 98	47 / 96	37** / 76	21 / 100	23 / 110	22 / 105	19 / 90
10-11	11 / 100	9 / 82	10 / 91	8 / 73	-1 / -	1 / -	-1 / -	4* / -
0-13	274 / 100	267 / 97	277 / 101	242 / 88	111 / 100	113 / 102	118 / 106	110 / 99

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Food consumption and efficiency

At the high dose, a significant decrease in food consumption was noted in both sexes at week 1. This change is not considered adverse because it is most likely due to palatability since it disappears at later time points.

Table 6.8.1-3: Summary of food consumption (% of control) of rats administered 3'-OH-S-2840 in the diet for 90 days.

Week	Sex and dose level (ppm)					
	Male			Female		
	500	2000	4000	500	2000	4000
1	97	91*	79**	107	99	73**
10	99	100	97	108	112*	103
12	100	101	97	107	114**	101
13	99	100	96	102	113**	103

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Haematology

At the high dose, a statistically significant decrease in mean corpuscular volume (MCV - 4% reduction compared to control) was observed in females. From the mid dose, there was a statistically significant increase in platelet count (>10%) in females. These changes are not considered adverse given the low magnitude of the effect and because there were no significant changes in other indicators of anaemia (haematocrit, haemoglobin concentration, and erythrocyte count).

A statistically significant increase in mean activated partial thromboplastin time (APTT – 7% increase compared to control) was seen in females at the top dose. This is

considered treatment related and adverse and may be related to the liver toxicity observed (see below).

Overall, an adverse effect on a coagulation parameter (APTT) was noted at the top dose.

Table 6.8.1-4: Summary of significant changes at haematology [mean ± SD (% of controls)] in rats administered 3'-OH-S-2840 in the diet for 90 days.

Para-meter	Sex and dose level (ppm)								
	Male				Female				
	0	500	2000	4000	0	500	2000	4000	HC#
MCV (fL)	49.1 ± 1.6	48.6 ± 1.3 (99)	48.7 ± 2.0 (99)	49.6 ± 1.5 (101)	53.9 ± 2.1	52.8 ± 1.3 (98)	52.3 ± 1.9 (97)	51.8 ± 1.8* (96) **(↓4%)**	
APTT (sec)	21.3 ± 0.9	21.5 ± 0.6 (101)	21.8 ± 0.6 (102)	21.8 ± 1.1 (102)	17.7 ± 0.8	17.8 ± 1.1 (101)	18.1 ± 1.1 (102)	18.9 ± 0.9* (107) **(↑7%)**	
WBC (10^3/µL)	3.98 ± 0.95	4.14 ± 0.77 (104)	3.94 ± 0.68 (99)	3.81 ± 0.47 (96)	2.11 ± 0.31	2.79 ± 0.49** (132) **(↑32%)**	2.57 ± 0.57 (122)	2.21 ± 0.52 (105)	
Lymph (10^3/µL)	3.19 ± 0.76	3.23 ± 0.61 (101)	3.13 ± 0.62 (98)	3.08 ± 0.46 (97)	1.73 ± 0.33	2.24 ± 0.56* (129) **(↑29%)**	2.00 ± 0.32 (116)	1.65 ± 0.35 (95)	
PLT## (10^3/µL)	930 ± 67	924 ± 102 (99)	936 ± 119 (101)	952 ± 71 (102)	961 ± 78 *832-1098*	970 ± 74 (101) *864-1113*	1082 ± 110* (113) **(↑13%)** *893-1220*	1072 ± 125* (112) **(↑12%)** *928-1285*	1029 ± 172 *710-1488*

#: historical control data (150 female animals; studies conducted between 2007 and 2016); ##: in italic: range of individual values; * and **: p≤0.05 and p≤0.01(compared with the concurrent controls, Dunnett's test or Dunnett-type test)

Clinical chemistry parameters

Statistically significant and dose dependent increases in γ-glutamyl transpeptidase (GGTP) (by 286% at 4000 ppm in males; by 175% and 425% at 2000 and 4000 ppm respectively in females) and total cholesterol (by 55% and 86% at 2000 and 4000 ppm respectively in females) were observed compared to controls. From 2000 ppm, there were statistically significant increases in total protein and globulin levels in females. In addition, there was a significant decrease in albumin/globulin ratio in females from the mid dose. Given the statistical significance, dose dependency and the magnitude of the changes, these alterations were considered treatment related and adverse.

A decrease in total bilirubin (by 33% and 17% at 2000 and 4000 ppm in males; by 29%, 43% and 43% at 500, 2000 and 4000 ppm respectively in females) was not considered to be toxicologically significant given the direction of change (a decrease rather than an increase) and the lack of dose-response in males. The increases in calcium (by 4%) and potassium (by 6%) seen in the high dose females were also considered treatment related and adverse.

Overall, adverse effects on clinical chemistry parameters indicative of liver damage were noted in males at 4000 ppm and in females from 2000 ppm.

Table 6.8.1-5: Summary of significant changes at haematology [mean ± SD (% of controls)] in rats administered 3'-OH-S-2840 in the diet for 90 days.

Para-meter	Sex and dose level (ppm)							
	Male				Females			
	0	500	2000	4000	0	500	2000	4000
GGTP (U/L)	0.7 ± 0.2	0.7 ± 0.1 (100)	1.3 ± 0.7 (186)	2.7 ± 1.1** (386) **(↑286%)**	0.8 ± 0.4	1.0 ± 0.4 (125)	2.2 ± 0.8** (275) **(↑175%)**	4.2 ± 1.5** (525) **(↑425%)**
T.Chol (mg/dL)	53 ± 13	51 ± 9 (96)	52 ± 9 (98)	57 ± 9 (108)	44 ± 11	47 ± 7 (107)	68 ± 13** (155) **(↑55%)**	82 ± 13** (186) **(↑86%)**
T.Bil (mg/dL)	0.06 ± 0.01	0.05 ± 0.01 (83)	0.04 ± 0.01** (67) **(↓33%)**	0.05 ± 0.01* (83) **(↓17%)**	0.07 ± 0.01	0.05 ± 0.01* (71) **(↓29%)**	0.04 ± 0.01** (57) **(↓43%)**	0.04 ± 0.01** (57) **(↓43%)**
Ca## (mg/dL)	9.9 ± 0.2	9.7 ± 0.3 (98)	9.9 ± 0.2 (100)	9.8 ± 0.4 (99)	9.7 ± 0.4 *9.0-10.3*	9.7 ± 0.4 (100) *9.1-10.5*	10.1 ± 0.3 (104) *9.4-10.5*	10.1 ± 0.4* (104) **(↑4%)** *9.3-10.6*
K## (mEq/L)	3.72 ± 0.11	3.70 ± 0.21 (99)	3.83 ± 0.21 (103)	3.70 ± 0.20 (99)	3.35 ± 0.19 *3.03-3.69*	3.42 ± 0.12 (102) *3.28-3.68*	3.51 ± 0.11 (105) *3.37-3.60*	3.55 ± 0.18* (106) **(↑6%)** *3.22-3.88*
Cl## (mEq/L)	107.6 ± 1.0	107.5 ± 0.7 (100)	107.3 ± 0.9 (100)	106.6 ± 1.4 (99)	110.2 ± 1.3 *107.7-112.7*	109.7 ± 1.1 (100) *108.3-111.9*	109.3 ± 0.8 (99) *108.0-110.3*	108.3 ± 0.7** (98) **(↓2%)** *107.1-109.2*

TP (g/dL)	6.02 ± 0.18	5.80 ± 0.17 (96)	6.00 ± 0.26 (100)	5.92 ± 0.28 (98)	6.12 ± 0.38	6.12 ± 0.22 (100)	6.61 ± 0.32** (108) **(↑8%)**	6.61 ± 0.45** (108) **(↑8%)**
Alb (g/dL)	4.11 ± 0.16	3.98 ± 0.17	4.03 ± 0.16	4.12 ± 0.22	4.75 ± 0.27	4.64 ± 0.25	4.87 ± 0.36	4.86 ± 0.44
Glob## (g/dL)	1.91 ± 0.21	1.82 ± 0.24 (95)	1.97 ± 0.22 (103)	1.80 ± 0.16 (94)	1.36 ± 0.16 *1.06-1.54*	1.48 ± 0.14 (109) *1.19-1.64*	1.74 ± 0.17** (128) **(↑28%)** *1.52-1.98*	1.75 ± 0.22** (129) **(↑29%)** *1.50-2.18*
A/G	2.18 ± 0.26	2.22 ± 0.33 (102)	2.06 ± 0.24 (94)	2.30 ± 0.21 (106)	3.52 ± 0.39	3.18 ± 0.43 (90)	2.84 ± 0.40** (81) **(↓19%)**	2.83 ± 0.47** (80) **(↓20%)**

Gross pathology

At necropsy, dark-coloured (3 of 10 in males) livers were observed at 4000 ppm.

Organ weights

At 2000 and 4000 ppm, an adverse increase (≥25%) in relative liver weight was observed in females. This correlated with the coagulation, clinical chemistry and histopathological changes (see below) observed at these doses.

At 2000 and 4000 ppm, increases in absolute and relative (statistically significant at 4000 ppm) adrenal weights were noted in females. The effect on the weights of the adrenals at the top dose is considered treatment related and adverse due to the associated histopathological changes.

Increased testes weight observed in the top dose males is not considered adverse due to the lack of associated histopathological findings.

Overall, adverse increases in liver weight were seen in females from 2000 ppm. In addition, adverse effects on adrenal weights were seen at 4000 ppm in females.

Table 6.8.1-6: Summary of significant changes in absolute and relative organ weights in rats administered 3'-OH-S-2840 in the diet for 90 days.

Organ	Sex and dose level (ppm)							
	Male				Female			
	0	500	2000	4000	0	500	2000	4000

Terminal body weight (g)	408 ± 24	402 ± 37 (99)	410 ± 23 (100)	378 ± 34 (93)	229 ± 13	232 ± 15 (101)	235 ± 11 (103)	226 ± 6 (99)
Liver – abs (g)	9.90 ± 0.86	10.15 ± 1.05 (103)	10.84 ± 1.11 (109)	9.98 ± 0.94 (101)	5.58 ± 0.39	5.89 ± 0.32 (106)	7.17 ± 0.58** (128) **(↑28%)**	7.10 ± 0.55** (127) **(↑27%)**
Liver - rel	2.42 ± 0.11	2.52 ± 0.11 (104)	2.64 ± 0.17** (109) **(↑9%)**	2.64 ± 0.09** (109) **(↑9%)**	2.44 ± 0.12	2.54 ± 0.12 (104)	3.06 ± 0.27** (125) **(↑25%)**	3.15 ± 0.28** (129) **(↑29%)**
Spleen – abs (mg)	597 ± 64	660 ± 42* (111) **(↑11%)**	626 ± 35 (105)	583 ± 85 (98)	426 ± 39	464 ± 38 (109)	456 ± 32 (107)	425 ± 47 (100)
Spleen – rel	0.15 ± 0.02	0.17 ± 0.02* (113) **(↑13%)**	0.15 ± 0.01 (100)	0.15 ± 0.02 (100)	0.19 ± 0.02	0.20 ± 0.01 (105)	0.19 ± 0.02 (100)	0.19 ± 0.02 (100)
Adrenals – abs (mg)	62.4 ± 7.6	65.9 ± 5.8 (106)	68.8 ± 7.7 (110)	63.4 ± 6.6 (102)	68.2 ± 6.3	72.5 ± 9.8 (106)	79.0 ± 9.5* (116) **(↑16%)**	77.5 ± 6.1* (114) **(↑14%)**
Adrenals – rel	0.015 ± 0.002	0.016 ± 0.002 (107)	0.017 ± 0.002 (113)	0.017 ± 0.002 (113)	0.30 ± 0.004	0.031 ± 0.003 (103)	0.034 ± 0.004 (113)	0.034 ± 0.003* (113) **(↑13%)**
Testes – rel	0.84 ± 0.06	0.84 ± 0.10 (100)	0.84 ± 0.04 (100)	0.95 ± 0.10* (113) **(↑13%)**				

* and **: p≤0.05 and p≤0.01 (Dunnett's test or Dunnett-type test)

Histopathology

Hypertrophy in the hepatocytes of female animals was observed at 2000 and 4000 ppm. At 4000 ppm, increased fine vacuolisation of cortical cells of the adrenal gland was observed in both males and females. Vacuolation of the interstitial gland of the ovary was observed in females at 2000 and 4000 ppm.

Overall, histopathological findings were observed in the liver and ovary of females from 2000 ppm and in the adrenal gland of males and females at 4000 ppm.

Table 6.8.1-7: Selected histopathological findings in rats administered 3'-OH-S-2840 in the diet for 90 days.

Organ & lesion	Sex and dose level (ppm)	
	Male	Female

	0	500	2000	4000	0	500	2000	4000
Liver – No. examined	10	0	0	10	10	10	10	10
Centrilobular hepatocyte hypertrophy	0	0	0	0	0	0	3	5*
Adrenal – No. examined	10	10	10	10	10	10	10	10
Increased cortical cell vacuolation	1	0	1	6	0	0	0	4
Ovary – No. examined					10	10	10	10
Interstitial gland vacuolation					0	0	2	5*

*: p≤0.05 (Fisher's exact probability test)

Conclusion

In conclusion, in a GLP and guideline study, dietary administration of 3'-OH-S-2840 for 90 days to male and female Wistar Hannover rats at dietary concentrations of 0, 500, 2000, or 4000 ppm (mean substance intakes: 0, 32.2, 128 and 258 mg/kg bw/day for males and 0, 37.9, 157 and 291 mg/kg bw/day for females) caused adverse effects from 2000 ppm (157 mg/kg bw/day) mainly in females, including effects on clinical-chemistry parameters indicative of liver damage, increased liver weights and histopathological findings of the liver and ovary. In addition, there were adverse effects on bodyweight, coagulation parameters, and adrenal weights at 4000 ppm (258 mg/kg bw/day) in both sexes.

Overall, a NOAEL of 500 ppm (37.9 mg/kg bw/day) can be established from this study based on the effects observed on clinical-chemistry parameters indicative of liver damage, increased liver weight and, histopathological findings of the liver and ovary at the LOAEL of 2000 ppm (157 mg/kg bw/day).

[redacted] (2018)

3.Reverse mutation test of 3'-OH-S-2840 in bacterial systems

Reference:	KCA 5.8.1.1/03
Report Title:	3'-OH-S-2840: Bacterial Reverse Mutation Test
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. IET 16-0064. The Institute of Environmental Toxicology, Japan
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160520-1 Purity: 99.6%

Method of analysis:	Not required
Guideline(s):	OECD 471 (1997)
Deviations from current guideline:	For the WP2*uvr*A strain, AF-2 was used as positive control without S9. AF-2 is the positive control recommended in the test guideline for the strains with plasmids whereas WP2*uvr*A does not contain any plasmids (Sugiyama et al., 2016[4]). No justification available in the study report.
Impact of the deviation:	The justification provided by the applicant is acceptable with reference to a published paper for the use of AF-2 as the positive control for the strain WP2*uvr*A[5].Therefore, the observed deviation does not impact the integrity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The mutagenic potential of 3'-OH-S-2840 was investigated in an OECD and guideline Ames test using *Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (strain WP2uvrA). The test was performed following the pre-incubation method. 3'-OH-S-2840 in DMSO was tested in the presence and absence of S9 from 313 - 5000 µg/plate for all the strains. Concentrations were chosen based on the results of a range-finding assay. Appropriate positive controls were used.

Results

In the range-finding assay, precipitation of 3'-OH-S-2840 was observed at and above 150 µg/plate in the absence of S9 and at and above 1500 µg/plate in the presence of S9. No cytotoxicity was observed in any of the strains at any of the tested concentrations. The results are summarized in table 6.8.1-9. No increase in revertant colonies were seen in any strain with or without metabolic activation.

[4] The strains recommended for use in the bacterial reverse mutation test (OECD guideline 471) can be certified as non-genetically modified organisms (biomedcentral.com)

[5] Negative and positive control ranges in the bacterial reverse mutation test: JEMS/BMS collaborative study - PMC (nih.gov).

Table 6.8.1-8: Reverse mutation test of 3'-OH-S-2840 - Range finding assay

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	WP2*uvr*A	TA98	TA1537
Without metabolic activation					
Vehicle control	125 ± 21	8 ± 3	16 ± 3	14 ± 4	6 ± 2
1.50	110 ± 18	8 ± 3	18 ± 5	17 ± 1	6 ± 3
5	105 ± 11	8 ± 2	15 ± 6	13 ± 7	5 ± 2
15	103 ± 3	6 ± 2	17 ± 8	12 ± 3	4 ± 2
50	116 ± 15	6 ± 1	18 ± 4	12 ± 3	9 ± 2
150 [a]	107 ± 3	5 ± 3	20 ± 3	16 ± 2	4 ± 1
500 [a]	103 ± 14	9 ± 4	19 ± 3	12 ± 5	4 ± 1
1500 [a]	116 ± 9	6 ± 2	18 ± 4	15 ± 3	3 ± 2
5000 [a]	120 ± 7	8 ± 4	14 ± 2	17 ± 4	4 ± 1
Positive control	585 ± 43	501 ± 9	169 ± 7	443 ± 13	570 ± 290
With metabolic activation					
Vehicle control	98 ± 4	6 ± 4	17 ± 4	24 ± 7	11 ± 1
1.50	118 ± 9	8 ± 4	14 ± 1	19 ± 4	9 ± 2
5	93 ± 3	5 ± 1	21 ± 1	14 ± 0	9 ± 2
15	115 ± 5	4 ± 3	19 ± 10	23 ± 3	11 ± 6
50	118 ± 16	7 ± 1	17 ± 2	22 ± 3	12 ± 5
150	124 ± 15	6 ± 3	23 ± 6	22 ± 5	9 ± 6
500	88 ± 10	7 ± 2	25 ± 7	20 ± 7	11 ± 2
1500 [a]	104 ± 26	7 ± 5	19 ± 4	21 ± 6	9 ± 1
5000 [a]	116 ± 16	4 ± 0	20 ± 4	24 ± 6	7 ± 1
Positive control	1228 ± 56	128 ± 8	180 ± 31	251 ± 10	66 ± 11

[a] Precipitation of the test substance was observed; The results were shown as Mean ± SD (n=3).

In the confirmatory assay, there was no statistically significant, concentration dependent increase in the number of revertant colonies after 3'-OH-S-2840 treatment in the presence or absence of S9. Positive controls showed the expected results. The results are summarized in the table below.

Table 6.8.1-9: Reverse mutation test of 3'-OH-S-2840- pre-incubation method

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	WP2*uvr*A	TA98	TA1537
Without metabolic activation					
Vehicle control	124 ± 21	16 ± 2	17 ± 3	16 ± 3	6 ± 4
313 [a]	131 ± 8	12 ± 2	16 ± 3	19 ± 2	4 ± 2
625 [a]	125 ± 6	12 ± 3	19 ± 6	11 ± 3	4 ± 3
1250 [a]	121 ± 20	9 ± 2	14 ± 4	13 ± 1	2 ± 2
2500 [a]	106 ± 3	14 ± 2	18 ± 7	15 ± 5	3 ± 1
5000 [a]	128 ± 13	15 ± 5	13 ± 9	15 ± 2	5 ± 0
Positive control	665 ± 44	472 ± 19	164 ± 27	469 ± 7	573 ± 206
With metabolic activation					
Vehicle control	99 ± 6	11 ± 2	24 ± 6	21 ± 4	8 ± 2

313	127 ± 23	8 ± 3	15 ± 7	22 ± 2	11 ± 4
625	121 ± 20	12 ± 1	21 ± 4	19 ± 4	7 ± 3
1250 [a]	120 ± 20	10 ± 1	20 ± 9	18 ± 3	6 ± 3
2500 [a]	123 ± 15	10 ± 0	16 ± 3	16 ± 4	10 ± 2
5000 [a]	114 ± 21	8 ± 1	20 ± 2	18 ± 2	8 ± 3
Positive controls	1240 ± 89	142 ± 13	233 ± 10	235 ± 20	75 ± 13
[a] Precipitation of the test substance was observed; The results were shown as Mean ± SD (n=3).					

Conclusion

Under the conditions of this GLP and guideline Ames test, 3'-OH-S-2840 did not induce an increase in revertant colonies with or without metabolic activation up to the limit concentration or concentrations causing precipitation. Therefore, it is concluded that 3'-OH-S-2840 is not mutagenic in bacteria.

[redacted] (2017a)

4. In vitro gene mutation test for 3'-OH-S-2840 in Chinese hamster lung fibroblast cells (V79)

Reference:	KCA 5.8.1.1/04
Report Title:	3'-OH-S-2840: Gene Mutation Assay in Chinese Hamster V79 Cells In Vitro (V79/HPRT)
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. 1813701 Envigo CRS GmbH, Germany,
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160520-1 Purity: 99.6%
Method of analysis:	Not required
Guideline(s):	OECD 476 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The potential of 3'-OH-S-2840 to induce gene mutations at the HPRT locus was investigated in the presence and absence of metabolic activation (S9) in a GLP and guideline study in cultured Chinese hamster V79 cells. The appropriate concentrations for the gene mutation assay were selected based on the relative survival (RS) (measured as cloning efficiency) of the treated cells and precipitation of 3'-OH-S-2840 in a preliminary assay. In both the first and second main experiments, the cells were exposed to 3'-OH-S-2840 in DMSO at 3.8-120 µg/mL for 4 h with and without S9. Mutant frequencies were evaluated at the concentrations of 3.8, 7.5, 15, 30 and 60 µg/mL. All the investigations required by the guideline were performed and appropriate controls were used.

Results

A preliminary cytotoxicity assay was performed at concentrations ranging from 3.8-480 µg/mL to set the top concentrations for the gene mutation assay. In all the treatment groups, precipitation was observed at and above 30 µg/mL (microscopically) and there was no cytotoxicity (RS<50%) observed up to the highest tested concentration both in the presence and absence of S9.

In the main mutation assay, 3'-OH-S-2840 treatment did not produce any biologically relevant, reproducible (between cultures) or concentration-related increases in the number of mutant colonies in the presence or absence of metabolic activation compared to the concurrent negative control. Cytotoxicity was not observed, but precipitation was noted from 30 or 60 µg/mL with or without S9. Positive and negative controls produced the expected mutant colonies, and these were within the laboratory HCD ranges. Results from the mutation assays are presented in table 6.8.1-10.

Table 6.8.1-10: Summary of results of Experiment I and II for gene mutations at the HPRT locus in Chinese hamster V79 cells treated with 3'-OH-S-2840.

Conc. µg/mL	S9 mix	Rel. clon. eff. I %	Rel. cell density %	Relative adjusted CE1 %	Mutant colonies / 10^6 cells	Rel. clon. eff. I %	Rel. cell density %	Relative adjusted CE1 %	Mutant colonies / 10^6 cells
Exp. I / 4 h treatment		Culture I				Culture II			
Neg C	-	96.4	111.0	107.0	11.1	97.7	77.8	76.0	13.9
Solv C	-	100.0	100.0	100.0	18.8	100.0	100.0	100.0	22.8
3.8	-	102.9	107.3	110.3	20.1	98.2	81.8	80.4	11.4
7.5	-	82.5	106.0	87.5	13.4	102.1	57.0	58.2	24.4

15	-	99.9	104.4	104.3	12.5	97.9	69.7	68.3	21.6
30Pm	-	105.0	125.5	131.8	11.8	101.3	104.8	106.2	16.9
60PM Pm	-	93.3	112.9	105.4	20.5	101.7	67.2	68.4	23.8
120P M Pm	-	87.5	117.7	103.0	#	100.2	77.6	77.8	#
Pos C	-	77.7	141.8	110.2	369.2	100.1	82.8	82.9	213.6
Exp. I / 4 h treatment		**Culture I**				**Culture II**			
Neg C	+	100.0	119.1	119.1	18.0	94.4	113.8	107.3	21.9
Solv C	+	100.0	100.0	100.0	15.1	100.0	100.0	100.0	18.1
3.8	+	100.8	114.2	115.1	28.3	97.9	102.2	100.1	24.5
7.5	+	42.6	133.2	56.7	24.3	100.7	109.8	110.6	18.3
15	+	46.2	91.1	42.1	16.9	100.6	60.8	61.1	29.3
30Pm PMI	+	91.3	91.1	83.2	28.5	106.1	55.9	59.3	31.1
60 PM Pm	+	112.4	54.5	61.2	18.1	102.3	96.9	99.1	21.2
120 PM Pm	+	83.7	58.3	48.8	#	106.4	106.0	112.8	#
Pos C	+	87.7	116.2	101.9	174.8	95.3	91.9	87.6	150.9
Exp. II / 4 h treatment		**Culture I**				**Culture II**			
Neg C	-	113.8	103.9	118.3	10.5	97.0	84.0	81.5	15.7
Solv C	-	100.0	100.0	100.0	25.4	100.0	100.0	100.0	20.6
3.8	-	105.1	93.9	98.7	20.0	101.8	66.3	67.5	16.5
7.5	-	116.3	89.8	104.5	17.6	103.8	68.8	71.4	10.1
15	-	94.1	86.2	81.1	24.4	102.2	81.2	82.9	29.0
30	-	67.4	93.4	63.0	9.3	95.3	65.5	62.5	7.1
60 PM Pm	-	107.0	86.8	92.9	0.9	106.4	107.8	114.7	16.6
120 PM Pm	-	90.7	76.6	69.5	#	100.8	57.8	58.3	#
Pos C	-	116.3	91.7	106.7	205.9	93.9	99.5	93.5	196.9
Exp. II / 4 h treatment		**Culture I**				**Culture II**			
Neg C	+	108.9	125.7	137.0	23.6	112.4	89.7	100.8	11.1
Solv C	+	100.0	100.0	100.0	16.2	100.0	100.0	100.0	20.2
3.8	+	81.6	117.6	96.0	17.6	102.5	102.7	105.3	21.9
7.5	+	78.6	126.3	99.3	15.5	94.0	77.9	73.2	19.0
15	+	106.8	107.0	114.3	22.7	101.9	109.1	111.1	21.1
30	+	75.9	92.4	70.2	20.8	98.4	95.9	94.3	27.2
60 PM Pm	+	70.2	83.1	58.4	19.6	91.8	99.7	91.5	22.7

120 PM Pm	+	138.0	63.1	87.1	#	88.3	98.9	87.3	#
Pos C	+	67.8	137.1	92.9	61.4	103.4	111.1	114.8	56.9

Rel. clon. eff.: relative cloning efficiency; Neg C: negative control – medium; Solv C: solvent control – DMSO; Pos C: positive control – EMS without S9 mix or DMBA with S9 mix;
PM: precipitation visible to the naked eye at the end of treatment; Pm: precipitation visible microscopically at the end of treatment; PMI: precipitation observed only in Culture I; #: culture was not continued to avoid analysis of too many precipitating concentrations

Conclusion

In this GLP and OECD compliant *in vitro* mammalian gene mutation study, 3'-OH-S-2840 was negative up to concentrations causing precipitation. Therefore, 3'-OH-S-2840 is non-mutagenic in this assay.

[redacted] (2017a)

5. In vitro Chromosomal Aberration test for 3'-OH-S-2840 in Cultured Mammalian Cells

Reference:	KCA 5.8.1.1/05
Report Title:	3'-OH-S-2840: Chromosome Aberration Test in Cultured Mammalian Cells
Author(s) & Year:	[redacted] (2017b)
Document No, Authority registration No	Study No. IET 16-0065 The Institute of Environmental Toxicology, Japan
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160520-1 Purity: 99.6%
Method of analysis:	Not required
Guideline(s):	OECD 473 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP

Acceptability:	Yes
Study relied upon:	Yes

Methods

The clastogenic potential of 3'-OH-S-2840 in the presence and absence of metabolic activation (S9) was investigated in a GLP and guideline study using Chinese hamster lung (CHL/IU) cells. Cytotoxicity tests were performed to choose the relevant concentrations for the chromosomal aberration tests. The relative increase in cell count (RICC) was used as the measure of cytotoxicity. The lowest concentration at which the RICC was 50% or lower was chosen as the maximum concentration for the chromosomal aberration test. In the initial chromosomal aberration test (Experiment I- short term treatment method), CHU/IL cells were exposed to inpyrfluxam in DMSO at concentrations of 25, 50, 100 and 200 µg/mL with S9, and 12.5, 25, 50 and 50 µg/mL without S9 for 6 h followed by an 18 h recovery period. In the second experiment (Experiment II- continuous treatment method), cells were treated continuously with the test substance for 24 h in the absence of S9 at concentrations of 3.13, 6.25, 12.5, 25 and 50 µg/mL. All the investigations required by the guideline were performed and appropriate controls were used.

Results

In the preliminary cytotoxicity test, precipitation was observed at and above 200 µg/mL both in the presence and absence of S9. Marked cytotoxicity (≤ 50% in RICC) was observed at and above 400 µg/mL in the absence of metabolic activation and at 800 µg/mL in the presence of metabolic activation in the short-term treatment, and at and above 100 µg/mL in the continuous treatment. The results are summarised in the table 6.8.1-11.

Table 6.8.1-11: Preliminary cytotoxicity test of 3'-OH-S-2840 in Chinese hamster lung (CHL/IU) cells

Concentration (µg/mL)	% Relative increase in cell count (RICC) compared to control		
	6 h treatment		24 h treatment
	-S9	+S9	-S9
Solvent control	100	100	100
3.13	90	107	100
6.25	93	103	88
12.5	96	94	82
25	92	98	63
50	82	96	58 [a]
100	56 [a]	92	46 [a]
200	57 [b]	68 [b]	49 [b]
400	-2 [b]	75 [b]	-59 [b]

800	-65 [b]	16 [b]	-102 [b]

[a]: precipitate was observed at the end of treatment; [b]: precipitate was observed at the beginning and at the end of treatment

In the main chromosome aberration test, no statistically significant or biologically relevant increase in structural alterations (excluding gaps) or polyploid and endoreduplicated cells was observed in the treated groups compared to controls in the presence and absence of metabolic activation in experiment I and in the absence of metabolic activation in experiment II up to concentrations causing precipitation and occasionally cytotoxicity.

All negative and positive controls gave the expected results which were within the laboratory HCD. Tables 6.8.1-12 to 6.8.1-14 summarise the results of the chromosomal aberration tests performed.

Table 6.8.1-12: Experiment I - Cytogenetic assay of 3'-OH-S-2840 in CHL/IU cells without metabolic activation (-S9) – 6 h treatment and 18 h recovery.

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (No. (%))	Cells with structural aberrations (No. (%))	
				With gaps	Without gaps
Solvent control	300	100	2 (0.7)	5 (1.7)	2 (0.7)
25 µg/mL	300	93	0 (0)	2 (0.7)	2 (0.7)
50 µg/mL	300	80	2 (0.7)	2 (0.7)	0 (0)
100 µg/mL [a]	300	61	0 (0)	2 (0.7)	2 (0.7)
Positive control (MMC)	300	37	0 (0)	191 (63.7)	186 (62.0)**

[a]: precipitate was observed at the end of treatment; **: $p \leq 0.01$

Table 6.8.1-13: Experiment I - Cytogenetic assay of 3'-OH-S-2840 in CHL/IU cells with metabolic activation (+S9) - 6 h treatment and 18 h recovery.

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (No. (%))	Cells with structural aberrations (No. (%))	
				With gaps	Without gaps
Solvent control	300	100	0 (0)	1 (0.3)	0 (0)
50 µg/mL	300	98	0 (0)	4 (1.3)	4 (1.3)
100 µg/mL	300	79	2 (0.7)	3 (1.0)	1 (0.3)
200 µg/mL [a]	300	62	3 (1.0)	2 (0.7)	2 (0.7)

Positive control (B[a]P)	300	45	2 (0.7)#	58 (19.3)	56 (18.7)**

[a]: precipitate was observed at the beginning and at the end of treatment; #: endoreduplication**: p ≤ 0.01

Table 6.8.1-14: Experiment II - Cytogenetic assay of 3'-OH-S-2840 in CHL/IU cells without metabolic activation (-S9) - 24 h treatment

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (No. (%))	Cells with structural aberrations (No. (%))	
				With gaps	Without gaps
Solvent control	300	100	1 (0.3)	1 (0.3)	1 (0.3)
12.5 µg/mL	300	75	0 (0)	1 (0.3)	1 (0.3)
25 µg/mL	300	62	1 (0.3)	3 (1.0)	2 (0.7)
50 µg/mL [a]	300	45	1 (0.3)	5 (1.7)	2 (0.7)
Positive control (MMC)	300	43	0 (0)	193 (64.3)	189 (63.0)**

[a]: precipitate was observed at the end of treatment; **: p ≤ 0.01

Conclusion

In this GLP and guideline in vitro chromosomal aberration test, 3'-OH-S-2840 was negative up to concentrations causing precipitation. Therefore, it is concluded that 3'-OH-S-2840 is not a clastogen in this assay.

[redacted] (2017b)

6. In vitro micronucleus test for 3'-OH-S-2840 in Cultured Mammalian Cells

Reference:	KCA 5.8.1.1/06
Report Title:	In Vitro Micronucleus (MNvit) Test of 3'-OH-S-2840 in Human Lymphoblast Cell Line (TK6)

Author(s) & Year:	[redacted] (2020a)
Document No, Authority registration No	Study No. J352 (028-225) Biosafety Research Center Inc (BSRC)
Substance used:	Test Material: 3'-OH-S-2840 Lot/Batch: 089-160914-1 Purity: 99.7%
Method of analysis:	Not required
Guideline(s):	OECD 487 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The potential of 3'-OH-S-2840 to induce micronuclei in vitro was investigated in a GLP and guideline study using a human lymphoblast cell line (TK6). Cell growth inhibition tests were performed to choose the relevant concentrations for the micronucleus test. The replication index (RI) was used to calculate the cell growth inhibition (% cytostasis = 100-RI). Cytotoxicity is considered sufficiently addressed when % cytostasis is ≥ 50%.

In the initial chromosomal aberration test (Experiment I- short term treatment), TK6 cells were exposed to 3'-OH-S-2840 in DMSO at concentrations of 12.5, 25.0, 50.0 and 100 µg/mL in the presence and absence of S9 for 3 h followed by a 24 h recovery period. In the second experiment (Experiment II- continuous treatment), cells were treated continuously with the test substance for 24 h in the absence of S9 at concentrations of 6.25, 12.5, 25.0, 37.5, 50.0, 75.0 and 100 µg/mL. Micronucleus analysis was conducted at 25.0, 50.0 and 100 µg/mL for the short term treatment assays and at 6.25, 25.0 and 50.0 µg/mL for the continuous treatment assay. All the investigations required by the guideline were performed and appropriate controls were used.

Results

In the preliminary cytotoxicity test, cell growth inhibition ≥ 50% was observed at and above 50.0 µg/mL in the 24 h assay. In the short-term assays in the presence and absence of S9, there was no cell growth inhibition ≥ 50% at any of the tested concentrations. At the start and end of the treatment, precipitation of the test substance was observed at 100 µg/mL in all assays.

In the micronucleus test, no statistically significant or biologically relevant increase in the number of micronucleated binucleate cells (MNBN) was observed in the treated groups compared to controls in the presence and absence of metabolic activation in experiment I up to precipitation and in the absence of metabolic activation in experiment II up to an appropriate level of cytotoxicity and precipitation.

All negative and positive controls gave expected results which were within the laboratory HCD. Tables 6.8.1-15 to 6.8.1-17 summarise the results of the micronulei tests performed.

Table 6.8.1-15: Experiment I – Micronucleus test of 3'-OH-S-2840 in TK6 cells without metabolic activation (-S9) – 3 h treatment and 24 h recovery.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
						Individual	mean
DMSO	0	-	1	1000	5	0.50	0.55
			2	1000	6	0.60	
3'-OH-S-2840	12.5	-2.9	1	NA	-	-	-
			2	NA	-	-	
	25.0	2.8	1	1000	7	0.70	0.55
			2	1000	4	0.40	
	50.0	1.9	1	1000	5	0.50	0.65
			2	1000	8	0.80	
	100 +	19.9	1	1000	9	0.90	0.75
			2	1000	6	0.60	
MMC	0.3	52.6	1	1000	21	2.10	2.55*
			2	1000	30	3.00	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL) b
i-N: Binucleate, MNBN: Micronucleated binucleate cells
MMC: Positive control (Mitomycin C)
NA: Not analyzed
+: Precipitation was observed under microscope at the end of treatment period.
*: Significant difference from negative control (Fisher's exact test):p<0.025

Table 6.8.1-16: Experiment I – Micronucleus test of 3'-OH-S-2840 in TK6 cells with metabolic activation (+S9) – 3 h treatment and 24 h recovery.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
						Individual	mean
DMSO	0	-	1	1000	8	0.80	0.80
			2	1000	8	0.80	
3'-OH-S-2840	12.5	2.3	1	NA	-	-	-
			2	NA	-	-	
	25.0	3.2	1	1000	9	0.90	0.70
			2	1000	5	0.50	
	50.0	5.5	1	1000	8	0.80	0.65
			2	1000	5	0.50	
	100 +	20.4	1	1000	8	0.80	0.65
			2	1000	5	0.50	
CP	8	65.9	1	1000	22	2.20	2.40*
			2	1000	26	2.60	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL) b
i-N: Binucleate, MNBN: Micronucleated binucleate cells
CP: Positive control (Cyclophosphamide)
NA: Not analyzed
+: Precipitation was observed under microscope at the end of treatment period.
*: Significant difference from negative control (Fisher's exact test): $p<0.025$

Table 6.8.1-17: Experiment II – Micronucleus test of 3'-OH-S-2840 in TK6 cells without metabolic activation (-S9) –24 h treatment.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
						Individual	Mean
DMSO	0	-	1	1000	7	0.70	0.80
			2	1000	9	0.90	
3'-OH-S-2840	6.25	9.0	1	1000	6	0.60	0.65
			2	1000	7	0.70	
	12.5	17.9	1	NA	-	-	-
			2	NA	-	-	
	25.0	33.6	1	1000	7	0.70	0.65
			2	1000	6	0.60	
	37.5	44.1	1	NA	-	-	-
			2	NA	-	-	
	50.0	53.9	1	1000	9	0.90	0.85
			2	1000	8	0.80	
	75.0 +	64.2	1	NA	-	-	-
			2	NA	-	-	
	100 +	65.4	1	NA	-	-	-
			2	NA	-	-	
COL	0.005	12.0	1	1000	23	2.30	2.35*
			2	1000	24	2.40	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL)
bi-N: Binucleate, MNBN: Micronucleated binucleate cells
COL: Positive control (Colchicine)
NA: Not analyzed
+: Precipitation was observed under microscope at the end of treatment period.
*: Significant difference from negative control (Fisher's exact test):p<0.025

Conclusion

In this GLP and guideline in vitro micronucleus test, 3'-OH-S-2840 was negative up to cytotoxic/precipitating concentrations. Therefore, it is concluded 3'-OH-S-2840 is not clastogenic or aneugenic in this assay.

[redacted] (2020a)

Summary data for 3'-OH-S-2840

3'-OH-S-2840 was not acutely toxic by the oral route (LD50 > 2000 mg/kg bw); therefore compared to inpyrfluxam (LD50 = 180 mg/kg bw), 3'-OH-S-2840 is less acutely toxic than the parent substance by the oral route. 3'-OH-S-2840 showed a qualitatively and quantitatively similar toxicological profile to that of the parent substance in a 90-day rat study (NOAEL of 500 ppm - 31.7 mg/kg bw/day based on liver effects for inpyrfluxam). A NOAEL of 500 ppm (37.9 mg/kg bw/day) was identified for 3'-OH-S-2840 based mainly on liver effects at the LOAEL of 2000 ppm (157 mg/kg bw/day). 3'-OH-S-2840 was not mutagenic in an appropriate package of in vitro tests.

1'-COOH-S-2840

1.Acute oral toxicity study of 1'-COOH-S-2840 in rats

Reference:	KCA 5.8.1.2/01
Report Title:	Acute Oral Toxicity Study of 1'-COOH-S-2840 in Rats
Author(s) & Year:	[redacted] (2017c)
Document No, Authority registration No	Study No. 4369 [redacted]
Substance used:	Test Material: 1'-COOH-S-2840 Lot/Batch: 16SC8535304 Purity: 99.8%
Method of analysis:	Not required
Guideline(s):	OECD 423 (2001)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes
Study relied upon:	Yes

The acute oral toxicity of 1'-COOH-S-2840 was assessed in a GLP and guideline study using the toxic class method. Two groups (first and second) of three female RccHan Wistar rats were administered 2000 mg/kg bw of 1'-COOH-S-2840 via oral gavage. All the investigations required by the guideline were performed.

The dose level was selected based on a previous oral toxicity study where there were no mortalities or clinical signs of toxicity at 50 mg/kg bw or 300 mg/kg bw (not submitted).

No mortality, clinical signs of toxicity, macroscopic pathological findings or changes in bodyweight or body weight gain were noted in any of the groups.

Under the conditions of this GLP and guideline study, the oral LD50 of 1'-COOH-S-2840 was >2000 mg/kg bw in female RccHan:WIST rats. 1'-COOH-S-2840 is not acutely toxic via oral route and does not meet the criteria for classification for acute oral toxicity according to Regulation 1272/2008 as it applies in GB.

[redacted] (2017b)

2.Reverse mutation test of 1'-COOH-S-2840 in bacterial systems

Reference:	KCA 5.8.1.2/02
Report Title:	1'-COOH-S-2840: Bacterial Reverse Mutation Test
Author(s) & Year:	[redacted] (2017)
Document No, Authority registration No	Study No. 4366. Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material: 1'-COOH-S-2840 Lot/Batch: 16SC8535304 Purity: 99.8%
Method of analysis:	Not required
Guideline(s):	OECD 471 (1997)
Deviations from current guideline:	For the WP2*uvr*A strain, AF-2 was used as positive control without S9. AF-2 is the positive control recommended in the test guideline for the strains with plasmids whereas WP2*uvr*A does not contain any plasmids (Sugiyama et al., 2016[6]). No justification available in the study report.
Impact of the deviation:	The justification provided by the applicant is acceptable with reference to a published paper for the use of AF-2 as the positive

[6] The strains recommended for use in the bacterial reverse mutation test (OECD guideline 471) can be certified as non-genetically modified organisms (biomedcentral.com)

	control for the strain WP2*uvr*A[7].Therefore, the observed deviation does not impact the integrity of the study.
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The mutagenic potential of 1'-COOH-S-2840 was investigated in a GLP and guideline Ames test using *Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (strain WP2uvrA). The test was performed following the pre-incubation method. 1'-COOH-S-2840 in DMSO was tested in the presence and absence of S9 from 156 - 5000 µg/plate for all the strains. Concentrations were chosen based on the results of a range-finding assay. Appropriate positive controls were used.

Results

In the range-finding assay, precipitation of 1'-COOH-S-2840 was observed at and above 2500 µg/plate in the presence of S9. No cytotoxicity was observed in any of the strains at any of the tested concentrations. The results are summarized in table 6.8.1-26. No increase in revertant colonies were seen in any strain with or without metabolic activation.

Table 6.8.1-18: Reverse mutation test of 1'-COOH-S-2840 - Range finding assay

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	WP2*uvr*A	TA98	TA1537
Without metabolic activation					
Vehicle control	144 ± 1.5	9 ± 3.1	25 ± 2.5	35 ± 8.1	8 ± 2.5
156	139 ± 4.9	8 ± 3.0	27 ± 2.9	36 ± 6.1	4 ± 1.2
313	146 ± 17.5	8 ± 4.4	26 ± 2.6	33 ± 1.5	5 ± 1.2
625	144 ± 1.5	10 ± 4.7	26 ± 1.5	35 ± 9.5	8 ± 3.1
1250	144 ± 22.5	8 ± 1.5	19 ± 1.5	35 ± 12.6	10 ± 3.2
2500	133 ± 9.8	9 ± 3.8	17 ± 5.8	35 ± 4.7	11 ± 2.5
5000	136 ± 13.5	3 ± 1.5	17 ± 5.2	28 ± 5.9	10 ± 3.1
Positive controls					
Compound	AF-2	SA	AF-2	AF-2	9-AA
Dose (µg/plate)	0.01	0.5	0.01	0.1	80
Revertants/plate	871 ± 22.0	403 ± 30.1	107 ± 6.8	368 ± 27.0	581 ± 237.5

[7] Negative and positive control ranges in the bacterial reverse mutation test: JEMS/BMS collaborative study - PMC (nih.gov).

With metabolic activation					
Vehicle control	140 ± 17.0	7 ± 1.5	24 ± 3.8	44 ± 8.1	15 ± 2.1
156	138 ± 5.9	8 ± 3.5	32 ± 8.2	43 ± 1.7	13 ± 0.6
313	140 ± 3.5	10 ± 3.5	26 ± 4.4	45 ± 4.9	14 ± 1.5
625	133 ± 11.0	8 ± 2.5	22 ± 3.5	41 ± 2.1	12 ± 0.6
1250	131 ± 19.1	4 ± 1.0	23 ± 5.8	47 ± 12.7	15 ± 4.6
2500 [a]	120 ± 11.4	13 ± 1.7	33 ± 7.5	44 ± 5.3	16 ± 3.6
5000 [a]	124 ± 16.1	10 ± 3.6	25 ± 5.2	43 ± 4.7	11 ± 4.9
Positive controls					
Compound	2-AA	2-AA	2-AA	2-AA	2-AA
Dose (µg/plate)	1	2	10	0.5	2
Revertants/plate	903 ± 117.6	253 ± 13.2	637 ± 25.5	322 ± 18.6	141 ± 11.6
[a] Precipitation of the test substance was observed					

In the confirmatory assay, there was no statistically significant, concentration dependent increase in the number of revertant colonies after 1'-COOH-S-2840 treatment in the presence or absence of S9. Positive controls showed the expected results. The results are summarized in the table below.

Table 6.8.1-19: Reverse mutation test of 1'-COOH-S-2840 – Confirmatory (main) assay

Dose level	Mean number of revertant colonies per plate				
(µg/plate)	TA100	TA1535	WP2*uvr*A	TA98	TA1537
Without metabolic activation					
Vehicle control	138 ± 10.0	13 ± 3.5	20 ± 3.1	34 ± 1.7	10 ± 5.5
156	142 ± 9.5	11 ± 3.6	21 ± 1.2	33 ± 3.1	8 ± 3.8
313	151 ± 4.2	7 ± 3.0	18 ± 4.7	36 ± 1.5	12 ± 2.6
625	145 ± 2.9	11 ± 2.3	22 ± 2.0	36 ± 4.7	7 ± 3.5
1250	145 ± 11.4	5 ± 1.0	18 ± 4.4	28 ± 2.6	7 ± 3.2
2500	137 ± 6.1	13 ± 1.5	19 ± 3.8	37 ± 7.2	7 ± 2.5
5000	135 ± 7.6	9 ± 3.6	22 ± 7.2	30 ± 7.1	7 ± 3.0
Positive controls					
Compound	AF-2	SA	AF-2	AF-2	9-AA
Dose (µg/plate)	0.01	0.5	0.01	0.1	80
Revertants/plate	893 ± 6.2	417 ± 21.4	104 ± 10.0	471 ± 7.5	879 ± 152.2
With metabolic activation					
Vehicle control	143 ± 14.0	13 ± 4.0	29 ± 4.5	40 ± 11.6	12 ± 1.5
156	126 ± 12.7	8 ± 2.3	24 ± 6.4	48 ± 3.0	11 ± 3.1
313	137 ± 20.8	15 ± 2.3	24 ± 2.6	42 ± 6.7	9 ± 5.6
625	121 ± 21.7	7 ± 4.0	23 ± 4.0	44 ± 9.0	13 ± 6.0
1250	128 ± 11.1	13 ± 2.1	26 ± 7.1	44 ± 6.5	11 ± 4.2
2500 [a]	114 ± 7.0	8 ± 2.9	24 ± 5.0	38 ± 7.0	9 ± 5.1
5000 [a]	121 ± 10.4	6 ± 2.1	17 ± 3.5	40 ± 8.2	8 ± 4.0
Positive controls					

Compound	2-AA	2-AA	2-AA	2-AA	2-AA
Dose (μg/plate)	1	2	10	0.5	2
Revertants/plate	851 ± 43.3	235 ± 9.0	544 ± 30.3	278 ± 20.4	134 ± 8.5
[a] Precipitation of the test substance was observed					

Conclusion

Under the conditions of this GLP and guideline Ames test, 1'-COOH-S-2840 was negative with or without metabolic activation up to the limit concentration or concentrations causing precipitation. Therefore, it is concluded that 1'-COOH-S-2840 is not mutagenic in bacteria.

[redacted] (2017)

3. In vitro gene mutation test for 1'-COOH-S-2840 in Chinese hamster lung fibroblast cells (V79)

Reference:	KCA 5.8.1.2/03
Report Title:	1'-COOH-S-2840: Gene Mutation Assay in Chinese Hamster V79 Cells In Vitro (V79/HPRT)
Author(s) & Year:	[redacted] (2017b)
Document No, Authority registration No	Study No. 1813801 Envigo CRS GmbH, Germany,
Substance used:	Test Material: 1'-COOH-S-2840 Lot/Batch: 16SC8535304 Purity: 99.8%
Method of analysis:	Not required
Guideline(s):	OECD 476 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes

Study relied upon:	Yes

Methods

The potential of 1'-COOH-S-2840 to induce gene mutations at the HPRT locus was investigated in the presence and absence of metabolic activation (S9) in a GLP and guideline study using cultured Chinese hamster V79 cells. The appropriate concentrations for the gene mutation assay were selected based on the relative survival (RS) (measured as cloning efficiency) of the treated cells and precipitation of 1'-COOH-S-2840 in a preliminary cytotoxicity test. In both the first and second main experiments, the cells were exposed to 1'-COOH-S-2840 in DMSO at 62.5-2000 µg/mL for 4 h with and without S9. Mutant frequencies were evaluated at the concentrations of 125, 250, 500 and 1000 µg/mL. All the investigations required by the guideline were performed and appropriate controls were used.

Results

A preliminary cytotoxicity assay was performed at concentrations ranging from 15.6-2000 µg/mL to set the top concentrations for the gene mutation assay. In all the treatment groups, precipitation was observed at 2000 µg/mL (microscopically) and there was no cytotoxicity (RS<50%) observed up to the highest tested concentration both in the presence and absence of S9.

In the main gene mutation assay, 1'-COOH-S-2840 treatment did not produce any biologically relevant, reproducible (between cultures) or concentration-related increases in the number of mutant colonies in the presence or absence of metabolic activation compared to the concurrent negative control. Cytotoxicity was not observed, but precipitation was noted from 1000 µg/mL in all cultures with and without S9. Positive and negative controls produced the expected mutant colonies, and these were within the laboratory HCD ranges. Results from the mutation assays are presented in table 6.8.1-20.

Table 6.8.1-20: Summary of results of Experiment I and II for gene mutations at the HPRT locus in Chinese hamster V79 cells treated with 1'-COOH-S-2840.

Conc. µg/mL	S9 mix	Rel. clon. eff. I %	Rel. cell density %	Relative adjusted CE1 %	Mutant colonies / 10^6 cells	Rel. clon. eff. I %	Rel. cell density %	Relative adjusted CE1 %	Mutant colonies / 10^6 cells
Exp. I / 4 h treatment		**Culture I**				**Culture II**			
Neg C	-	89.9	97.3	87.5	20.5	104.8	84.1	88.1	33.4

Solv C	-	100.0	100.0	100.0	9.2	100.0	100.0	100.0	27.6
62.5	-	72.7	110.0	80.0	#	103.4	95.4	98.6	#
125	-	72.6	89.7	65.1	10.6	100.8	63.8	64.3	20.0
250	-	104.2	70.1	73.1	15.9	104.8	57.9	60.6	17.1
500	-	77.6	83.7	64.9	22.6	107.2	58.4	62.6	20.9
1000[P]	-	91.5	90.6	82.9	22.8	102.0	66.4	67.7	32.7
2000[P]	-	79.7	96.7	77.1	12.3	99.8	82.3	82.1	14.0
Pos C	-	81.6	90.9	74.2	202.4	96.9	68.2	66.1	297.5
Exp. I / 4 h treatment		**Culture I**				**Culture II**			
Neg C	+	96.7	121.8	117.8	22.5	112.5	90.2	101.5	24.6
Solv C	+	100.0	100.0	100.0	28.0	100.0	100.0	100.0	24.3
62.5	+	93.0	107.4	99.9	#	113.7	83.5	94.9	#
125	+	94.1	84.9	79.9	22.5	103.9	80.6	83.7	27.4
250	+	95.5	80.7	77.1	23.1	93.2	100.0	93.2	23.0
500	+	91.4	84.9	77.6	27.3	97.1	77.3	75.1	32.1
1000[P]	+	94.8	89.4	84.7	31.1	102.1	68.6	70.0	18.1
2000[P]	+	85.1	92.6	78.7	25.0	97.0	71.4	69.3	42.4
Pos C	+	94.0	95.0	89.3	201.0	106.0	84.8	89.9	135.1
Exp. II / 4 h treatment		**Culture I**				**Culture II**			
Neg C	-	80.1	105.7	84.7	14.7	129.9	92.4	120.0	9.1
Solv C	-	100.0	100.0	100.0	8.2	100.0	100.0	100.0	16.5
62.5	-	81.0	95.6	77.4	#	153.0	81.7	125.0	#
125	-	76.1	78.7	59.9	10.6	179.7	84.0	151.1	21.3
250	-	81.7	83.7	68.4	10.9	176.3	83.3	146.9	20.9
500	-	76.2	107.6	82.0	12.4	138.2	80.2	110.9	20.7
1000[P]	-	72.9	80.7	58.9	14.6	167.5	74.8	125.4	16.5
2000[P]	-	74.0	81.1	60.1	##	150.8	69.8	105.3	##
Pos C	-	79.4	129.4	102.7	153.1	151.9	84.3	128.1	317.4
Exp. II / 4 h treatment		**Culture I**				**Culture II**			
Neg C	+	102.5	112.1	114.9	12.8	100.0	99.4	99.4	19.9
Solv C	+	100.0	100.0	100.0	23.5	100.0	100.0	100.0	19.2
62.5	+	105.6	86.7	91.6	#	104.4	90.2	94.1	#
125	+	103.1	98.2	101.2	17.1	100.0	99.6	99.6	27.8
250	+	117.8	100.7	118.7	18.1	97.2	98.6	95.9	17.7
500	+	104.7	95.5	100.0	18.1	102.8	113.9	117.1	26.9
1000[P]	+	107.9	83.6	90.2	27.2	108.0	102.4	110.6	24.9
2000[P]	+	109.5	82.0	89.7	##	104.6	112.2	117.4	##
Pos C	+	107.6	116.7	125.6	276.7	96.5	97.2	93.8	188.3

Rel. clon. eff.: relative cloning efficiency; Neg C: negative control – medium; Solv C: solvent control – DMSO; Pos C: positive control – EMS without S9 mix or DMBA with S9 mix;
[P]: precipitation visible to the naked eye at the beginning and the end of treatment; #: culture was not continued as only four analysable concentrations were required; ##: culture was not continued to avoid analysis of too many precipitating concentrations

Conclusion

In this GLP and guideline in vitro mammalian cell gene mutation study, 1'-COOH-S-2840 was negative up to concentrations causing precipitation. Therefore, 1'-COOH-S-2840 is non-mutagenic in this assay.

[redacted] (2017b)

4. In vitro Chromosomal Aberration test for 1'-COOH-S-2840 in Cultured Mammalian Cells

Reference:	KCA 5.8.1.2/04
Report Title:	In vitro chromosomal aberration test on 1'-COOH-S-2840 in Chinese hamster lung cells (CHL/IU)
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. 4363 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material: 1'-COOH-S-2840 Lot/Batch: 16SC8535304 Purity: 99.8%
Method of analysis:	Not required
Guideline(s):	OECD 473 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The clastogenic potential of 1'-COOH-S-2840 was investigated in a GLP and guideline study in the presence and absence of metabolic activation (S9) using Chinese hamster lung (CHL/IU) cells. Cytotoxicity tests were performed to choose the relevant concentrations for the chromosomal aberration tests. The relative increase in cell count (RICC) was used as the measure of cytotoxicity. The lowest concentration at

which the RICC was 50% or lower was chosen as the maximum concentration for the chromosomal aberration test. In the initial chromosomal aberration test (Experiment I- short term treatment), CHU/IL cells were exposed to inpyrfluxam in DMSO at concentrations of 250, 500, 1000 and 2000 µg/mL with and without S9 for 6 h followed by an 18 h recovery period. In the second experiment (Experiment II- continuous treatment method), cells were treated continuously with the test substance for 24 h in the absence of S9 at concentrations of 125, 250, 500, 1000 and 1250 µg/mL. All the investigations required by the guideline were performed and appropriate controls were used.

Results

In the preliminary cytotoxicity test, precipitation was observed at 2000 µg/mL both in the presence and absence of S9. No cytotoxicity (≤ 50% in RICC) was observed at 2000 µg/mL in the presence and absence of metabolic activation in the short term treatment and at and above 1000 µg/mL in the continuous treatment without metabolic activation. The results are summarised in the table 6.8.1-21.

Table 6.8.1-21: Preliminary cytotoxicity test of 1'-COOH-S-2840 in Chinese hamster lung (CHL/IU) cells

Concentration (µg/mL)	% Relative increase in cell count (RICC) compared to control		
	6 h treatment		24 h treatment
	-S9	+S9	-S9
Solvent control	100	100	100
7.81	97.9	94.9	94.0
15.6	96.6	91.3	97.3
31.3	97.8	94.6	100.4
62.5	105.1	93.5	93.4
125	110.0	91.3	92.8
250	96.6	90.6	85.6
500	96.2	85.4	74.9
1000	80.2	66.5	48.0
2000 [a]	50.8	45.1	10.0

[a]: precipitate was observed at the beginning of treatment

In the main test, no statistically significant or biologically relevant increase in structural alterations (excluding gaps) or polyploid and endoreduplicated cells was observed in the treated groups compared to controls in the presence and absence of metabolic activation in experiment I and in the absence of metabolic activation in experiment II up to concentrations causing precipitation or an appropriate level of cytotoxicity. All negative and positive controls gave the expected results which were within the laboratory HCD. Tables 6.8.1-22 to 6.8.1-24 summarise the results of the chromosomal aberration test performed.

Table 6.8.1-22: Experiment I - Cytogenetic assay of 1'-COOH-S-2840 in CHL/IU cells without metabolic activation (-S9) – 6 h treatment and 18 h recovery.

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Solvent control	300	100	0.7	1.0	1.0
500 µg/mL	300	112.5	1.7	0.7	0.7
1000 µg/mL	300	87.9	0.7	0.7	0.7
2000 µg/mL [a]	300	55.3	1.7	1.0	0.7
Positive control (MMC)	300	84.5	0.0	15.7	15.7*

[a]: precipitate was observed at the beginning of treatment; *: $p < 0.01$

Table 6.8.1-23: Experiment I - Cytogenetic assay of 1'-COOH-S-2840 in CHL/IU cells with metabolic activation (+S9) - 6 h treatment and 18 h recovery.

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Solvent control	300	100	0.0	1.0	1.0
500 µg/mL	300	84.3	0.0	1.7	1.0
1000 µg/mL	300	62.3	0.3	0.3	0.3
2000 µg/mL [a]	300	33.1	0.7	0.7	0.7
Positive control (CP)	300	51.6	0.7	14.7	14.7*

[a]: precipitate observed at the beginning of treatment; *: $p < 0.01$

Table 6.8.1-24: Experiment II - Cytogenetic assay of 1'-COOH-S-2840 in CHL/IU cells without metabolic activation (-S9) - 24 h treatment

Dose group	Number of metaphases scored	Relative increase in cell count (%)	Polyploid and endoreduplicated cells (%)	Cells with structural aberrations (%)	
				With gaps	Without gaps
Solvent control	300	100	1.0	1.3	1.3
500 µg/mL	300	78.4	0.3	1.3	1.3
1000 µg/mL	300	51.8	0.7	2.0	2.0
1250 µg/mL [a]	300	31.6	0.0	2.0	2.0
Positive control (MMC)	300	67.4	0.0	14.0	13.7*

[a]: precipitate observed at the beginning of treatment; *: p < 0.01

Conclusion

In this GLP and guideline in vitro chromosomal aberration test, 1'-COOH-S-2840 was negative up to concentrations causing precipitation or cytotoxicity. Therefore, it is concluded that 1'-COOH-S-2840 is not a clastogen in this assay.

[redacted] (2017a)

5. In vitro micronucleus test for 1'-COOH-S-2840 in Cultured Mammalian Cells

Reference:	KCA 5.8.1.2/05
Report Title:	In Vitro Micronucleus (MNvit) Test of 1'-COOH-S-2840 in Human Lymphoblast Cell Line (TK6)
Author(s) & Year:	[redacted] (2020b)
Document No, Authority registration No	Study No. J351 (028-224) Biosafety Research Center Inc (BSRC)
Substance used:	Test Material: 1'-COOH-S-2840 Lot/Batch: 16SC8535304 Purity: 99.8%
Method of analysis:	Not required
Guideline(s):	OECD 487 (2016)
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	Yes - GLP
Acceptability:	Yes
Study relied upon:	Yes

Methods

The potential of 1'-COOH-S-2840 to induce micronuclei in vitro was investigated in a GLP and guideline study using a human lymphoblast cell line (TK6). Cell growth inhibition tests were performed to choose the relevant concentrations for the micronucleus test. The replication index (RI) was used to calculate the cell growth

inhibition (% cytostasis = 100-RI). Cytotoxicity is considered sufficiently addressed when % cytostasis is ≥ 50%.

In the initial chromosomal aberration test (Experiment I- short term treatment), TK6 cells were exposed to 1'-COOH-S-2840 in DMSO at concentrations of 250, 500, 1000 and 2000 µg/mL in the presence and absence of S9 for 3 h followed by a 24 h recovery period. In the second experiment (Experiment II- continuous treatment), cells were treated continuously with the test substance for 24 h in the absence of S9 at concentrations of 125, 250, 500, 750, 1000,1250,1500, 1750 and 2000 µg/mL. Micronucleus analyses were conducted at 500,1000 and 2000 µg/mL for the -S9 and +S9 assays and at 250, 500 and 1000 µg/mL for the 24 h assay. All the investigations required by the guideline were performed and appropriate controls were used.

Results

In the preliminary cytotoxicity test, cell growth inhibition ≥ 50% was observed at 2000 µg/mL in the 24 h assay. In the short-term assays in the presence and absence of S9, there was no cell growth inhibition ≥ 50% at any of the tested concentrations.

In the main test, no statistically significant or biologically relevant increase in the number of micronucleated binucleate cells (MNBN) was observed in the treated groups compared to controls in the presence and absence of metabolic activation in experiment I up to the limit concentration and in the absence of metabolic activation in experiment II up to an appropriate level of cytotoxicity. All negative and positive controls gave expected results which were within the laboratory HCD. Tables 6.8.1-25 to 6.8.1-27 summarise the results of the micronuleus test performed.

Table 6.8.1-25: Experiment I – Micronucleus test of 1'-COOH-S-2840 in TK6 cells without metabolic activation (-S9) – 3 h treatment and 24 h recovery.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
							mean
DMSO	0	-	1	1000	11	1.10	0.80
			2	1000	5	0.5	
1'-COOH-S-2840	250	-1.0	1	NA			
			2	NA			
	500	-0.4	1	1000	5	0.50	0.85
			2	1000	12	1.20	
	1000	-0.9	1	1000	12	1.20	0.85
			2	1000	5	0.50	
	2000	-3.2	1	1000	9	0.9	0.95
			2	1000	10	1.00	
MMC	0.3	52.9	1	1000	20	2.00	2.30*
			2	1000	26	2.60	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL) b
i-N: Binucleate, MNBN: Micronucleated binucleate cells

MMC: Positive control (Mitomycin C)
NA: Not analyzed
*: Significant difference from negative control (Fisher's exact test):p<0.025

Table 6.8.1-26: Experiment I – Micronucleus test of 1'-COOH-S-2840 in TK6 cells with metabolic activation (+S9) – 3 h treatment and 24 h recovery.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
							mean
DMSO	0	-	1	1000	8	0.80	0.80
			2	1000	8	0.80	
1'-COOH-S-2840	250	2.6	1	NA			
			2	NA			
	500	2.1	1	1000	8	0.80	0.75
			2	1000	7	0.70	
	1000	-6.1	1	1000	10	1.00	0.90
			2	1000	8	0.80	
	2000	-3.4	1	1000	7	0.70	0.85
			2	1000	10	1.00	
CP	8	68.6	1	1000	26	2.60	2.55*
			2	1000	25	2.50	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL) b
i-N: Binucleate, MNBN: Micronucleated binucleate cells
CP: Positive control (Cyclophosphamide)
NA: Not analyzed
*: Significant difference from negative control (Fisher's exact test): p<0.025

Table 6.8.1-27: Experiment II – Micronucleus test of 1'-COOH-S-2840 in TK6 cells without metabolic activation (-S9) –24 h treatment.

Substance	Conc. (µg/mL)	Cytostasis (%)	Culture	Number of bi-N cells analyzed	Number of MNBN	MNBN (%)	
							Mean
DMSO	0	-	1	1000	11	1.10	0.90
			2	1000	7	0.70	
1'-COOH-S-2840	125	6.1	1	NA			
			2	NA			
	250	11.1	1	1000	10	1.00	0.95
			2	1000	9	0.9	
	500	31.2	1	1000	10	1.00	0.95
			2	1000	9	0.9	
	750	40.3	1	NA			
			2	NA			
	1000	51.6	1	1000	8	0.80	0.85
			2	1000	9	0.90	
	1250	60.1	1	NA			
			2	NA			
	1500	69.0	1	NA			
			2	NA			
	1750	75.9	1	NA			
			2	NA			
	2000	81.9	1	NA			
			2	NA			
COL	0.005	12.3	1	1000	25	2.50	2.65*
			2	1000	28	2.80	

DMSO: Negative control (Dimethyl sulfoxide, 10 µL/mL)
bi-N: Binucleate, MNBN: Micronucleated binucleate cells
COL: Positive control (Colchicine)
NA: Not analyzed
*: Significant difference from negative control (Fisher's exact test):p<0.025

Conclusion

In this GLP and guideline in vitro micronucleus test, 1'-COOH-S-2840 did not induce micronuclei in TK6 cells up to the limit concentration or cytotoxic concentrations. Therefore, it is concluded 1'-COOH-S-2840 is not clastogenic or aneugenic in this assay.

[redacted] (2020a)

Summary of data for 1'-COOH-S-2840

1'-COOH-S-2840 was not acutely toxic by the oral route (LD50 > 2000 mg/kg bw); therefore compared to inpyrfluxam (LD50 = 180 mg/kg bw), 1'-COOH-S-2840 is less acutely toxic than the parent substance by the oral route. 1'-COOH-S-2840 was not mutagenic in an adequate battery of in vitro tests.

Summary information on 3'-OH-S-2840, 1'-COOH-S-2840, DFPA, N-des-Me-DFPA and DFPA-$CONH_2$

A summary of the studies available for metabolites 3'-OH-S-2840 and 1'-COOH-S-2840 is presented in the table below. An overview of the available information on the other plant metabolites (i.e. DFPA, N-des-Me-DFPA and DFPA-CONH2) that are common to other succinate dehydrogenase inhibitor (SDHI) fungicides is also summarised in the table.

Table 6.8.1-28: Summary of available studies on metabolites

Data point/ Study *Acceptability*	Test system / dosages	Results	Reference
3'-OH-S-2840			
KCA 5.8.1.1/01 Acute oral (OECD 423) *Acceptable*	Female RccHan:WIST rats 2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	[redacted] (2017b)
KCA 5.8.1.1/02 90-day dietary toxicity study (OECD 408) *Acceptable*	RccHan:WIST rats 0, 500, 2000, and 4000 ppm (0, 32.2, 128 and 258 mg/kg bw/day for males, and 0, 37.9, 157 and 291 mg/kg bw/day for females)	**NOAEL: 500 ppm (32.2 mg/kg bw/day)** based on clinical-chemistry findings indicative of liver damage, histopathological findings in the liver and ovary at the **LOAEL: 2000 ppm (157 mg/kg bw/day)**	[redacted] (2018)
KCA 5.8.1.1/03 Ames test (OECD 471)	*Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2*urv*A) / 1.5 to	Negative (± S9 Mix)	[redacted] (2017a)

Data point/ Study *Acceptability*	Test system / dosages	Results	Reference
Acceptable	5000 µg/plate (± S9 Mix)		
KCA 5.8.1.1/04 Mammalian cells gene mutation (OECD 476) *Acceptable*	Chinese hamster V79 cells - HPRT locus / 3.8 to 60 µg/mL (± S9 Mix)	Negative (± S9 Mix)	[redacted] (2017a)
KCA 5.8.1.1/05 Chromosome aberration (OECD 473) *Acceptable*	Chinese hamster CHL/IU lung cells25 to 100 µg/mL (short-term treatment, -S9 Mix), 50 to 200 µg/mL (short-term treatment, +S9 Mix) and 12.5 to 50 µg/mL (continuous treatment, -S9 Mix)	Negative (± S9 Mix)	[redacted] (2017b)
KCA 5.8.1.1/06 In Vitro Micronucleus (MNvit) (OECD 487) *Acceptable*	Human lymphoblast cell line (TK6) / 12.5 to 100 µg/mL (short-term treatment, -S9 Mix), 12.5 to 100 µg/mL (short-term treatment, +S9 Mix) and 6.25 to 100 µg/mL (continuous treatment, -S9 Mix)	Negative (± S9 Mix)	[redacted] (2020a)
1'-COOH-S-2840			
KCA 5.8.1.2/01 Acute oral (OECD 423) *Acceptable*	Female RccHan:WIST rats2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	[redacted] (2017c)
KCA 5.8.1.2/02 Ames test (OECD 471) *Acceptable*	*Salmonella typhimurium* (strains TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2*urv*A) / 156 to 5000 µg/plate	Negative (± S9 Mix)	[redacted] (2017)

Data point/ Study *Acceptability*	Test system / dosages	Results	Reference
	(± S9 Mix)		
KCA 5.8.1.2/03 Mammalian cells gene mutation (OECD 476) *Acceptable*	Chinese hamster V79 cells - HPRT locus / 125 to 2000 µg/mL (±S9 Mix, experiment I) and 125 to 1000 µg/mL (±S9 Mix, experiment II)	Negative (± S9 Mix)	[redacted] (2017b)
KCA 5.8.1.2/04 Chromosome aberration (OECD 473) *Acceptable*	Chinese hamster CHL/IU lung cells500 to 2000 µg/mL (short-term treatment, ±S9 Mix) and 500 to 1250 µg/mL (continuous treatment, -S9 Mix)	Negative (± S9 Mix)	[redacted] (2017a)
KCA 5.8.1.1/06 In Vitro Micronucleus (MNvit) (OECD 487) *Acceptable*	Human lymphoblast cell line (TK6) 12.5 to 100 µg/mL (short-term treatment, -S9 Mix), 12.5 to 100 µg/mL (short-term treatment, +S9 Mix) and 6.25 to 100 µg/mL (continuous treatment, -S9 Mix)	Negative (± S9 Mix)	[redacted] (2020b)
Metabolites common to other succinate dehydrogenase inhibitor (SDHI) fungicides			
Type of study		**Results**	**Reference**
DFPA			
Acute oral, Rat		LD_{50} >2000 mg/kg bw	European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance fluxapyroxad (BAS 700 F). EFSA Journal 2012;10(1):2522
Mutagenicity Ames test *In vitro* mammalian gene cell mutation *In vitro* chromosome aberration *In vivo* micronucleus test		Negative	
90-day subchronic (Rat) Method of analysis not acceptably validated but there is confidence in the results. In addition, study only used in a semi-quantitative manner to determine		NOAEL: 1000 mg/kg bw/day (Highest Dose Level Tested)	

Data point/ Study *Acceptability*	Test system / dosages	Results	Reference
relative potency to the parent substance.			Also EFSA Journal 2015;13(3):4043 – benzovindiflupyr
Pre-natal developmental toxicity (Rabbit) Method of analysis not acceptably validated but there is confidence in the results. In addition, study only used in a semi-quantitative manner to determine relative potency to the parent substance.		NOAEL – maternal and developmental: 250 mg/kg bw/day (Highest Dose Level Tested; severe maternal toxicity at ≥ 500 mg/kg bw/day in range-finding studies)	
N-Des-Me-DFPA			
Acute oral, Rat		LD_{50} >2000 mg/kg bw	European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance fluxapyroxad (BAS 700 F). EFSA Journal 2012;10(1):2522 and/or European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance isopyrazam EFSA Journal 2012;10(3):2600 and/or European Food Safety Authority; Conclusion on the peer review of the pesticide risk assessment of the active substance
Mutagenicity Ames test *In vitro* mammalian gene cell mutation *In vitro* chromosome aberration *In vivo* micronucleus test		Negative	
90-day subchronic (Rat) Method of analysis not acceptably validated but there is confidence in the results. In addition, study only used in a semi-quantitative manner to determine relative potency to the parent substance.		NOAEL: 1000 mg/kg bw/day (Highest Dose Level Tested)	
Pre-natal developmental toxicity (Rabbit) Method of analysis not acceptably validated but there is confidence in the results. In addition, study only used in a semi-quantitative manner to determine relative potency to the parent substance.		NOAEL maternal: 300 mg/kg bw/day, based on reduction of body weight gain and food consumption; NOAEL developmental: 1000 mg/kg/day (Highest Dose Level Tested)	

Data point/ Study *Acceptability*	Test system / dosages	Results	Reference
			sedaxane. EFSA Journal 2012;10(7):2823
DFPA-CONH2			
Acute oral, Rat		LD_{50} >500 and <2000 mg/kg bw (Acute Cat 4)	EFSA Conclusion: on the peer review of the pesticide risk assessment of the active substance pydiflumetofen, EFSA Journal 2019; 17(10): 5821
Mutagenicity Ames test *In vitro* mammalian gene cell mutation *In vitro* chromosome aberration *In vivo* rat micronucleus test		Negative Ames and mammalian cell gene mutation, but positive in vitro chromosome aberration. However, negative in vivo micronucleus with demonstration of bone marrow exposure by subsequent blood analysis	
28-day dietary (Rat) with validated method of analysis		NOAEL = 500 ppm (37.4 mg/kg bw/day) Reductions in body weight and food consumption.	

Summary of information on DFPA

DFPA presented low oral acute (LD50 > 2000 mg/kg bw/day) and short-term toxicity, with no adverse effects observed up to 1000 mg/kg bw/day (limit dose) in a 90-day dietary study in rats; no adverse effects were observed in a developmental toxicity study in rabbits up to the highest dose tested of 250 mg/kg bw/day, but DFPA produced high maternal toxicity at 500 mg/kg bw/day in a developmental range-finding study. DFPA was negative for genotoxicity in Ames test, mammalian cell gene mutation assay and chromosome aberration test, and no alert for aneugenicity was triggered in a comparative QSAR analysis (see below). EFSA (2012; fluxapyroxad) set an ADI of 0.25 mg/kg bw/day for DFPA based on the NOAEL of 250 mg/kg bw/day from the rabbit developmental study and the application of an assessment factor of 1000. HSE notes that DFPA is significantly less toxic than the parent substance (oral LD50 = 180 mg/kg bw; NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects) therefore the parent's dietary reference values would be appropriate. These are more conservative that the metabolite-specific ADI and more appropriate for a plant and livestock metabolite for the purposes of the residue definition (RD) for risk assessment.

Summary of information on N-Des-Me-DFPA

N-Des-Me-DFPA presented low oral acute (LD50 > 2000 mg/kg bw/day) and short-term toxicity, with no adverse effects observed up to 1000 mg/kg bw/day (limit dose) in a 90-day dietary study in rats; in a developmental toxicity study in rabbits no adverse effects were observed on the development of the foetus up to 1000 mg/kg bw/day (limit dose), while the maternal NOAEL was 300 mg/kg bw/day based on reduction of maternal body weight gain and decreased food intake. N-Des-Me-DFPA was negative for genotoxicity in Ames test, mammalian cell gene mutation assay and chromosome aberration test, and no alert for aneugenicity was triggered in a comparative QSAR analysis (see below). EFSA (2012, fluxapyroxad) set an ADI of 0.3 mg/kg bw/day for N-Des-Me-DFPA based on the maternal NOAEL of 300 mg/kg bw/day from the rabbit developmental study and the application of an assessment factor of 1000. HSE notes that N-Des-Me-DFPA is significantly less toxic than the parent substance (oral LD50 = 180 mg/kg bw; NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects), therefore the parent's dietary reference values would be appropriate. These are more conservative that the metabolite-specific ADI and more appropriate for a plant and livestock metabolite for the purposes of the RD for risk assessment.

Summary of information on DFPA-CONH2

DFPA-CONH2 presented moderate acute oral toxicity (LD50 > 500 and < 2000 mg/kg bw) and, hence, is still less acutely toxic than inpyrfluxam (LD50 = 180 mg/kg bw). In a 28-day dietary study in rats, a NOAEL of 37.4 mg/kg bw/day was identified based on decreases in body weight and food consumption. These data suggest that DFPA-CONH2 is of similar or slightly lower toxicity than the parent substance (NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects). DFPA-CONH2 was negative in an Ames test and mammalian cell gene mutation assay, but was positive in an in vitro chromosome aberration test. However, it was negative in a follow-up in vivo micronucleus study in which exposure of the bone marrow was demonstrated by subsequent blood analysis. Overall, DFPA-CONH2 is not mutagenic and all relevant genotoxicity endpoints have been investigated. EFSA (2019, pydiflumetofen) set an ADI and ARfD of 0.04 mg/kg bw/day for DFPA-CONH2 based on the NOAEL of 37.4 mg/kg bw/day from the rat 28-day study and the application of an assessment factor of 1000. HSE notes that DFPA-CONH2 is of similar or slightly lower toxicity than the parent substance, therefore the parent's dietary reference values would be adequate. These are more appropriate for a plant and livestock metabolite for the purposes of the RD for risk assessment.

B.6.8.1.1. Comparative QSAR analysis for genotoxicity and acute oral toxicity of groundwater metabolites 1'-keto-S-2840 and 1'-COOH-S-2840

One metabolite, 1'-COOH-S-2840, has been estimated to occur in groundwater at levels above 0.1 µg/L and hence requires a relevance assessment (see Vol 1, section 2.10). Metabolite 1'-keto-S-2840 has the potential to occur in groundwater, but at levels much lower than 1'-COOH-S-2840. These levels have not been estimated

because they would be covered by the exposure assessment performed for 1'-COOH-S-2840. However, the relevance assessment of 1'-COOH-S-2840 would also cover that of 1'-keto-S-2840 only if the toxicological profile of 1'-keto-S-2840 is similar to that of 1'-COOH-S-2840. To exclude the toxicological relevance of 1'-COOH-S-2840 in accordance with the SANCO/221/2000 – rev.11 Guidance Document, information on the genotoxicity and acute oral toxicity potential of the metabolite is required. 1'-COOH-S-2840 is not genotoxic in an adequate range of studies and it is of low acute oral toxicity. Based on these results, it can be concluded that 1'-COOH-S-2840 is not a relevant groundwater metabolite (see Vol 1, section 2.10). Metabolite 1'-keto-S-2840 is structurally very similar to 1'-COOH-S-2840; therefore read-across of the toxicological relevance assessment of 1'-COOH-S-2840 to 1'-keto-S-2840 has been proposed. To support the read-across, the applicant has provided a comparative QSAR analysis of 1'-keto-S-2840 and 1'-COOH-S-2840 for the two key toxicological endpoints (genotoxicity and acute oral toxicity) underpinning the relevance assessment of 1'-COOH-S-2840.

Genotoxicity

Method

The applicant assessed the target metabolite 1'-keto-S-2840 and the substances identified as potential read across candidates, inpyrfluxam, 1'-CH_2OH-S-2840, 1'-COOH-S-2840, and 3'-OH-S-2840 for their genotoxicity using acceptable QSAR models, one being knowledge-based, one being statistical-based, and the third one used for structural profiling (Pellizzaro, 2025; Report No. 2006362.UK0 – 8326). The following (Q)SAR models were used:

- Derek Nexus (v.6.4.0), an expert system for prediction of general toxicity and genotoxicity; implemented in Nexus (v.2.7.0); using Derek KB 2024 1.0. – knowledge-based model
- OECD QSAR Toolbox (v4.7) – hybrid model
- Leadscope Inc non-human genetic toxicity model suite (2023.0.2-4)- statistical model

The alerts flagged for 1'-keto-S-2840 by the (Q)SAR models used were compared to the alerts returned for inpyrfluxam, 1'-CH_2OH-S-2840, 1'-COOH-S-2840, and 3'-OH-S-2840. To note, the genotoxicity potential of inpyrfluxam and the metabolites, 1'-COOH-S-2840 and 3'-OH-S-2840 is well established on the basis of experimental data. 1'-CH_2OH-S-2840 is the aglycone of the major rat metabolite, the glucuronide conjugate of 1'-CH_2OH-S-2840, for which the genotoxicity profile is considered to be covered by the parent, inpyrfluxam. Neither the parent nor these metabolites are genotoxic.

Results

In Derek Nexus, 1'-keto-S-2840 was predicted to be negative for mutagenicity (in vitro, in vivo) and chromosome damage (in vitro and in vivo). These predictions were identical to the predictions obtained for inpyrfluxam, 1'-CH_2OH-S-2840, 1'-COOH-S-2840, and 3'-OH-S-2840.

Table 6.8.1.1-1: Derek Nexus genotoxicity predictions for 1'-keto-S-2840 and the read across candidates

Name	Mutagenicity *in vitro*	Mutagenicity *in vivo*	Chromosome damage *in vitro*	Chromosome damage *in vivo*
1'-keto-S-2840	Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
Inpyrfluxam	Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
1'-CH_2OH-S-2840	Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
1'-COOH-S-2840	Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
3'-OH-S-2840	Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated

In the Leadscope Bacterial Mutation model, 1'-keto-S-2840 returned an indeterminate outcome (0.454 predicted value). The structural features that contribute to the prediction are the pyrazole, carboxamide and fluoro aryl moieties. All of these features are present in inpyrfluxam, 1'-CH_2OH-S-2840, 1'-COOH-S-2840 and 3'-OH-S-2840, where they are known to be of no concern for genotoxicity based on experimental data. Therefore, the indeterminate prediction for 1'-keto-S-2840 is considered to be of no concern.

Table 6.8.1.1-2: Leadscope genotoxicity predictions for 1'-keto-S-2840 and the read across candidates

Name	Bacterial Mutation v2	*In vivo* Micronuc Mouse v2
1'-keto-S-2840	Indeterminate 0.454	Negative 0.466
Inpyrfluxam	Negative 0.349	Negative 0.384
1'-CH_2OH-S-2840	Indeterminate 0.422	Not in domain 0.412
1'-COOH-S-2840	Not in domain 0.136	Not in domain 0.271
3'-OH-S-2840	Not in domain 0.422	Not in domain 0.412

1'-keto-S-2840 returned one alert (H-acceptor-path3-H-acceptor) for the in vivo micronucleus test by the ISS profiler in the OECD QSAR Toolbox. This alert is activated by the pyrazole functional group, which is also present in inpyrfluxam, 1'-CH_2OH-S-2840, 1'-COOH-S-2840 and 3'-OH-S-2840. Therefore, using read across, this alert is considered to be of no concern for 1'-keto-S-2840.

Table 6.8.1.1-3: OECD QSAR Toolbox genotoxicity profiling for 1'-keto-S-2840 and the read across candidates

Profiler	DNA binding by OASIS	DNA binding by OECD	DNA alerts for AMES, CA and MNT by OASIS	*in vitro* mutagenicity (Ames test) alerts by ISS	*in vivo* mutagenicity (Micronucleus) alerts by ISS	Protein binding alerts for Chromosomal aberration by OASIS
1'-keto-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
Inpyrfluxam	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
1'-CH_2OH-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
1'-COOH-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
3'-OH-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found

Conclusion

Overall, using a combination of *in silico* predictions and read across methods, it can be concluded that 1'-keto-S-2840 is not genotoxic.

Acute oral toxicity

Method

1'-keto-S-2840 was out of domain in many models that predict acute oral toxicity (TEST, Leadscope, Danish QSAR models, OECD QSAR Toolbox, VEGA). Therefore, read across was used to predict the acute oral toxicity of 1'-keto-S-2840.

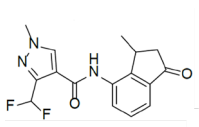
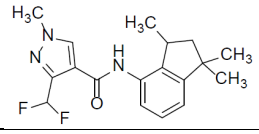
As for genotoxicity above, candidates for read across were inpyrfluxam and its metabolites, 1'-COOH-S-2840 and 3'-OH-S-2840. In addition, substances containing the pyrazole functional group were identified using the substructure search in the OECD QSAR Toolbox. Chemical structure similarity of the read across candidates to 1'-keto-S-2840 was evaluated using the OECD QSAR Toolbox.

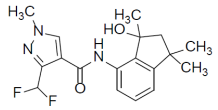
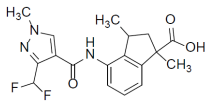
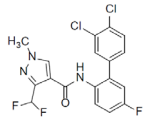
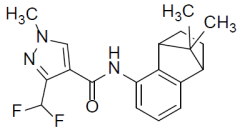
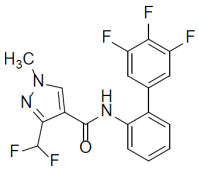
Results

Six read across candidates were identified: inpyrfluxam, 3'-OH-S-2840, 1'-COOH-S-2840, bixafen, isopyrazam, and fluxapyroxad. The acute oral toxicity LD50 of inpyrfluxam is 180 mg/kg bw. For all other read across candidates, the acute oral toxicity LD50 is >2000 mg/kg bw.

All read across candidates had >70% chemical structure similarity to 1'-keto-S-2840 and were bioavailable, with predicted logP values between 1 and 5. All substances, except 1'-COOH-S-2840, had a predicted pKa value of ~9. Therefore, it is expected that all substances (except 1'-COOH-S-2840) will be in the same state of ionization and will have similar bioavailability at physiological pH.

Table 6.8.1.1-4: Chemical structure similarity of the read across candidates to 1'-keto-S-2840

Name	Chemical structure similarity to 1'-keto-S-2840	logP	acidic pKa (OASIS Electric)
1'-keto-S-2840 	100.0%	1.64	9.32
Inpyrfluxam 	88.89%	3.87	9.54

3'-OH-S-2840 	81.08%	2.36	9.38
1'-COOH-S-2840 	84.21%	2.26	4.46
Bixafen 	74.29%	4.36	8.73
Isopyrazam 	88.89%	4.28	9.59
Fluxapyroxad 	78.79%	3.47	8.82

Conclusion

Given the very high (~84%) chemical structure similarity between 1'-keto-S-2840 and 1'-COOH-S-2840, their acute toxicity profile is considered to be similar. Using read across it is expected that the acute oral toxicity LD50 value for 1'-keto-S-2840 would be >2000 mg/kg bw.

Overall conclusion

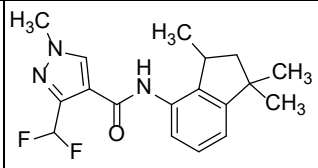
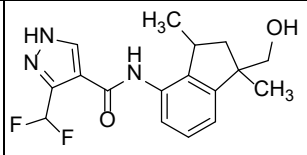
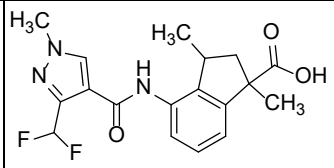
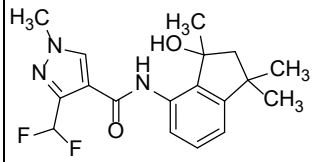
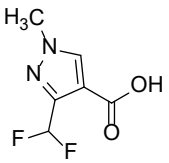
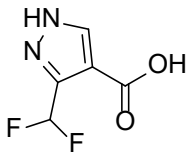
Overall, based on a combination of in silico predictions and read-across methods, it can be concluded that 1'-keto-S-2840 is not genotoxic and is not expected to have greater acute oral toxicity than 1'-COOH-S-2840 (LD50 > 2000 mg/kg bw). Therefore, the groundwater relevance assessment conducted for 1'-COOH-S-2840 (see Vol 1, section 2.10) is considered to adequately cover the relevance assessment of 1'-keto-S-2840.

B.6.8.1.2. Toxicological characterisation of plant and livestock metabolites for the purposes of the residue definition (RD) for risk assessment

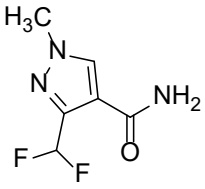
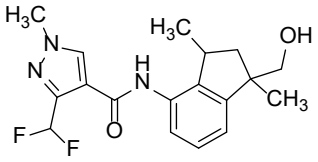
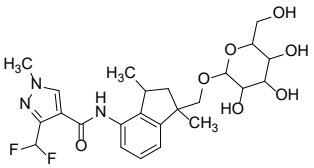
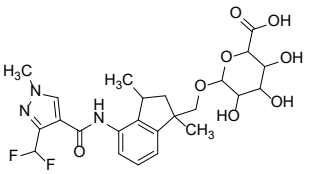
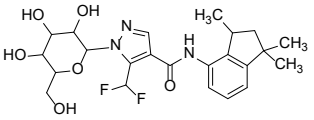
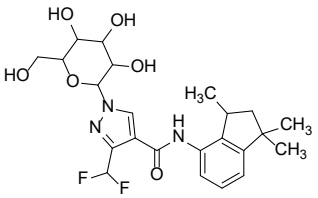
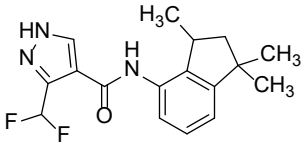
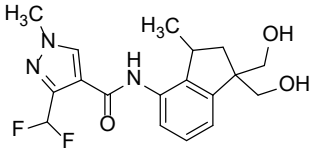
The plant and livestock metabolism studies available for inpyrfluxam show that inpyrfluxam is extensively metabolised in these organisms.

The applicant has carried out a toxicological assessment of significant plant and livestock metabolites of inpyrfluxam selected for potential inclusion in the residue definitions following the principles of the un-noted 2016 EFSA guidance[8] on the residue definition for risk assessment; the assessment has been reviewed by HSE, and the results are presented below. The significant plant and livestock metabolites considered by HSE are listed below:

Table 6.8.1.1-5: List of significant plant and livestock metabolites of inpyrfluxam

Name, code, and smiles	Structure
Parent **Inpyrfluxam** -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CN(C)N=C3C(F)F	
N-des-Me-1'-CH_2OH-S-2840 -- O=C(NC1=CC=CC2=C1C(CC2(C)CO)C)C3=CNN=C3C(F)F	
1'-COOH-S-2840 (A and B isomers) -- O=C(NC1=CC=CC2=C1C(CC2(C(O)=O)C)C)C3=CN(C)N=C3C(F)F	
3'-OH-S-2840 -- O=C(NC1=CC=CC2=C1C(C)(O)CC2(C)C)C3=CN(C)N=C3C(F)F	
DFPA -- O=C(O)C1=CN(C)N=C1C(F)F	
***N*-des-Me-DFPA** -- O=C(O)C1=CNN=C1C(F)F	

[8] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2016. Guidance on the establishment of the residue definition for dietary risk assessment. EFSA Journal 2016;14(12):4549, 129 pp. doi:10.2903/j.efsa.2016.4549

Name, code, and smiles	Structure
DFPA-CONH$_2$ -- O=C(N)C1=CN(C)N=C1C(F)F	
1'-CH_2OH**-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)CO)C)C3=CN(C)N=C3C(F)F	
Gly-1'-CH_2OH**-S-2840** **(sugar conjugate of 1'-**CH_2OH**-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)COC(C(C(O)C3O)O)OC3CO)C)C4=CN(N=C4C(F)F)C	
Glu-1'-CH_2OH**-S-2840** **(Glucuronide of 1'-**CH_2OH**-S-2840)** -- O=C(NC1=CC=CC2=C1C(CC2(C)COC(C(C(O)C3O)O)OC3C(O)=O)C)C4=CN(N=C4C(F)F)C	
Glc-NDM-S-2399B -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=C(N(N=C3)C4C(C(O)C(O)C(CO)O4)O)C(F)F	
Glc-NDM-S-2399A -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CN(N=C3C(F)F)C4OC(CO)C(O)C(O)C4O	
***N*-des-Me-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CNN=C3C(F)F	
1',1'-bis-(CH_2OH**)-S-2840)** -- O=C(NC1=CC=CC2=C1C(CC2(CO)CO)C)C3=CN(C)N=C3C(F)F	

Some metabolites are major rat metabolites and hence, covered by parent. For other metabolites, genotoxicity and general toxicity data are available. For metabolites for which no toxicological information is available, a comparative QSAR analysis for

genotoxicity and general toxicity (Pellizzaro, 2025; Report No. 2006362.UK0 – 6462) has been performed by the applicant.

1. Presence of the selected metabolites in rat ADME studies

It is well accepted that the toxicity profile of plant/livestock metabolites which are major rat metabolites is considered to be 'covered' by the toxicity profile of the parent (i.e. active substance). A major rat metabolite is a metabolite contributing to 10 % or more of the administered dose (AD) in terms of total radioactive material recovered in urine (or other matrices) in both sexes as detected in ADME studies (un-noted 2016 guidance on the establishment of the residue definition for dietary risk assessment - EFSA Journal 2016;14(12):4549).

The 3 major rat metabolites identified from the ADME studies conducted with inpyrfluxam were 1'-COOH-S-2840 and N-des-Me-1'-CH_2OH-S-2840 in urine and 1'-CH_2OH-S-2840 glucuronide in bile (see section B.6.1). Metabolite 1'-CH_2OH-S-2840 is the upstream metabolite of its glucuronide conjugate in the rat. The glucuronide conjugate is a major rat metabolite. Therefore, its upstream metabolite (1'-CH_2OH-S-2840) must have been present in the rat at > 10% of the AD, and hence, it can also be considered a major rat metabolite. The toxicological properties of the major rat metabolites of inpyrfluxam can be considered to have been intrinsically tested in the toxicological studies undertaken with inpyrfluxam and thus these metabolites can be considered of **equivalent toxicity to the parent substance** and potential candidates for inclusion in the RD from a toxicological perspective. The **dietary reference values of inpyrfluxam** could be used for these metabolites for risk assessment purposes.

2. Metabolites with available toxicity data

Toxicological data on genotoxicity and general toxicity are available for metabolites, 3'-OH-S-2840, DFPA, N-des-Me-DFPA and DFPA-CONH2.

3'-OH-S-2840

For the metabolite, 3'-OH-S-2840, acute (oral) and 90-day (oral) toxicity studies in rats and in vitro genotoxicity studies (covering gene mutations, clastogenicity and aneugenicity potential) are available (see B6.8.1).

3'-OH-S-2840 was not acutely toxic by the oral route (LD50 > 2000 mg/kg bw); therefore compared to inpyrfluxam (LD50 = 180 mg/kg bw), 3'-OH-S-2840 is less acutely toxic than the parent substance by the oral route. 3'-OH-S-2840 showed a qualitatively and quantitatively similar toxicological profile to that of the parent substance in a 90-day rat study (NOAEL of 500 ppm - 31.7 mg/kg bw/day based on liver effects for inpyrfluxam). A NOAEL of 500 ppm (37.9 mg/kg bw/day) was identified for 3'-OH-S-2840 based mainly on liver effects at the LOAEL of 2000 ppm (157 mg/kg bw/day). 3'-OH-S-2840 was not mutagenic in an appropriate package of in vitro tests.

Based on these findings, the toxicity profile of 3'-OH-S-2840 is **comparable to that of the parent compound**. Therefore, the **dietary reference values of inpyrfluxam** could be used for this metabolite for risk assessment purposes.

DFPA

DFPA presented low oral acute (LD50 > 2000 mg/kg bw/day) and short-term toxicity, with no adverse effects observed up to 1000 mg/kg bw/day (limit dose) in a 90-day dietary study in rats; no adverse effects were observed in a developmental toxicity study in rabbits up to the highest dose tested of 250 mg/kg bw/day, but DFPA produced high maternal toxicity at 500 mg/kg bw/day in a developmental range-finding study. DFPA was negative for genotoxicity in Ames test, mammalian cell gene mutation assay and chromosome aberration test, and no alert for aneugenicity was triggered in a comparative QSAR analysis (see below). EFSA (2012; fluxapyroxad) set an ADI of 0.25 mg/kg bw/day for DFPA based on the NOAEL of 250 mg/kg bw/day from the rabbit developmental study and the application of an assessment factor of 1000. HSE notes that **DFPA is significantly less toxic than the parent substance** (oral LD50 = 180 mg/kg bw; NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects) therefore the **parent's dietary reference values** would be appropriate. These are more conservative that the metabolite-specific ADI and more appropriate for a plant and livestock metabolite for the purposes of the RD for risk assessment.

N-Des-Me-DFPA

N-Des-Me-DFPA presented low oral acute (LD50 > 2000 mg/kg bw/day) and short-term toxicity, with no adverse effects observed up to 1000 mg/kg bw/day (limit dose) in a 90-day dietary study in rats; in a developmental toxicity study in rabbits no adverse effects were observed on the development of the foetus up to 1000 mg/kg bw/day (limit dose), while the maternal NOAEL was 300 mg/kg bw/day based on reduction of maternal body weight gain and decreased food intake. N-Des-Me-DFPA was negative for genotoxicity in Ames test, mammalian cell gene mutation assay and chromosome aberration test, and no alert for aneugenicity was triggered in a comparative QSAR analysis (see below). EFSA (2012, fluxapyroxad) set an ADI of 0.3 mg/kg bw/day for N-Des-Me-DFPA based on the maternal NOAEL of 300 mg/kg bw/day from the rabbit developmental study and the application of an assessment factor of 1000. HSE notes that **N-Des-Me-DFPA is significantly less toxic than the parent substance** (oral LD50 = 180 mg/kg bw; NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects), therefore the **parent's dietary reference values** would be appropriate. These are more conservative that the metabolite-specific ADI and more appropriate for a plant and livestock metabolite for the purposes of the RD for risk assessment.

DFPA-CONH2

DFPA-CONH2 presented moderate acute oral toxicity (LD50 > 500 and < 2000 mg/kg bw) and, hence, is still less acutely toxic than inpyrfluxam (LD50 = 180 mg/kg bw). In

a 28-day dietary study in rats, a NOAEL of 37.4 mg/kg bw/day was identified based on decreases in body weight and food consumption. These data suggest that DFPA-CONH2 is of similar or slightly lower toxicity than the parent substance (NOAEL 90 day study 31.7 mg/kg bw/day based on liver effects). DFPA-CONH2 was negative in an Ames test and mammalian cell gene mutation assay, but was positive in an in vitro chromosome aberration test. However, it was negative in a follow-up in vivo micronucleus study in which exposure of the bone marrow was demonstrated by subsequent blood analysis. Overall, DFPA-CONH2 is not mutagenic and all relevant genotoxicity endpoints have been investigated. EFSA (2019, pydiflumetofen) set an ADI and ARfD of 0.04 mg/kg bw/day for DFPA-CONH2 based on the NOAEL of 37.4 mg/kg bw/day from the rat 28-day study and the application of an assessment factor of 1000. HSE notes that **DFPA-CONH2 is of similar or slightly lower toxicity than the parent substance**, therefore the **parent's dietary reference values** would be adequate. These are more appropriate for a plant and livestock metabolite for the purposes of the RD for risk assessment.

3. Metabolites with available genotoxicity and general toxicity QSAR predictions

The applicant assessed inpyrfluxam and all of the 13 selected plant/livestock metabolites for their genotoxicity and general toxicity potential using acceptable QSAR models, including a knowledge-based model, a statistical-based model, and a third model applied for structural profiling (Pellizzaro, 2025; Report No. 2006362.UK0 – 6462). The following (Q)SAR models were used:

- Derek Nexus (v.6.4.0), an expert system for prediction of general toxicity and genotoxicity; implemented in Nexus (v.2.7.0); using Derek KB 2024 1.0.1 – knowledge-based model – for both genotoxicity and general toxicity
- Leadscope Inc non-human genetic toxicity model suite (2023.0.2-4) - statistical model - for genotoxicity alone
- OECD QSAR Toolbox (v4.7) – hybrid model - for both genotoxicity and general toxicity

The alerts flagged by the metabolites by the (Q)SAR models used were compared to the alerts returned by inpyrfluxam, major rat metabolites and metabolites with experimental data.

With regard to the evaluation of chemical similarity of the selected plant/livestock metabolites to inpyrfluxam, major rat metabolites or metabolites with experimental data, for read-across purposes, HSE followed the general principles detailed in the EFSA Scientific Opinion on Evaluation of the Toxicological Relevance of Pesticide Metabolites for Dietary Risk Assessment (EFSA Journal 2012;10(07):2799) and the un-noted EFSA Guidance on the Establishment of the Residue Definition for Dietary Risk Assessment (EFSA Journal 2016;14(12):4549), taking into consideration:

1. The metabolic steps that most likely do not lead to additional toxicity of metabolites:
    - Simple demethylation of the ring or side chain
    - Simple hydroxylation of the ring system without cleavage of the ring
    - Hydroxylation of another ring position
    - Conjugation of a metabolite with amino acid

2. If the metabolite is a conjugated metabolite; in general, conjugated metabolites (glucuronic acid conjugates, glycoside conjugates or glycoside-malonic acid conjugates) are considered to be of similar or lower toxicity compared to their respective aglycon compounds (due to cleavage under acidic conditions in the human gastrointestinal tract).

The QSAR predictions returned by the different models for inpyrfluxam and the selected plant/livestock metabolites to determine their genotoxicity and general toxicity are presented below:

Table 6.8.1.2-1: Derek Nexus genotoxicity predictions for inpyrfluxam and its crop/livestock metabolites

Name	Mutagenicity in vitro	Mutagenicity in vivo	Chromosome damage in vitro	Chromosome damage in vivo
Inpyrfluxam	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
1'-CH_2OH-S-2840	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
Gly-1'-CH_2OH-S-2840	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
Glu-1'-CH_2OH-S-2840	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
1'-COOH-S-2840	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
3'-OH-S-2840	**Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**

1',1'-bis-(CH_2OH)-S-2840	Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
N-des-Me-S-2840	Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
Glc-NDM-S-2399B	Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
Glc-NDM-S-2399A	Mutagenicity in vitro in bacterium is INACTIVE No misclassified or unclassified features	No alerts activated	No alerts activated	No alerts activated
N-des-Me-1'-CH_2OH-S-2840	**Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
DFPA	**Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
N-des-Me-DFPA	**Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**
DFPA-CONH2	**Mutagenicity *in vitro* in bacterium is INACTIVE No misclassified or unclassified features**	**No alerts activated**	**No alerts activated**	**No alerts activated**

Bold = substances that have been adequately tested for genotoxicity

Table 6.8.1.2-2: Leadscope genotoxicity predictions for inpyrfluxam and its crop/livestock metabolites

Name	Bacterial Mutation v2	*In vivo* Micronuc Mouse v2
Inpyrfluxam	**Negative;0.349**	**Negative; 0.384**
1'-CH_2OH-S-2840	**Indeterminate;0.422**	**Not in domain;0.412**
Gly-1'-CH_2OH-S-2840	**Negative;0.223**	**Negative ;0.413**
Glu-1'-CH_2OH-S-2840	**Negative;0.075**	**Negative; 0.251**
1'-COOH-S-2840	**Not in domain;0.136**	**Not in domain;0.271**
3'-OH-S-2840	**Not in domain;0.422**	**Not in domain;0.412**
1',1'-bis-(CH_2OH)-S-2840	Negative;0.255	Not in domain;0.408
N-des-Me-S-2840	Negative;0.348	Negative;0.361
Glc-NDM-S-2399B	Negative;0.181	Negative;0.403
Glc-NDM-S-2399A	Negative;0.181	Negative;0.403
N-des-Me-1'-CH_2OH-S-2840	**Indeterminate;0.421**	**Not in domain;0.399**
DFPA	**Negative;0.300**	**Negative;0.432**

N-des-Me-DFPA	**Negative;0.299**	**Negative;0.423**
DFPA-CONH2	**Indeterminate;0.449**	**Positive; 0.631**

Bold = substances that have been adequately tested for genotoxicity

Table 6.8.1.2-3: OECD QSAR Toolbox genotoxicity profiling for inpyrfluxam and its crop/livestock metabolites

Profiler	DNA binding by OASIS	DNA binding by OECD	DNA alerts for AMES, CA and MNT by OASIS	*in vitro* mutagenicity (Ames test) alerts by ISS	*in vivo* mutagenicity (Micronucleus) alerts by ISS	Protein binding alerts for Chromosomal aberration by OASIS
Inpyrfluxam	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
1'-CH_2OH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
Gly-1'-CH_2OH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
Glu-1'-CH_2OH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
1'-COOH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
3'-OH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
1',1'-bis-(CH_2OH)-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
N-des-Me-S-2840	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
Glc-NDM-S-2399B	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
Glc-NDM-S-2399A	No alert found	No alert found	No alert found	No alert found	H-acceptor-path3-H-acceptor	No alert found
N-des-Me-1'-CH_2OH-S-2840	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**

DFPA	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
N-des-Me-DFPA	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**
DFPA-CONH2	**No alert found**	**No alert found**	**No alert found**	**No alert found**	**H-acceptor-path3-H-acceptor**	**No alert found**

Bold = substances that have been adequately tested for genotoxicity.

Table 6.8.1.2-4: Derek Nexus general toxicity predictions for inpyrfluxam and its crop/livestock metabolites

Name	Derek Nexus output
Inpyrfluxam	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
1'-CH_2OH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
Gly-1'-CH_2OH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
Glu-1'-CH_2OH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
1'-COOH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
3'-OH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
1',1'-bis-(CH_2OH)-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Alert matched: RapidPrototype059 1,3-Dihydroxypropane or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features

N-des-Me-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
Glc-NDM-S-2399B	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
Glc-NDM-S-2399A	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
N-des-Me-1'-CH_2OH-S-2840	Nephrotoxicity in mammal is EQUIVOCAL Alert matched: RapidPrototype044 Indan or derivative Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
DFPA	Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
N-des-Me-DFPA	Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features
DFPA-CONH2	Skin sensitisation in mammal is NON-SENSITISER No misclassified or unclassified features

Table 6.8.1.2-5: OECD QSAR Toolbox general toxicity profiling for inpyrfluxam and its crop/livestock metabolites

Profiler	**Output for** **Inpyrfluxam** **1'-CH_2OH-S-2840** **Gly-1'-CH_2OH-S-2840** **Glu-1'-CH_2OH-S-2840** **1'-COOH-S-2840** **3'-OH-S-2840** **1',1'-bis-(CH_2OH)-S-2840** **N-des-Me-S-2840** **Glc-NDM-S-2399B** **Glc-NDM-S-2399A** **N-des-Me-1'-CH_2OH-S-2840**	**Output for** **DFPA** **N-des-Me-DFPA** **DFPA-CONH2**
Acute Oral Toxicity	Not categorized	Not categorized
Carcinogenicity (genotox and nongenotox) alerts by ISS	No alert found	No alert found
DART scheme	Not known precedent reproductive and developmental toxic potential	Not known precedent reproductive and developmental toxic potential
Estrogen Receptor Binding	Non binder	Non binder

Eye irritation/corrosion Exclusion rules by BfR	Undefined	Undefined
Eye irritation/corrosion Inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met
Oncologic Primary Classification	Not classified	Not classified
Protein binding alerts for skin sensitization by OASIS	No alert found	No alert found
Protein binding by OASIS	Acylation >> Ester aminolysis >> Amides	No alert found
Protein binding by OECD	Acylation >> Direct Acylation Involving a Leaving group >> Acetates	No alert found
Repeated dose (HESS)	Not categorized	Not categorized
Retinoic Acid Receptor Binding	Not possible to classify according to these rules	Not possible to classify according to these rules
Skin irritation/corrosion Exclusion rules by BfR	Undefined	Undefined
Skin irritation/corrosion Inclusion rules by BfR	Inclusion rules not met	Inclusion rules not met
Toxic hazard classification by Cramer	High (Class III);1N,2N,3Y,4N	High (Class III);1N,2N,3Y,4N
rtER Expert System - USEPA	No alert found	No alert found

Inpyrfluxam and all metabolites, apart from DFPA, N-Des-Me-DFPA and DFPA-CONH2, activated the nephrotoxicity prototype in Derek Nexus. Similarly, excluding DFPA, N-Des-Me-DFPA and DFPA-CONH2, all metabolites and the parent activated the protein binding alerts, normally associated with skin sensitisation potential, in the OECD QSAR Toolbox. Leadscope genotoxicity predictions indicated that 1'-CH_2OH-S-2840, DFPA-CONH2 and N-des-Me-1'-CH_2OH-S-2840 were indeterminate for bacterial mutation while DFPA-CONH2 returned a positive alert for in vivo micronucleus (mouse). The predicted nephrotoxicity, along with the indeterminate and positive genotoxicity alerts, are not considered relevant, as sufficient experimental and read across data confirm that none of the selected plant/livestock metabolites pose genotoxicity or greater general toxicity concerns than the parent compound. The skin sensitisation alerts are also not considered relevant to dietary metabolites.

In silico predictions and read-across were relied upon for the evaluation of the following metabolites: Gly-1'-CH_2OH-S-2840, 1',1'-bis-(CH_2OH)-S-2840, N-des-Me-S-2840, Glc-NDM-S-2399A and Glc-NDM-S-2399B due to the absence of sufficient toxicological data.

<u>Gly-1'-CH_2OH-S-2840</u>

Gly-1'-CH_2OH-S-2840 was predicted to be non-genotoxic and had no additional alerts for general toxicity than the parent across all in silico models used. Gly-1'-CH_2OH-S-2840 is a sugar conjugate of 1'-CH_2OH-S-2840, which is considered to be a major rat metabolite, covered by parent. The sugar moiety will be cleaved in the gastrointestinal

tract, releasing the aglycone, which is a major rat metabolite, **covered by parent**. Therefore, the **dietary reference values of inpyrfluxam** could be used for Gly-1'-CH_2OH-S-2840 for risk assessment purposes.

1',1'-bis-(CH_2OH)-S-2840

1',1'-bis-(CH_2OH)-S-2840 was predicted to be non-genotoxic and had no additional alerts for general toxicity than the parent across all in silico models used. The chemical structures of 1',1'-bis-(CH_2OH)-S-2840 and the major rat metabolite, 1'-CH_2OH-S-2840 glucuronide are similar, both containing similar functional groups except for a different number of alcohols. Due to the structural similarity among these compounds, their metabolism and toxicity profiles are expected to be comparable. Read across from 1'-CH_2OH-S-2840 glucuronide, a major rat metabolite covered by parent, further supports the absence of genotoxicity and higher general toxicity for 1',1'-bis-(CH_2OH)-S-2840. Overall, the toxicity profile of 1',1'-bis-(CH_2OH)-S-2840 can be considered to be comparable **to the major rat metabolite,** 1'-CH_2OH-S-2840 glucuronide and the **parent substance**. Therefore, the **dietary reference values of inpyrfluxam** could be used for 1',1'-bis-(CH_2OH)-S-2840 for risk assessment purposes.

N-des-Me-S-2840

N-des-Me-S-2840 was predicted to be non-genotoxic and had no additional alerts for general toxicity than the parent across all in silico models used. N-des-Me-S-2840 has a similar chemical structure to inpyrfluxam, but with the N-methyl group removed. Given the high degree of structural similarity with the parent substance, supported by the comparative QSAR predictions, it is reasonable to anticipate that their toxicity profiles will be essentially identical. Overall, the toxicity profile of N-des-Me-S-2840 can be considered to be **equivalent to the parent substance**. Therefore, the **dietary reference values of inpyrfluxam** could be used for risk assessment purposes.

Glc-NDM-S-2399A and Glc-NDM-S-2399B

Glc-NDM-S-2399A and Glc-NDM-S-2399B were predicted to be non-genotoxic and had no additional alerts for general toxicity than the parent across all in silico models used. Glc-NDM-S-2399A and Glc-NDM-S-2399B are sugar conjugates of N-des-Me-S-2840. Therefore, the toxicity profile of Glc-NDM-S-2399A and Glc-NDM-S-2399B are considered to be covered by the assessment of N-des-Me-S-2840. Overall, the toxicity profiles of Glc-NDM-S-2399A and Glc-NDM-S-2399B can be considered to be **equivalent to that of the parent**. Therefore, the **dietary reference values of inpyrfluxam** could be used for risk assessment purposes.

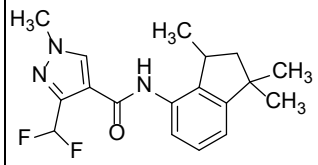
Overall conclusion on the toxicological assessment of selected plant and livestock metabolites of inpyrfluxam

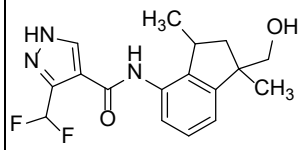
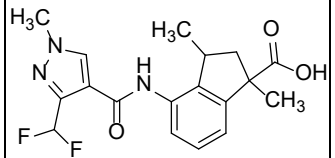
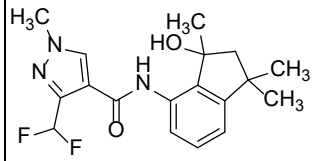
Overall, all of the plant and livestock metabolites of inpyrfluxam have been assigned the parent's dietary reference values, because either they are major rat metabolites covered by parent (1'-COOH-S-2840, N-des-Me-1'-COOH-S-2840, 1'-CH_2OH-S-2840 glucuronide and 1'-CH_2OH-S-2840), or are no more toxic than parent based on experimental data (1'-COOH-S-2840, 3'-OH-S-2840, DFPA, N-des-Me-DFPA and DFPA-CONH2) or a comparative QSAR analysis and read-across approach (Gly-1'-CH_2OH-S-2840, 1',1'-bis-(CH_2OH)-S-2840, N-des-Me-S-2840, Glc-NDM-S-2399A and Glc-NDM-S-2399B).

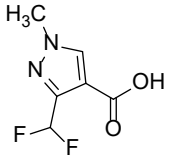
No toxicological information is available for a postulated low-level metabolite in wheat and barley grain processed products, dehydrate of 3'-OH-S-2840. Therefore, currently no toxicological reference values (TRVs) can be established for this metabolite.

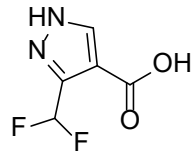
A summary table of the toxicological characterisation of the plant/livestock metabolites of inpyrfluxam is presented below.

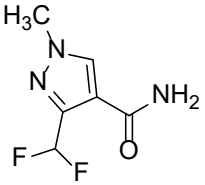
Table 6.8.1.1-14: Summary table of the toxicological characterisation of plant/livestock metabolites of inpyrfluxam for the purposes of the RD for risk assessment

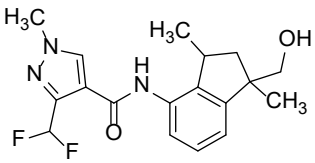
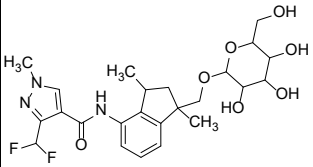
Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
Parent **Inpyrfluxam** **--** O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CN(C)N=C3C(F)F		Parent	**Non-genotoxic** <u>Full genotoxicity data package</u> Ames test (negative) Mammalian cells gene mutation assay (negative) Chromosome aberration assay (negative) *In vivo* micronucleus assay (negative)	**Classification** Acute tox Cat 3, H301: Toxic of swallowed <u>Full data package</u> No evidence of carcinogenicity, reproductive and developmental toxicity, or neurotoxicity. Repeat dose exposure resulted in liver changes in all test species with secondary thyroid findings in rats, mice and dogs; in adrenal gland changes in rats and dogs; and in ovary changes in rats.	**ADI = 0.06 mg/kg/day** **ARfD = 0.3 mg/kg bw**

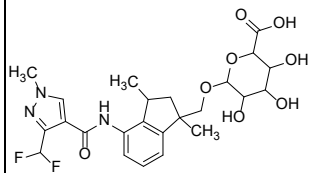
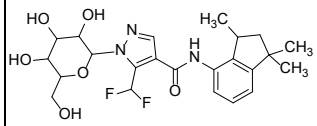
Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
N-des-Me-1'-CH_2OH**-S-2840** **--** O=C(NC1=CC=CC2=C1C(CC2(C)CO)C)C3=CNN=C3C(F)F		Yes	**Non-genotoxic** Covered by parent (major rat metabolite)	**Covered by parent** (major rat metabolite)	**Parent's TRVs**
1'-COOH-S-2840 (A and B isomers) **--** O=C(NC1=CC=CC2=C1C(CC2(C(O)=O)C)C)C3=CN(C)N=C3C(F)**F**		Yes (>10 % of the AD, urine, both sexes)	**Non-genotoxic** Covered by parent (major rat metabolite) + Negative adequate genotoxicity package	**Covered by parent** (major rat metabolite) + Data Acute oral study: LD_{50} > 2000 mg/kg bw	**Parent's TRVs**
3'-OH-S-2840 **--** O=C(NC1=CC=CC2=C1C(C)(O)CC2(C)C)C3=CN(C)N=C3C(F)F		No	**Non-genotoxic** Negative adequate genotoxicity package	**Not more toxic than parent** Data Acute oral study: LD_{50} > 2000 mg/kg bw 90-day dietary toxicity study in rat: NOAEL = 500 ppm	**Parent's TRVs**

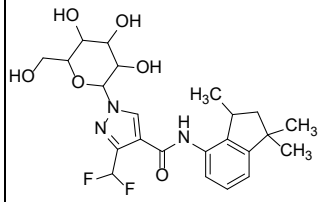
Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
				(37.9 mg/kg/day) based on histopathological findings in the liver and ovary	
DFPA **--** O=C(O)C1=CN(C)N=C1C(F)F		No	**Non-genotoxic** <u>Negative genotoxicity package</u> Ames test (negative) Mammalian cells gene mutation assay (negative) Chromosome aberration assay (negative) + negative QSAR prediction for in vivo micronucleus	**Not more toxic than parent** <u>Data</u> Acute oral study: LD_{50} > 2000 mg/kg bw 90-day dietary toxicity study in rat: NOAEL = 1000 mg/kg bw/day (no effect at the highest dose) Pre-natal developmental toxicity in rabbit: NOAEL (maternal and developmental) = 250 mg/kg bw/day	**Parent's TRVs**

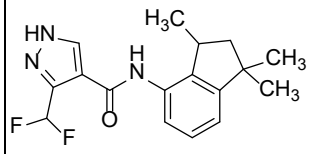
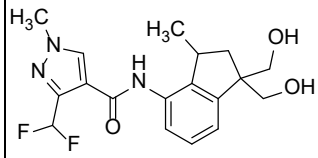
Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
				(highest dose; severe maternal toxicity at ≥ 500 mg/kg/day in range-finding study)	
***N*-des-Me-DFPA** **--** O=C(O)C1=CNN=C1C(F)F		No	**Non-genotoxic** <u>Negative genotoxicity package</u> Ames test (negative) Mammalian cells gene mutation assay (negative) Chromosome aberration assay (negative) + negative QSAR prediction for in vivo micronucleus	**Not more toxic than parent** <u>Data</u> Acute oral study: LD_{50} > 2000 mg/kg bw 28-day dietary toxicity study in rat: NOAEL = 1018 mg/kg bw/day (no effect at the limit dose) 90-day dietary toxicity study in rat: NOAEL = 1000 mg/kg bw/day (no effect at the limit dose)	**Parent's TRVs**

Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
				Pre-natal developmental toxicity in rabbit: NOAEL (maternal) = 300 mg/kg bw/day (reduction of body weight gain and food consumption) NOAEL (developmental) = 1000 mg/kg bw/day (no effect at the limit dose)	
DFPA-CONH$_2$ -- O=C(N)C1=CN(C)N=C1C(F)F		No	**Non-genotoxic** <u>Negative adequate genotoxicity package</u> Ames test (negative) Mammalian cells gene mutation assay (negative) Chromosome aberration assay (positive) *In vivo* micronucleus assay (negative)	**Not more toxic than parent** <u>Data</u> Acute oral study: LD_{50} > 500 and < 2000 mg/kg bw 28-day dietary toxicity study in rat: NOAEL = 37.4 mg/kg bw/day (based on lower body weight,	**Parent's TRVs**

Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
				body weight gain and food consumption)	
1'-CH_2OH**-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)CO)C)C3=CN(C)N=C3C(F)F		Yes (its direct downstream glucuronide conjugate, Glu-1'-CH_2OH-S-2840 is a major rat metabolite)	**Non-genotoxic** Covered by parent (major rat metabolite)	**Covered by parent** (major rat metabolite)	**Parent's TRVs**
Gly-1'-CH_2OH**-S-2840 (sugar conjugate of 1'-**CH_2OH**-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)COC(C(C(O)C3O)O)OC3CO)C)C4=CN(N=C4C(F)F)C		No	**Non-genotoxic** As the sugar conjugate of 1'-CH_2OH-S-2840, a predicted major rat metabolite, covered by parent – the sugar will be cleaved in the GI tract releasing the predicted major rat metabolite	**Covered by parent** As the sugar conjugate of 1'-CH_2OH-S-2840, a predicted major rat metabolite, covered by parent – the sugar will be cleaved in the GI tract releasing the predicted major rat metabolite	**Parent's TRVs**

Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
Glu-1'-CH_2OH-S-2840 (Glucuronide of 1'-CH_2OH-S-2840) -- O=C(NC1=CC=CC2=C1C(CC2(C)COC(C(C(O)C3O)O)OC3C(O)=O)C)C4=CN(N=C4C(F)F)C		Yes (>10% of the AD, urine + bile)	**Non-genotoxic** Covered by parent (major rat metabolite)	**Covered by parent** (major rat metabolite)	**Parent's TRVs**
Glc-NDM-S-2399B -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=C(N(N=C3)C4C(C(O)C(O)C(CO)O4)O)C(F)F		No	**Non-genotoxic** Aas the conjugated form of the metabolite *N*-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form *N*-des-Me-S-2840; no concern for genotoxicity was identified in the comparative QSAR analysis of *N*-des-Me-S-2840 compared to inpyrfluxam	**Not more toxic than parent** Aas the conjugated form of the metabolite *N*-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form *N*-des-Me-S-2840; no additional relevant alert for general toxicity was identified in the comparative	**Parent's TRVs**

Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
				QSAR analysis of *N*-des-Me-S-2840 compared to inpyrfluxam	
Glc-NDM-S-2399A **--** O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CN(N=C3C(F)F)C4OC(CO)C(O)C(O)C4O		No	**Non-genotoxic** As the conjugated form of the metabolite *N*-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form *N*-des-Me-S-2840; no concern for genotoxicity was identified in the comparative QSAR analysis of *N*-des-Me-S-2840 compared to inpyrfluxam	**Not more toxic than parent** Aas the conjugated form of the metabolite *N*-des-Me-S-2840, the toxicity profile of this metabolite is expected to be comparable / less severe compared to its aglycon form *N*-des-Me-S-2840; no additional relevant alert for general toxicity was identified in the comparative QSAR analysis of *N*-des-Me-S-2840 compared to inpyrfluxam	**Parent's TRVs**

Name, code, and smiles	Structure	Major rat metabolite (%)?	Genotoxicity conclusion and basis	General toxicity conclusion and basis	Reference values for dietary risk assessment
***N*-des-Me-S-2840** -- O=C(NC1=CC=CC2=C1C(CC2(C)C)C)C3=CNN=C3C(F)F		No	**Non-genotoxic** No concern for genotoxicity was identified in the comparative QSAR analysis compared to inpyrfluxam	**Not more toxic than parent** No additional relevant alert for general toxicity was identified in the comparative QSAR analysis compared to inpyrfluxam	**Parent's TRVs**
1',1'-bis-(CH_2OH)-S-2840) -- O=C(NC1=CC=CC2=C1C(CC2(CO)CO)C)C3=CN(C)N=C3C(F)F		No	**Non-genotoxic** No concern for genotoxicity was identified in the comparative QSAR analysis compared to the predicted major rat metabolite 1'-CH_2OH-S-2840	**Not more toxic than parent** No additional relevant alert for general toxicity was identified in the comparative QSAR analysis compared to the predicted major rat metabolite 1'-CH_2OH-S-2840	**Parent's TRVs**

B.6.8.2. Supplementary studies on the active substance

Not available

B.6.8.3. Studies on endocrine disruption

Inpyrfluxam was evaluated for any potential of endocrine activity using a battery of non-GLP in vitro assays. There were 5 in vitro studies in mammalian cells including estrogen and androgen receptor transactivation, steroidogenesis, thyroid hormone alpha receptor transactivation, sodium/iodide symporter (NIS) assays, and the thyroperoxidase (TPO) assay in rat thyroid microsomes.

Further, two in vivo mechanistic studies have been performed with inpyrfluxam in rats and mice to investigate the mode of action behind the adverse effects noted in liver and thyroid in rats, mice and dogs.

In vitro studies

1. In vitro estrogen and androgen receptor/s transactivation assay

Reference:	KCA 5.8.3/01
Report Title:	Evaluation of effects of S-2399 on human estrogen receptor alpha and human androgen receptor using in vitro reporter gene assay
Author(s) & Year:	[redacted] (2017b)
Document No, Authority registration No	Study No. RGA-124 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: LS-02122 Purity: 99.9%
Method of analysis:	Not required
Guideline(s):	Follow principles of OECD 455 (2015); OECD 458 (2016)
Deviations from current guideline:	*hERα Assay:* • A single run was performed instead of duplicate runs for detecting estrogenic and anti-estrogenic effects. • A single positive control, 17β-estradiol (E2), was used for the agonist assay, and 4-hydroxytamoxifen (OHT) for the antagonist assay (instead of multiple positive controls). • The concentration of E2 in the antagonist assay was slightly higher than that specified in the testing guidelines. *hAR Assay:*

	• The hAR-HeLa 4-11 cell line (stably transfected) was used instead of the cell line described in the test guideline. • A single run was performed to detect androgenic and anti-androgenic effects, instead of duplicate runs.
Impact of the deviation:	The observed deviations reduce the validity of the data: 1. *Single Positive Control:* The test systems were validated to detect both hERα and hAR transactivation effectively. 2. *Elevated E2 Levels in the hERα Antagonist Assay:* The positive control yielded the expected results, confirming assay reliability. 3. *Single Run Instead of Duplicate:* The test item showed no agonist or antagonist activity in either the hERα or hAR transactivation assays, while the positive controls produced the expected outcomes. 4. *Use of non recommended cell line:* The cell line was proved to express hAR and the positive controls produced the expected results
GLP or GEP:	No
Acceptability:	Yes- as supplementary/mechanistic study
Study relied upon:	Yes

Methods

In a non-GLP study, the estrogenic, anti-estrogenic, androgenic and anti-androgenic potential of inpyrfluxam was assessed in receptor transactivation assays using HeLa9903 cells stably expressing human estrogen receptor alpha (hERα) or HeLa 4-11 cells stably expressing human androgen receptor (hAR). The appropriate concentrations for the transactivation assays were selected based on the solubility and preliminary cytotoxicity assays. Inpyrfluxam in DMSO was tested across a concentration range from 100 pM to 100 µM, both in the presence and absence of the appropriate agonist (17β-Estradiol (E2) for the hERα assay and Dihydrotestosterone (DHT) for the hAR assay). E2 and 4-hydroxytamoxifen were used as positive controls in the agonist and antagonist hERα assays respectively. DHT and hydroxyflutamide were used as positive controls in the agonist and antagonist hAR assays respectively.

The study followed the principles of OECD test guidelines 455 and 458, with the exception of the aforementioned deviations.

Results

Solubility and preliminary cytotoxicity assay

Inpyrfluxam was soluble in cell culture medium up to 100 µM with no precipitation. A preliminary cytotoxicity assay was conducted with concentrations ranging from 100 pM-100 µM to determine the maximum concentration for the transactivation assays. Cytotoxicity (≥20%) was noted at and above 10 µM in both cell lines.

Agonist and antagonist assays for hERα and hAR

Inpyrfluxam did not show any agonistic or antagonistic effects on hERα or hAR, in the presence and absence of appropriate agonists (E2 or DHT). However, HSE notes the high SDs and the significant variability in the results. Cytotoxicity (≥20%) was noted at and above 10 µM in both cell lines. Precipitation was not observed at any of the tested concentrations.

Positive controls produced the expected results which confirmed the validity of the test system. The results are presented in table 6.8.3-1 and 6.8.3-2.

Table 6.8.3-1: Effect of Inpyrfluxam on agonistic and antagonistic activity in HeLa9903 cells stably expressing hERα.

Concentration	Relative transcriptional activity (%) (Mean± SD)			
	Agonistic activity		Antagonistic activity	
	Positive control (E2)	S-2399	Positive control (OHT)	S-2399
0	100 ± 25	100 ± 30	100 ± 21	100 ± 23
10 pM	185 ± 54 *	NT	117 ± 38	NT
100 pM	411 ± 96 *	93 ± 15	136 ± 33	110 ± 36
1 nM	588 ± 97 *	86 ± 13	136 ± 10	90 ± 24
10 nM	490 ± 111 *	97 ± 32	30 ± 5 **	104 ± 24
100 nM	504 ± 109 *	91 ± 15	19 ± 5 **	104 ± 32
1 µM	523 ± 68 *	120 ± 56	19 ± 3 **	110 ± 19
10 µM	320 ± 18 *	ND	13 ± 6 **	ND
100 µM	NT	ND	NT	ND

*: Agonistic activity observed (criteria: ≥ 149% as PC_{10} value, 10% or more relative activation of the average of transcriptional activity induced by 1 nM of E2)
**: Antagonistic activity observed (criteria: ≤ 70%, 30% or more relative inhibition of the average of transcriptional activity induced by 100 pM of 17β-estradiol)
E2: 17β-Estradiol NT: Not tested
OHT: 4-Hydroxytamoxifen ND: Not determined because of cytotoxicity

Table 6.8.3-2: Effect of inpyrfluxam on agonistic and antagonistic activity in HeLa 4-11 cells stably expressing hAR.

Concentration	Relative transcriptional activity (%) (Mean± SD)			
	Agonistic activity		Antagonistic activity	
	Positive control (DHT)	S-2399	Positive control (HF)	S-2399
0	100 ± 6	100 ± 4	100 ± 6	100 ± 5
10 pM	170 ± 11	NT	94 ± 5	NT
100 pM	946 ± 47 *	93 ± 5	91 ± 6	94 ± 3
1 nM	1587 ± 47 *	90 ± 2	90 ± 3	93 ± 6
10 nM	1637 ± 48 *	93 ± 5	70 ± 5	92 ± 5
100 nM	1635 ± 48 *	93 ± 4	24 ± 2 **	93 ± 5
1 µM	1596 ± 25 *	87 ± 3	6 ± 1 **	85 ± 6
10 µM	1622 ± 94 *	ND	31 ± 3 **	ND
100 µM	NT	ND	NT	ND

*: Agonistic activity observed (criteria: ≥ 249% as PC_{10} value, 10% or more relative activation of the average of transcriptional activity induced by 1 nM of DHT)
**: Antagonistic activity observed (criteria: ≤ 70%, 30% or more relative inhibition of the average of transcriptional activity induced by 100 pM of dihydrotestosterone)
DHT: Dihydrotestosterone NT: Not tested
HF: Hydroxyfultamide ND: Not determined because of cytotoxicity

Conclusion

In this non-guideline and non-GLP in vitro hERα and hAR transactivation assay using mammalian cells, inpyrfluxam did not exhibit estrogenic, anti-estrogenic, androgenic or anti-androgenic potential up to cytotoxic concentrations. Therefore, it is concluded that inpyrfluxam does not have any effect on either hERα or hAR-mediated transactivation in vitro. However, considering the deviations from the guidelines and the high variability in the results, the reliability of these results is reduced.

[redacted] (2017b)

2. In vitro steroidogenesis assay

Reference:	KCA 5.8.3/02
Report Title:	In vitro steroidogenesis assay of S-2399 with H295R cell line
Author(s) & Year:	[redacted] (2017c)
Document No, Authority registration No	Study No. HK002 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: LS-118-101206-1 Purity: 99.2%

Method of analysis:	Not required
Guideline(s):	Follow principles of OECD 456 (2011)
Deviations from current guideline:	An independent quality control plate was not tested and quality control parameters such as between-plate coefficients of variance for solvent controls were not calculated. Concurrent positive controls, forskolin and prochloraz, were tested in the same plate as the test substance instead of in an independent quality control plate. Replicate measures of the same sample were not performed.
Impact of the deviation:	The observed deviations reduce the validity of the results; although no changes in hormonal production were observed after inpyrfluxam treatment in both runs, the positive controls produced the expected outcomes, and all set quality control parameters within the plates were met, the reliability of the findings is diminished.
GLP or GEP:	No
Acceptability:	Yes- as supplementary/mechanistic study
Study relied upon:	Yes

Methods

In a non-GLP study, inpyrfluxam was evaluated for its potential to affect androgen or estrogen production in H295R cells in vitro. The appropriate concentrations for the steroidogenesis assay were selected based on the solubility, preliminary cytotoxicity and chemical interference tests. Inpyrfluxam in DMSO was tested across a concentration range from 300 pM – 3 µM in two experimental runs.

The study followed the principles of OECD test guidelines 456, with the exception of the aforementioned deviations.

Results

Solubility, cell viability and chemical interference tests

Inpyrfluxam was soluble in cell culture medium up to 100 µM with no precipitation.

A preliminary cytotoxicity assay was conducted with concentrations ranging from 1 µM -100 µM to determine the maximum concentration for the main assays. Cytotoxicity (≥20%) was observed at and above 10 µM.

No chemical interference (≥ 20%) was detected up to 3 µM, with more than 80% recovery of testosterone and 17β-estradiol.

Steroidogenesis assay

No statistically significant changes in testosterone or 17β-estradiol production were observed at any of the tested concentrations compared to the control, across both experimental runs. Additionally, there was no cytotoxicity (≥20% reduction in cell viability) or precipitation up to the highest tested concentration of 3 µM.

Positive controls produced expected results which confirmed the validity of the test system. The results are presented in table 6.8.3-3 and 6.8.3-4.

Table 6.8.3-3: Effect of Inpyrfluxam on testosterone or 17β-estradiol production in H295R cells – experiment 1

Inpyrfluxam concentration	Viability (%)	Testosterone (pg/mL)	Fold change	Stat.	Estradiol (pg/mL)	Fold change	Stat.
0	100 ± 6.74	3210 ± 233	1.00 ± 0.0725	-	147 ± 10.8	1.00 ± 0.0735	-
300 pM	101 ± 7.46	3250 ± 42.4	1.01 ± 0.0132	NS	161 ± 3.81	1.09 ± 0.0259	NS
3 nM	102 ± 2.85	3810 ± 713	1.18 ± 0.222	NS	167 ± 1.95	1.13 ± 0.0133	NS
30 nM	96.0 ± 4.33	3830 ± 169	1.19 ± 0.0527	NS	169 ± 6.17	1.15 ± 0.0419	NS
300 nM	98.3 ± 1.25	3610 ± 208	1.12 ± 0.0647	NS	168 ± 4.92	1.14 ± 0.0334	NS
3 µM	96.3 ± 0.981	3560 ± 424	1.11 ± 0.132	NS	162 ± 29.7	1.10 ± 0.202	NS
Positive controls							
Forskolin 10 µM	126 ± 5.90	5750 ± 252	1.79 ± 0.0783	NT	2830 ± 461	19.2 ± 3.13	NT
Prochloraz 1 µM	96.6 ± 1.98	106 ± 29.2	0.0330 ± 0.00910	NT	67.5 ± 10.3	0.458 ± 0.0697	NT

Stat.: results of statistical analysis; NS: not significant; NT: not tested

Table 6.8.3-4: Effect of Inpyrfluxam on testosterone or 17β-estradiol production in H295R cells – experiment 2

Inpyrfluxam concentration	Viability (%)	Testosterone (pg/mL)	Fold change	Stat.	Estradiol (pg/mL)	Fold change	Stat.
0	100 ± 3.37	1750 ± 208	1.00 ± 0.119	-	109 ± 13.0	1.00 ± 0.119	-
300 pM	104 ± 4.21	1990 ± 108	1.14 ± 0.0620	NS	113 ± 1.57	1.04 ± 0.0144	NS
3 nM	107 ± 0.597	1710 ± 122	0.975 ± 0.0696	NS	105 ± 9.15	0.965 ± 0.0839	NS
30 nM	101 ± 0.760	2030 ± 100	1.16 ± 0.0574	NS	110 ± 5.91	1.01 ± 0.0542	NS
300 nM	101 ± 1.42	1700 ± 231	0.972 ± 0.132	NS	108 ± 3.68	0.993 ± 0.0337	NS
3 µM	93.5 ± 2.55	1840 ± 274	1.05 ± 0.157	NS	92.9 ± 8.98	0.852 ± 0.0824	NS
Positive controls							
Forskolin 10 µM	130 ± 6.89	4280 ± 72.5	2.45 ± 0.0415	NT	2070 ± 133	19.0 ± 1.22	NT
Prochloraz 1 µM	95.3 ± 4.34	137 ± 32.2	0.0783 ± 0.0184	NT	<31.3*	<0.287	NT

Stat.: results of statistical analysis; NS: not significant; NT: not tested; *: measured optical density value was out of the linear range of the standard curve (below limit of quantification of 31.3 pg/mL)

Conclusion

In this non-guideline and non-GLP in vitro steroidogenesis assay using mammalian cells, inpyrfluxam did not affect testosterone or 17β-estradiol production up to the highest concentration showing no chemical interference. Therefore, it is concluded that inpyrfluxam does not have the potential to affect androgen or estrogen production in vitro. However, considering the deviations from the guideline, the reliability of these results is reduced.

[redacted] (2017c)

3. In vitro human thyroid hormone receptor alpha transactivation assay

Reference:	KCA 5.8.3/04
Report Title:	Evaluation of the effect of S-2399 Technical Grade on human thyroid hormone receptor alpha using in vitro reporter gene assay
Author(s) & Year:	[redacted] (2019)
Document No, Authority	Study No. RGA-147 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan

registration No	
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	None
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes- as supplementary/mechanistic study
Study relied upon:	Yes

Methods

In a non-GLP and non-guideline study, the agonistic or antagonistic potential of inpyrfluxam was assessed using HeLa TRa1 cells stably expressing human thyroid hormone receptor alpha (hTRα). The appropriate concentrations for the transactivation assay were selected based on the solubility and preliminary cytotoxicity assays. Inpyrfluxam in DMSO was evaluated across a concentration range from 1 nM to 100 µM, both in the presence and absence of 10 nM agonist (3,3’,5-Triiodo-L-thyronine (T3)). After 48 h exposure period, receptor transactivation was estimated by measuring the emitted luminescence. T3 and 3,3',5,5'-Tetrabromobisphenol A (TBBPA) were used as positive controls in the agonist and antagonist assays, respectively.

Results

Solubility and preliminary cytotoxicity assay

Inpyrfluxam was soluble in cell culture medium up to 100 µM with no precipitation.

Preliminary cytotoxicity assay was conducted with concentrations ranging from 1 nM - 100 µM to determine the maximum concentration for the transactivation assay. Cytotoxicity (≥20%) was observed at and above 10 µM.

Agonist and antagonist assays for hTRα

Inpyrfluxam did not show any agonistic or antagonistic effects on hTRα in the presence and absence of appropriate agonists (T3). Cytotoxicity (≥20%) was noted at and above 10 µM. Precipitation was not observed at any tested concentrations.

Positive controls produced the expected results which confirmed the validity of the test system. The results are presented in table 6.8.3-4 and 6.8.3-5.

Table 6.8.3-4: Effect of inpyrfluxam on agonistic activity in HeLa TRa1 cells stably expressing hTRα.

	Concentration	Cell viability (%) (Mean ± SD)	Relative transcriptional activity (%) (Mean ± SD)
Inpyrfluxam	0	100 ± 3.7	100 ± 8.7
	1 nM	101.0 ± 4.5	104.5 ± 10.9
	10 nM	100.9 ± 4.8	101.6 ± 9.6
	100 nM	100.5 ± 5.0	102.5 ± 11.3
	1 µM	95.7 ± 4.2	91.6 ± 11.7
	10 µM	60.4 ± 4.5†	ND
	100 µM	29.2 ± 1.8†	ND
Positive control (T3)	0	100 ± 5.7	100 ± 4.2
	10 pM	110.4 ± 4.6	113.7 ± 4.0
	100 pM	108.9 ± 2.3	113.7 ± 4.0
	1 nM	99.4 ± 2.8	230.9 ± 13.1*
	10 nM	93.2 ± 2.0	702.4 ± 39.5*
	100 nM	93.9 ± 1.9	789.8 ± 35.8*
	1 µM	93.2 ± 2.6	780.6 ± 51.4*
	10 µM	91.0 ± 4.7	746.4 ± 52.4*

†: Toxic effect was observed (criteria : ≦ 80% viability)
*: Agonistic activity was observed (criteria: ≧ 169.0% as PC_{10} value, 10% or more relative activation of the mean of transcriptional activity induced by 100 nM of T3)
T3: 3,3',5-Triiodo-L-thyronine
ND: Not determined because of cytotoxicity

Table 6.8.3-5: Effect of inpyrfluxam on antagonistic activity in HeLa TRa1 cells stably expressing hTRα.

	Concentration	Cell viability (%) (Mean ± SD)	Relative transcriptional activity (%) (Mean ± SD)
Inpyrfluxam	0	100 ± 0.9	100 ± 3.6
	1 nM	102.6 ± 1.2	100.6 ± 7.5
	10 nM	102.5 ± 0.9	101.3 ± 5.2
	100 nM	101.4 ± 1.1	101.3 ± 5.8
	1 µM	98.3 ± 1.0	94.6 ± 4.2
	10 µM	51.9 ± 4.7†	ND
	100 µM	30.0 ± 1.7†	ND
Positive control (TBBPA)	0	100 ± 11.7	100 ± 6.1
	8 µM	104.0 ± 3.3	95.8 ± 4.3
	10 µM	105.3 ± 4.8	95.8 ± 5.4
	20 µM	107.3 ± 3.6	83.3 ± 3.4

	40 µM	108.7 ± 2.8	51.0 ± 2.4*
	60 µM	107.3 ± 2.0	23.3 ± 2.3*
	80 µM	97.1 ± 2.4	8.8 ± 1.1*
	100 µM	74.9 ± 1.4†	ND

†: Toxic effect was observed (criteria : ≦ 80% viability)
*: Antagonistic activity was observed (criteria: ≦ 70%, 30% or more relative inhibition of the mean of transcriptional activity induced by 10 nM of 3,3',5-Triiodo-L-thyronine). The mean transcriptional of the spike in control (10 nM of 3,3',5-Triiodo-L-thyronine) was 615.4% in the assay plate of S-2399 Technical Grade.
TBBPA: 3,3',5,5'-Tetrabromobisphenol A
ND: Not determined because of cytotoxicity.

Conclusion

In this non-guideline and non-GLP in vitro hTRα transactivation assay using mammalian cells, inpyrfluxam did not show agonistic or antagonistic potential up to the cytotoxic concentrations. Therefore, it is concluded that inpyrfluxam does not have any effect on hTRα mediated transactivation in vitro. However, these results should be interpreted with caution given that such assay has not been validated.

[redacted] (2019)

4. In vitro sodium/iodide symporter (NIS) assay

Reference:	KCA 5.8.3/05
Report Title:	In vitro inhibition assay of sodium/iodide symporter with S-2399 TG
Author(s) & Year:	[redacted]2020)
Document No, Authority registration No	Study No. S2096 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	None
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	No, due to significant limitations
Study relied upon:	No

Methods

In a non-GLP and non-guideline study, the potential of inpyrfluxam to inhibit the sodium/iodide symporter (NIS) activity was assessed using hNIS-HEK293T-EPA cells. The appropriate concentrations for the NIS assay were selected based on the solubility and preliminary cytotoxicity assays. The hNIS-HEK293T-EPA cells were incubated with inpyrfluxam in DMSO (concentration range 0.1 µM to 30 µM) and $Na^{125}I$ (0.05µCi/well) for 45 minutes. After the incubation period, the cells were lysed with ethanol and the released ^{125}I was quantified. Three independent experiments in sixtuplets were run. Sodium perchlorate (NaClO4) was used as the positive control.

Results

Solubility and preliminary cytotoxicity assay

Inpyrfluxam was soluble in cell culture medium up to 100 µM with no precipitation.

A preliminary cytotoxicity assay was conducted with concentrations ranging from 0.1 µM -100 µM to determine the maximum concentration for the NIS assay. No cytotoxicity (≥20%) was noted up to 100 µM.

Table 6.8.3-6: Effect of inpyrfluxam on viability of hNIS-HEK293T-EPA cells after 45 min treatment

Inpyrfluxam	Viability (%)				
	1st Run		2nd Run		Average
	Mean	SD	Mean	SD	
0 µM	100	7.9	100	5	100
1 µM	103.5	4.7	100	4.4	101.75
3 µM	104.9	2.4	97.8	4.8	101.35
10 µM	103.7	2.5	98.6	5.6	101.15
30 µM	101.8	3.7	92.7*	5	97.25
100 µM	77.5	11.4	87.8**	2.3	82.65

*: Statistically different from the control group at the 0.05 level; **: Statistically different from the control group at the 0.01 level

Sodium/iodide symporter assay

At 30 µM, inpyrfluxam exhibited slight inhibitory effects (~13%) on NIS activity in hNIS-HEK293T-EPA cells. However, no concurrent cytotoxicity assessment or statistical analysis was done. In addition, the assay was not performed up to the highest soluble and non cytotoxic concentration of 100 µM.

Positive control produced expected results which confirmed the validity of the test system. The results are presented in table 6.8.3-7.

Table 6.8.3-7: Effect of inpyrfluxam on NIS activity in hNIS-HEK293T-EPA cells after 45 min treatment

Test Material	Concentration	^{125}I uptake (%)			
		(Mean ± SD)			Average
		1ST Run#	2ND Run#	3RD Run#	
Inpyrfluxam	0 µM	100.0 ± 2.4	100.0 ± 1.9	100.0 ± 2.5	100.0
	0.1 µM	98.7 ± 3.3	94.6 ± 3.7	105.5 ± 1.3	99.6
	0.3 µM	117.2 ± 2.2	100.8 ± 3.2	116.0 ± 3.0	111.3
	1 µM	99.9 ± 0.9	103.4 ± 2.0	104.7 ± 3.4	102.7
	3 µM	98.8 ± 1.9	104.2 ± 4.1	112.0 ± 4.8	105.0
	10 µM	97.2 ± 2.8	106.5 ± 2.3	99.1 ± 1.4	100.9
	30 µM	81.4 ± 0.5	85.6 ± 1.3	94.7 ± 4.0	87.2
$NaClO_4$	0 µM	100.0 ± 2.4	100.0 ± 1.9	100.0 ± 2.5	100.0
	0.001 µM	103.1 ± 2.5	85.4 ± 0.8	91.0 ± 4.3	93.2
	0.01 µM	98.2 ± 2.9	96.0 ± 2.4	91.9 ± 3.6	95.4
	0.1 µM	64.2 ± 2.1	69.2 ± 2.4	62.9 ± 2.3	65.4
	1 µM	21.1 ± 1.1	26.9 ± 1.2	17.3 ± 0.9	21.8
	10 µM	5.5 ± 0.0	4.0 ± 0.1	4.0 ± 0.2	4.5
	100 µM	0.7 ± 0.0	0.8 ± 0.0	1.8 ± 0.3	1.1

only quadruplet data were used for evaluation of the concentrations that showed higher variation in radioiodide data (CPM)

Conclusion

In this non-guideline and non-GLP in vitro NIS assay using mammalian cells, inpyrfluxam showed slight inhibitory potential at 30 µM. However, no concurrent cytotoxicity assessment or statistical analysis was performed. Additionally, the assay was not conducted up to the highest soluble and non cytotoxic concentration. Therefore, the assay is considered to be of limited value.

[redacted] (2020)

5. In vitro thyroperoxidase (TPO) assay

Reference:	KCA 5.8.3/06
Report Title:	In vitro thyroperoxidase inhibition assay with S-2399TG
Author(s) & Year:	[redacted] (2019)

Document No, Authority registration No	Study No. S2017 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Japan
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not required
Guideline(s):	None
Deviations from current guideline:	No
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes
Study relied upon:	Yes

Methods

In a non-GLP and non-guideline study, the potential of inpyrfluxam to inhibit thyroperoxidase (TPO) was assessed using rat thyroid microsomes. The rat thyroid microsomes (n=3) were incubated with inpyrfluxam in DMSO (concentration range - 0.001 µM to 300 µM) and hydrogen peroxide (300 µM) in the presence of a fluorescent probe, amplex red (25 µM). After 30 minutes of incubation, TPO activity was estimated by measuring the emitted fluorescence. 6-propyl-2-thiouracil (PTU) was used as the positive control.

The assay followed the principles of the publication by Paul K. B et al., 2014[9].

Results

Thyroperoxidase assay

Precipitation was noted at 100 and 300 µM in the total assay mix, therefore, these two concentrations were excluded from the evaluation. There was no decrease in the fluorescence signal up to 30 µM.

[9] Katie B. Paul, Joan M. Hedge, Daniel M. Rotroff, Michael W. Hornung, Kevin M. Crofton, and Steven O. Simmons (2014). Development of a thyroperoxidase inhibition assay for high-throughput screening." Chem Res Toxicol 27(3):387-399. DOI: 10.1021/tx400310w

The positive control produced the expected results which confirmed the validity of the test system. The results are presented in table 6.8.3-8.

Table 6.8.3-8: Effect of inpyrfluxam on TPO activity in rat microsomes after 30 min incubation

Test Material	Concentration (µm)	% of vehicle control			
		Microsome A	Microsome B	Microsome C	Mean ± SD
Inpyrfluxam	0.001	137.3	116.6	77.4	110.4 ± 30.42
	0.003	101.9	105.9	73	93.6 ± 17.95
	0.01	108.6	111.2	68.4	96.1 ± 24
	0.03	128.5	114.2	81	107.9 ± 24.37
	0.1	133.3	121.5	75.1	110 ± 30.77
	0.3	109.8	110.1	92.8	104.2 ± 9.9
	1	105.2	117.5	68.9	97.2 ± 25.27
	3	108.3	116	71.8	98.7 ± 23.61
	10	113.3	112.1	71.2	98.9 ± 23.97
	30	115.1	124.2	73	104.1 ± 27.32
PTU	0.001	103.5	101.1	101.3	102 ± 1.33
	0.003	112.8	110.4	70.4	97.9 ± 23.82
	0.01	104.9	108.1	71.8	94.9 ± 20.1
	0.03	110.5	107	73.9	97.1 ± 20.2
	0.1	88.8	86.4	64.3	79.8 ± 13.51
	0.3	62.1	52.6	47.8	54.2 ± 7.28
	1	37	34.1	29.3	33.5 ± 3.89
	3	24.2	21.5	18.5	21.4 ± 2.85
	10	18.4	16.1	14.7	16.4 ± 1.87
	30	13.3	12.7	10.4	12.1 ± 1.53
	100	12.3	11.6	10.9	11.6 ± 0.7
	300	10.5	9	8.6	9.4 ± 1

Conclusion

In this non-guideline and non-GLP in vitro TPO assay in rat microsomes, inpyrfluxam did not show inhibitory effect up to the precipitating concentrations. Therefore, it is concluded that inpyrfluxam does not have any effect on TPO in vitro.

[redacted] (2019)

Overall conclusion on in vitro EATS assays

Inpyrfluxam was evaluated for any potential of endocrine activity for the EATS (estrogen, androgen, thyroid, steroidogenesis) modalities using a battery of non- GLP in vitro assays. Inpyrfluxam did not have any effect on androgen or estrogen production, hERα or hAR or hTRα -mediated transactivation and TPO activity invitro.

The NIS assay is not considered acceptable due to the lack of concurrent cytotoxicity measurement and statistical analysis. Additionally, the assay was not conducted up to the highest soluble and non cytotoxic concentration.

In vivo mechanism of action studies

Two mechanistic studies have been performed with the active substance in rats and mice to investigate the mode of action behind the adverse effects noted in liver and thyroid in rats, mice and dogs.

1. **Mode of Action Study for Rat Liver and Thyroid findings**

Reference:	KCA 5.5/04
Report Title:	Study for Mode of Action Analysis for Rat Liver and Thyroid findings by S-2399 Technical Grade
Author(s) & Year:	[redacted] (2017a)
Document No, Authority registration No	Study No. S1782 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not available
Guideline(s):	None
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes- as supplementary/mechanistic study
Study relied upon:	Yes

Method

In a non-guideline and non-GLP study, groups of ten male and female HarlanRccHanTM:WIST rats received inpyrfluxam by dietary administration at concentrations of 0, 1500 (females; mean substance intake: 107.1 – 120.1 mg/kg bw/day) or 2000 ppm (males; mean substance intake: 177.1 – 187.0 mg/kg bw/day), for periods of 7, 14 and 28 days. Each phase of the study also included a positive control (1000 ppm phenobarbital, NaPB) group, consisting of ten males and ten females. To investigate the mechanism of action (MOA) behind the liver and thyroid changes induced by inpyrfluxam in rats, hepatic CYP and UGT gene expression, liver

T4-UGT activity, serum thyroid hormones, and liver and thyroid histopathology were analysed.

The dose levels of inpyrfluxam selected were the same as the highest dose in the rat 2-year study ([redacted] 2017). HSE notes that at 1000 ppm there were no effects on liver and thyroid in the 28-day rat apical study, with effects noted only from 3000 ppm ([redacted] 2014). The dietary concentration of NaPB was set at 1000 ppm, equivalent to the dose level at which effects on the liver are normally seen (IARC, 2001).[10]

Results

Mortality, body weight and food consumption

There was no treatment related mortality noted in any group.

In the animals treated with inpyrfluxam, no treatment-related changes in body weight or weight gain were observed in males. However, females exhibited a decrease in both body weight (4%) and weight gain (9.8%) throughout the treatment phases.

The observed changes in bodyweight and body weight gain in females are considered to be due to the decreased food consumption (see below).

No treatment-related change in food consumption was observed in males, while females exhibited a decrease in food intake throughout the treatment phases.

Although mild suppression of body weight gain and food consumption was noted in treated females, no excessive toxicity was observed in any phase of the study. Therefore, treatment with inpyrfluxam did not confound the evaluation of the main target endpoints. *Gross pathology*

At necropsy, enlarged liver was observed in males treated with inprfluxam after 14- and 28-day treatment. This is considered treatment related. Enlargement of the liver was also noted in the positive control males and females across all the treatment phases. There were no gross pathology findings in the thyroid in any group.

Liver weights

Increases compared to controls in the absolute (3,16 and 6% for 7, 14 and 28 days respectively) and relative (7, 15 and 7% for 7, 14 and 28 days respectively) liver weights were noted in both sexes treated with inpyrfluxam. These are considered treatment related. Similarly, the positive control increased the absolute (29, 36 and 34% for 7, 14 and 28 days) and relative (27, 31, 31% for 7, 14 and 28 days) liver weights in both sexes.

[10] IARC (2001). Some thyrotropic agents: Phenobarbital and its sodium salt. IARC Monogr. Eval. Carcinog. Risks Hum. **79**, 161-288. (IARC Publications Website - Some Thyrotropic Agents)

Thyroid weights were not measured.

Histopathology

Histopathology of the liver was performed only after 7- and 14-day treatment intervals. In the animals treated with inpyrfluxam, diffuse hepatocyte hypertrophy was noted in males (10/10) after 14-day treatment and in females (3/10) at both exposure intervals. The positive control showed centrilobular hepatocyte hypertrophy (10/10) in both sexes and both exposure intervals.

Histopathology of the thyroid was performed only after 14- and 28-day treatment intervals in males and after the 28-day treatment interval in females. In the animals treated with inpyrfluxam, diffuse follicular cell hypertrophy of the thyroid was observed in males (7/10) after 14-day treatment and in both sexes (3/10) after 28-day treatment. Similar effects but with a higher incidence (10/10) were noted in both sexes that received the positive control after 14- and 28-day treatment.

Overall, there were histopathological findings in the liver and thyroid of the animals treated with inpyrfluxam. These findings showed a higher incidence in the rats treated with NaPB.

Hepatic cytochrome P450 (CYP) and UDP-glucuronosyl transferase (UGT) mRNA expression

In the animals treated with inpyrfluxam, there were statistically significant increases compared to controls in the mRNA expression levels of *Cyp2b1/2* (up to approx. 3.6-fold in males and females) *Cyp3a1*(up to approx. 7-fold in males and up to approx. 8-fold in females)*, Cyp3a2* (up to approx.1.5-fold in males and up to approx. 3.9-fold in females)*, Ugt1a1* (up to approx. 2.3-fold in males and females) and *Ugt2b1* (up to approx. 6.4-fold in males and up to approx. 1.9-fold in females) (not statistically significant after 7-day treatment in females)*,* in both sexes after all the three treatment intervals.

The positive control also demonstrated similar effects on the above-mentioned mRNA levels with the exception of the *Ugt1a1* increase above controls, which was not statistically significant after 14- and 28-day treatment intervals in females. The fold increases above controls were much higher for NaPB compared to inpyrfluxam, with the exception of the *Ugt1a1* increases, which appeared similar to those observed for inpyrfluxam.

T4-UDP-glucuronosyl transferase (T4-UGT) activity

T4-UGT activity in the liver was measured only in the 28-day treatment group. A statistically significant increase in T4-UGT activity was recorded in females, but not males treated with inpyrfluxam.

The positive control demonstrated a statistically significant increase in T4-UGT activity in both sexes.

Serum thyroid hormone levels

There were no changes in serum T3 and T4 levels in males and females treated with inpyrfluxam at any exposure interval. A slight, but not statistically significant increase compared to controls in serum TSH levels was recorded in inpyrfluxam-treated males only after 14- (7 vs 5.3 in controls) and 28-day (8.4 vs 6 in controls) treatment intervals.

For the NaPB-treated animals, there were no changes in serum T3 and T4 levels in either sex at any exposure interval. However, statistically significant increases compared to controls were seen in serum TSH levels in males and females.

Table 6.8.3-9: Detailed summary of results in male rats administered inpyrfluxam for 7-,14- and 28 days (Mean of 6-10 rats, unless otherwise specified)

Parameter	7-day treatment			14-day treatment			28-day treatment		
	Control	S-2399 TG	NaPB	Control	S-2399 TG	NaPB	Control	S-2399 TG	NaPB
	0 ppm	2000 ppm	1000 ppm	0 ppm	2000 ppm	1000 ppm	0 ppm	2000 ppm	1000 ppm
Liver weight									
Absolute (g)	9.32	9.61	12.02**	10.88	12.61**	14.83**	12.38	13.21	16.65**
Relative to body weight (g/100g)	4.80	5.13**	6.11**	4.59	5.27**	6.02**	4.06	4.36**	5.32**
Necropsy (No. of affected animals / No. of animals examined)									
Enlarged liver	0/10	0/10	10/10**	0/10	3/10	10/10**	0/10	4/10	10/10**
Thyroid: Not remarkable	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Histopathology (No. of affected animals / No. of animals examined)									
Liver									
Hypertrophy, hepatocyte, centrilobular	0/10	0/10	10/10**	0/10	0/10	10/10**	-		
Hypertrophy, hepatocyte, diffuse	0/10	0/10	0/10	0/10	10/10**	0/10			
Thyroid									
Hypertrophy, follicular cell, diffuse	-			0/10	7/10**	10/10**	0/10	3/10	10/10**
Gene expression (% of control)									
Cyp2b1/2	100	324**	34696**	100	207**	17888**	100	365**	57894**
Cyp3a1	100	588*	1191**	100	704**	1039**	100	413**	804**
Cyp3a2	100	139**	225**	100	148**	240**	100	137	222*
Ugt1a1	100	194**	184**	100	180**	188**	100	235**	230**

Ugt2b1	100	320**	1101**	100	270**	1351**	100	640**	2021**
Enzyme activity									
S9 protein (mg/g liver)	-			-			166	162	167
UGT (pmol/min/mg S9 protein)							0.17	0.19	0.42**
UGT (pmol/min/g liver)							28.83	31.10	69.75 **
UGT (pmol/min/liver)							354.00	407.33	1151.8 0**
Serum hormone									
TSH (ng/mL)	6.8	6.3	9.4	5.3	7.0	10.0*	6.0	8.4	13.9*
T3 (ng/mL)	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6
T4 (µg/dL)	3.91	3.75	3.63	4.12	4.13	4.49	4.31	4.20	4.76

Empty cells: not evaluated at that time point(s); *: p<0.05; **: p<0.01; shadowed cells: biologically significant changes

Table 6.8.3-10: Detailed summary of results in female rats administered inpyrfluxam for 7-,14- and 28 days (Mean of 6-10 rats, unless otherwise specified)

Parameter	7-day treatment			14-day treatment			28-day treatment		
	Control	S-2399 TG	NaPB	Control	S-2399 TG	NaPB	Control	S-2399 TG	NaPB
	0 ppm	1500 ppm	1000 ppm	0 ppm	1500 ppm	1000 ppm	0 ppm	1500 ppm	1000 ppm
Liver weight									
Absolute (g)	6.51	6.51	9.28**	6.90	7.10	8.96**	6.98	7.24	9.86**
Relative to body weight (g/100g)	4.42	4.65	5.78**	4.19	4.57*	5.20**	3.63	3.92*	4.76**
Necropsy (No. of affected animals / No. of animals examined)									
Liver: Enlarged	0/10	0/10	9/10**	0/10	0/10	7/10**	0/10	0/10	10/10**
Thyroid: Not remarkable	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Histopathology (No. of affected animals / No. of animals examined)									
Liver									
Hypertrophy, hepatocyte, centrilobular	0/10	0/10	9/10**	0/10	0/10	10/10**	-		
Hypertrophy, hepatocyte, diffuse	0/10	3/10	0/10	0/10	3/10	0/10			
Thyroid									
Hypertrophy, follicular cell, diffuse	-			-			0/10	3/10	10/10**
Gene expression (% of control)									
Cyp2b1/2	100	335	17853 **	100	172*	16738 **	100	268*	96471 **
Cyp3a1	100	832*	1023**	100	700**	907**	100	882**	1041**
Cyp3a2	100	394	3193*	100	174	4270*	100	327**	4450**

Ugt1a1	100	223**	154*	100	196**	126	100	225**	136
Ugt2b1	100	128	273**	100	172**	392**	100	192**	356**
Enzyme activity									
S9 protein (mg/g liver)							160	166	158
UGT (pmol/min/mg S9 protein)							0.12	0.36**	0.18*
UGT (pmol/min/g liver)							19.12	60.77**	28.68*
UGT (pmol/min/liver)							133.32	439.55**	288.34**
Serum hormone									
TSH (ng/mL)	4.5	3.8	6.6*	4.1	4.3	6.8**	5.2	5.5	6.3
T3 (ng/mL)	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
T4 (µg/dL)	2.52	2.47	2.38	2.95	2.79	3.22	2.99	2.96	3.34

Empty cells: not evaluated at that time point(s); *: $p<0.05$; **: $p<0.01$

Conclusion

In this non-GLP and non-guideline liver and thyroid mechanistic study, dietary administration of inpyrfluxam to Hannover Wistar rats at concentrations of 2000 ppm in males and 1500 ppm in females for up to 28 days caused increased liver weight, histopathological findings of the liver (diffuse hepatocyte hypertrophy) and thyroid (diffuse follicular cell hypertrophy), increases in hepatic *Cyp2b1/2, Cyp3a1, Cyp3a2, Ugt1a1 and Ugt2b1 mRNA* levels, increases in T4-UGT activity in females and slightly higher serum TSH levels in males. There were no changes in serum T3 and T4 levels. NaPB caused similar effects, but generally, the magnitude or the incidence of the effects was higher.

Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction in the rat. UGT induction may then be responsible for the thyroid follicular hypertrophy observed in the study. However, the study did not show a decrease in thyroid hormones and there was only a slight increase in TSH in males. It is possible the dose used in the study was not sufficiently high to affect thyroid hormone levels. However, as thyroid follicular hypertrophy, a very sensitive parameter of thyroid homeostasis disruption, occurred, thyroid hormone perturbations must also have occurred.

[redacted] (2017a)

2. Mode of Action Study for Mouse Liver and Thyroid findings

Reference:	KCA 5.5/05
Report Title:	Study for Mode of Action Analysis for Mouse Liver and Thyroid findings by S-2399 Technical Grade
Author(s) & Year:	[redacted] (2017b)

Document No, Authority registration No	Study No. S1767 [redacted]
Substance used:	Test Material:S-2399 Technical Grade (inpyrfluxam) Lot/Batch: 13CG0617G Purity: 95%
Method of analysis:	Not available
Guideline(s):	None
Deviations from current guideline:	N/A
Impact of the deviation:	N/A
GLP or GEP:	No
Acceptability:	Yes- as supplementary/mechanistic study
Study relied upon:	Yes

Method

In a non-guideline and non-GLP study, groups of ten male and female [redacted] mice received inpyrfluxam by dietary administration at concentrations of 0 or 7000 ppm (mean substance intake: 739.7 – 884.7 mg/kg bw/day in males; 788.9 – 979.5 mg/kg bw/day in females) for periods of 7- and 14- days. To investigate the MOA behind the liver and thyroid changes induced by inpyrfluxam in mice, hepatic CYP and UGT gene expression, liver T4-UGT activity, serum thyroid hormones, and liver and thyroid histopathology were analysed.

The dose level used in the study was the same as the highest dose in the mouse cancer study ([redacted] 2016a). HSE notes that in the 90-day mouse apical study, liver effects were seen from 3500 ppm and thyroid effects were noted at 7000 ppm ([redacted] 2016a).

Results

There were no treatment related mortalities or changes in body weight, body weight gain and food consumption in either sex.

Gross pathology

At necropsy, enlarged liver was observed in males (3/10) and females (3/10) after 7- and 14- day treatment intervals. No overt changes were noted in the thyroid.

Liver weights

Statistically significant increases in absolute and relative liver weights were noted in both sexes at both exposure intervals.

Histopathology

Centrilobular hepatocyte hypertrophy was noted in both males (10/10 at both exposure intervals) and females (2/10 after 7-day treatment and 5/10 after 14-day treatment).

There were no treatment related changes in the thyroid histopathology in either sex.

Hepatic cytochrome P450 (CYP) and UDP-glucuronosyl transferase (UGT) mRNA expression

There were statistically significant increases compared to controls in the liver mRNA levels of *Cyp2b10* (up to approx. 37-fold in males and up to approx. 2.5-fold in females), *Ugt1a1* (up to approx. 1.3-fold in males and up to approx. 1.4-fold in females) and *Ugt2b1* (up to approx. 1.5-fold in males and up to approx. 1.3-fold in females) in both sexes after 7- and 14-day treatment intervals.

T4-UDP-glucuronosyl transferase (T4-UGT) activity

Unexpectedly, there was a decrease compared to controls in liver T4-UGT activity in both males (after 7-and 14-day treatment) and females (after 7-day treatment). An increase was only seen in females after 14-day treatment.

Serum thyroid hormone levels

Compared to controls, decrease in serum T4 and T3 levels were noted in both males and females after 7- and 14-day treatment. In addition, there was a decrease in serum TSH level in females.

Table 6.8.3-10: Detailed summary of results in mice administered inpyrfluxam for 7- and14- days (Mean of 5-10 mice, unless otherwise specified)

	Male				Female			
	7-day treatment		14-day treatment		7-day treatment		14-day treatment	
	0 ppm	7000 ppm	0 ppm	7000 ppm	0 ppm	7000 ppm	0 ppm	7000 ppm
Liver weight								
Absolute (g)	1.98	2.37**	2.12	2.44**	1.59	2.01**	1.68	2.08**
Relative to body weight (g/100g)	5.25	6.24**	5.40	6.26**	4.95	6.32**	5.03	6.17**

Necropsy (No. of affected animals / No. of animals examined)								
Enlarged liver	0/10	3/10	0/10	3/10	0/10	3/10	0/10	3/10
Thyroid: Not remarkable	10/10	10/10	10/10	10/10	10/10	10/10	10/10	10/10
Histopathology (No. of affected animals / No. of animals examined)								
Liver								
Hypertrophy, hepatocyte, centrilobular	0/10	10/10**	0/10	10/10**	0/10	2/10	0/10	5/10*
Thyroid								
Not remarkable	10/10	10/10	10/10	10/10	9/10	10/10	8/10	9/10
Ectopic thymus	0/10	0/10	0/10	0/10	1/10	0/10	2/10	1/10
Gene expression (% of control)								
Cyp2b10	100	3471**	100	3699**	100	212**	100	254**
Ugt1a1	100	131*	100	121*	100	141**	100	124**
Ugt2b1	100	151*	100	154**	100	134*	100	104
Enzyme activity								
S9 protein(mg/g liver)	216	233**	222	222	229	219*	226	234
UGT (pmol/min/mg S9 protein)	0.27	0.23**	0.19	0.18	0.30	0.23**	0.19	0.19
UGT (pmol/min/g liver)	57.70	52.63	43.07	39.92	68.22	49.67**	43.27	44.35
UGT (pmol/min/liver)	114.51	125.28	91.46	97.13	108.20	99.50	72.31	90.78*
Serum hormone								
TSH (ng/mL)	4.3	4.4	3.8	3.7	10.6	3.1	5.5	2.9
T3 (ng/mL)	0.8	0.6	0.6	0.6	0.8	0.6*	0.7	0.6
T4 (µg/dL)	12.2	9.9	15.8	11.0**	13.5	8.9**	13.0	7.5**

*: p<0.05; **: p<0.01; shadowed cells: biologically significant changes

Conclusion

In this non-GLP and non-guideline liver and thyroid mechanistic study, dietary administration of inpyrfluxam to mice at concentrations of 0 or 7000 ppm for up to 14 days caused liver enlargement, increased liver weight and histopathological findings of the liver (centrilobular hepatocyte hypertrophy). In addition, there were increases in hepatic *Cyp2b10, Ugt1a1 and Ugt2b1 mRNA* levels, but liver T4-UGT activity was deceased (although an increase was seen in females after 14 days of treatment). Serum T3 and T4 levels were decreased in both sexes and TSH levels were also decreased but only in females.

Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction, at mRNA expression level, in the mouse. Thyroid hormones were decreased, but, unexpectedly TSH levels were also decreased in females and no follicular hypertrophy was observed in either sex. The findings of this study are difficult to interpret as some inconsistencies from the expected pattern of results were seen. However, it appears that any thyroid hormone disruption caused by inpyrfluxam in the mouse may be the consequence of liver enzyme induction.

[redacted] (2017b)

Overall conclusion of in vivo mode of action studies

Inpyrfluxam was evaluated for its mechanism of action underpinning the adverse effects in liver and thyroid seen in apical studies using two in vivo studies in rats and mice.

Rats

Dietary administration of inpyrfluxam to Hannover Wistar rats at concentrations of 2000 ppm in males and 1500 ppm in females for up to 28 days caused increased liver weight, histopathological findings of the liver (diffuse hepatocyte hypertrophy) and thyroid (diffuse follicular cell hypertrophy), increases in hepatic *Cyp2b1/2, Cyp3a1, Cyp3a2, Ugt1a1 and Ugt2b1 mRNA* levels, increases in T4-UGT activity in females and slightly higher serum TSH levels in males. There were no changes in serum T3 and T4 levels. NaPB caused similar effects, but generally, the magnitude or the incidence of the effects was higher. Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction in the rat. UGT induction may then be responsible for the thyroid follicular hypertrophy observed in the study. However, the study did not show a decrease in thyroid hormones and there was only a slight increase in TSH in males. It is possible the dose used in the study was not sufficiently high to affect thyroid hormone levels. However, as thyroid follicular hypertrophy, a very sensitive parameter of thyroid homeostasis disruption, occurred, thyroid hormone perturbations must also have occurred.

Mice

Dietary administration of inpyrfluxam to mice at concentrations of 0 or 7000 ppm for up to 14 days caused liver enlargement, increased liver weight and histopathological findings of the liver (centrilobular hepatocyte hypertrophy). In addition, there were increases in hepatic *Cyp2b10, Ugt1a1 and Ugt2b1 mRNA* levels, but liver T4-UGT activity was deceased (although an increase was seen in females after 14 days of treatment). Serum T3 and T4 levels were decreased in both sexes and TSH levels were also decreased but only in females. Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction, at mRNA expression level, in the mouse. Thyroid hormones were decreased, but, unexpectedly TSH levels were also decreased in females and no follicular hypertrophy was observed in either sex. The findings of this study are difficult to interpret as some inconsistencies from the expected pattern of results were seen. However, it appears that any thyroid hormone disruption caused by inpyrfluxam in the mouse may be the consequence of liver enzyme induction.

B.6.8.4. Endocrine disruption assessment

The definition of an endocrine disruptor (ED) is based on the WHO/IPCS (2002) definition and the assessment of endocrine disruption is based on the criteria for endocrine disruption (Commission Regulation (EU) 2018/605 of 19 April 2018 as it applies in GB). The criteria as listed in Annex II point 3.6.5 are as follows:

'From 20 October 2018, an active substance, safener or synergist shall be considered as having endocrine disrupting properties that may cause adverse effect in humans if, based on points (1) to (4) of the sixth paragraph, it is a substance that meets all of the following criteria, unless there is evidence demonstrating that the adverse effects identified are not relevant to humans:

1) *it shows an adverse effect in an intact organism or its progeny, which is a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;*
2) *it has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;*
3) *the adverse effect is a consequence of the endocrine mode of action.'*

For the evaluation of the first criterion, it has to be determined whether adverse effects potentially related to an endocrine mode of action (MOA) are observed. In this section the evaluation of effects on reproductive and endocrine related organs from all valid repeated-dose toxicity, long-term and reproductive toxicity studies are compiled, and the adversity and specificity of the observed effects is assessed. For the evaluation of the second criterion (if applicable), the available *in vitro* endocrine activity assays are evaluated. To fulfil the third part of the criteria, an assessment as to whether the determined adverse effects are a consequence of an endocrine-mediated mechanism is performed.

The present assessment for the new active substance inpyrfluxam follows the ECHA/EFSA/JRC guidance for the identification of endocrine disruptors in the context of Regulations (EU) 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311) and it considers the EATS (Estrogen, Androgen, Thyroid, Steroidogenesis) modalities. The guidance proposes a workflow for assessing the endocrine disrupting properties of pesticides and biocides, which starts by collecting all available data on inpyrfluxam and assembling them into lines of evidence in the format of an Excel File (Appendix E of the guidance). This is followed by the analysis of the evidence which includes a decision tree with different possible scenarios to conclude whether a substance does not meet the ED criteria, additional information is needed, or a MOA analysis is required to conclude on the ED properties. The MOA analysis step aims to establish if there is a biologically plausible link between observed adverse effects and endocrine activity. Finally, the conclusion as to whether the ED

criteria are met with respect to humans is drawn and transparently documented, including the remaining uncertainties.

Evaluation of inpyrfluxam toxicology– gather all information

Based on Commission Regulation (EU) No 2018/605 as it applies in GB, the assessment of the potential for endocrine disruption of a substance should be based on a Weight of Evidence (WoE) approach and information can be obtained from existing data, read-across from structurally similar chemicals, *in silico* tools, *in vitro* and *in vivo* screening assays and/or from mechanistic studies. The assays appropriate for use in the WoE determination are discussed in the OECD Conceptual Framework (CF) for Testing and Assessment of Endocrine Disruptors (OECD Revised Guidance Document 150, 2018b). Using combinations of Level 1- Level 5 assays, endocrine disruptors can be identified according to their adverse effects on apical endpoints (Level 4 and 5 studies), taking into account severity, specificity and consistency, and endocrine activity (Level 2 and 3 studies). Currently, the most complete testing battery exists for oestrogen, androgen, thyroid and steroidogenesis (EATS) modalities, while non-EATS modalities will require further development in the future to allow reliable assessments.

The Level 1 to Level 5 toxicological information available for inpyrfluxam regarding its potential for endocrine disruption is presented below:

1. OECD CF 150 Level 1 – existing or new non-test information

ADME

Inpyrfluxam was well absorbed, extensively distributed and metabolised and was excreted rapidly in rats after oral administration with the urine and the bile as the main route of excretion. Oral absorption was found to be 100 % but the post-hepatic systemic availability was estimated at 60% ([redacted] 2016a). There was no evidence of bioaccumulation following repeated oral dosing (14 days) in the rat ([redacted] 2016b). More details can be found in Section B.6.1 of the DAR.

QSAR

No QSAR data available.

2. OECD CF 150 Level 2 – in vitro mechanistic studies

There were 5 in vitro studies in mammalian cells including estrogen and androgen receptor transactivation, steroidogenesis, thyroid hormone alpha receptor transactivation, sodium/iodide symporter (NIS) assays, and the thyroperoxidase (TPO) assay in rat thyroid microsomes. Inpyrfluxam did not have any effect on androgen or

estrogen production, hERα or hAR or hTRα -mediated transactivation and TPO activity invitro.

The NIS assay is not considered acceptable due to the lack of concurrent cytotoxicity measurement and statistical analysis. Additionally, the assay was not conducted up to the highest soluble and non cytotoxic concentration.

Table 6.8.4-1: OECD CF 150 Level 2 – in vitro mechanistic studies

Type of studies	Study	System	OCED Test guideline No	Study ID matrix in Appendix E	Reference
In vitro assays	human estrogen receptor alpha and human androgen receptor transactivation assay	Mammalian cells	Not guideline	17 (a) 17 (b)	[redacted] (2017b), Study No. RGA 124
	steroidogenesis assay	Mammalian cells	Not guideline	18	[redacted] (2017c), Study No. HK002
	human thyroid hormone receptor alpha transactivation assay	Mammalian cells	No guideline available	23	[redacted] (2019), Study No. RGA 147
	sodium/iodide symporter assay	Mammalian cells	No guideline available	24	[redacted] (2020) Study No. S2096
	thyroperoxidase inhibition assay	Microsomes	No guideline available	25	[redacted] (2019), Study no. S2017

3. OECD CF 150 Level 3 – in vivo mechanistic studies

Two mechanistic studies have been performed with the active substance in rats and mice to investigate the mode of action behind the adverse effects noted in liver and thyroid - in rats, mice and dogs.

Dietary administration of inpyrfluxam to Hannover Wistar rats at concentrations of 2000 ppm in males and 1500 ppm in females for up to 28 days caused increased liver weight, histopathological findings of the liver (diffuse hepatocyte hypertrophy) and thyroid (diffuse follicular cell hypertrophy), increases in hepatic *Cyp2b1/2, Cyp3a1, Cyp3a2, Ugt1a1 and Ugt2b1 mRNA* levels, increases in T4-UGT activity in females and slightly higher serum TSH levels in males. There were no changes in serum T3 and T4 levels. NaPB caused similar effects, but generally, the magnitude or the incidence of the effects was higher. Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction in the rat. UGT induction may then be responsible for the thyroid follicular hypertrophy observed in the study. However, the study did not show a decrease in thyroid hormones and there was only a slight increase in TSH in males. It is possible the dose used in the study was not sufficiently high to affect thyroid hormone levels. However, as thyroid follicular hypertrophy, a very sensitive parameter of thyroid homeostasis disruption, occurred, thyroid hormone perturbations must also have occurred.

Dietary administration of inpyrfluxam to mice at concentrations of 0 or 7000 ppm for up to 14 days caused liver enlargement, increased liver weight and histopathological findings of the liver (centrilobular hepatocyte hypertrophy). In addition, there were increases in hepatic *Cyp2b10, Ugt1a1 and Ugt2b1 mRNA* levels, but liver T4-UGT activity was deceased (although an increase was seen in females after 14 days of treatment). Serum T3 and T4 levels were decreased in both sexes and TSH levels were also decreased but only in females. Overall, this study shows that inpyrfluxam causes liver enzyme induction, including UGT induction, at mRNA expression level, in the mouse. Thyroid hormones were decreased, but, unexpectedly TSH levels were also decreased in females and no follicular hypertrophy was observed in either sex. The findings of this study are difficult to interpret as some inconsistencies from the expected pattern of results were seen. However, it appears that any thyroid hormone disruption caused by inpyrfluxam in the mouse may be the consequence of liver enzyme induction.

Table 6.8.4-2: OECD CF 150 Level 3 – in vivo mechanistic studies

Type of studies	Study	Species	OCED Test guideline No	Study ID matrix in Appendix E	Reference
In vivo mechanistic assays	Mode of Action Analysis for Rat Liver and Thyroid findings	Rat	Not guideline	15 (a) - Male 15 (b)- Female	[redacted] (2017a), Study No. S1554

	Mode of Action Analysis for mouse Liver and Thyroid findings	Mouse	Not guideline	16	[redacted] (2017b),

4. OECD CF 150 Level 4 – Studies assessing mammalian endocrine sensitive endpoints

Level 4 studies included a 28-day oral repeated-dose toxicity study in the rat, 90 day oral repeated dose toxicity studies in rat, mouse and dog, a 12-month repeated-dose toxicity study in dog, prenatal developmental toxicity studies in the rat and the rabbit, and long-term toxicity studies in the rat and mouse. All studies were conducted according to the respective OECD guidelines and all of them were performed in accordance with GLP principles. The number of organs or tissues subject to evaluation (principally organ weight and/or histopathological assessment) depended on the study type conducted. The following organs were primarily considered for assessment in this context: liver, thyroid, pituitary, adrenal glands, and reproductive organs/tract (e.g., testes, ovaries and uterus).

Data were populated in the Excel template provided as Appendix E of the EFSA/ECHA guidance (2018). According to this template each study was given a unique identification number (Study ID Matrix) that is important for its identification in the data-matrix and Lines of Evidence (LoE) spreadsheets on Excel.

Table 6.8.4-3: OECD CF 150 Level 4 – Studies assessing mammalian endocrine sensitive endpoints

Type of studies	Study	Species	OCED Test guideline No	Study ID matrix in Appendix E	Reference
Repeated-dose toxicity studies *Section B.6.3*	One-month Oral Toxicity study (diet)	**Rat**	Not guideline	1	[redacted] (2014) Study No. S1554
	90-Day Oral Toxicity study (diet)	**Rat**	OECD TG 408 (1998)	2	[redacted] (2016) Study No. [redacted] 13-0069
	90-Day Oral Toxicity study (diet)	**Mouse**	OECD TG 408 (1998)	4	[redacted] (2016a)

					Study No. [redacted] 13-0068
	90-Day Oral Toxicity study (capsule)	**Dogs**	OECD TG 409 (1998)	6	[redacted] (2016) Study No. [redacted] 13-0106
	One-year Oral Toxicity study (capsule)	**Dogs**	OECD TG 452 (2009)	7	[redacted] (2017) Study No. [redacted] 14-0096
Long-term toxicity studies Section B.6.5	Combined Chronic Toxicity and Carcinogenicity Study (diet)	**Rat**	OECD TG 453 (2009)	3a (M) 3b (F)	[redacted] (2017) Study No. [redacted] 14-0046
	Carcinogenicity study (diet)	**Mouse**	OECD TG 451 (2009)	5	[redacted] (2017) Study No. [redacted] 14-0047
	Reproduction Toxicity Study	**Rat**	OECD TG 416 (2001)	9	[redacted] (2017) Study No. [redacted] 15-0018
Reproductive toxicity studies Section B.6.6	Teratogenicity Study	**Rat**	OECD TG 414 (2001)	11	[redacted] (2017a) Study No. [redacted] 14-0071
	Teratogenicity Study	**Rabbit**	OECD TG 414 (2001)	14	[redacted] (2017c) Study No. [redacted] 15-0017

5. OECD CF 150 Level 5 – in vivo Mammalian Assays providing more comprehensive data on Adverse Effects on Endocrine Relevant Endpoints over More Extensive Parts of Organism Life Cycles

Level 5 studies were conducted according to the current OECD guideline and included a two-generation reproductive toxicity study in the rat.

A summary of all Level 5 studies considered for mammalian toxicology, including the Study ID Matrix is outlined in the table below:

Table 6.8.4-4: OECD CF 150 Level 5 – in vivo Mammalian Assays providing more comprehensive data on Adverse Effects on Endocrine Relevant Endpoints over More Extensive Parts of Organism Life Cycles

Type of studies	Study	Species	OCED Test guideline No	Study ID matrix in Appendix E	Reference
Reproductive toxicity studies Section B.6.6	Reproduction Toxicity Study	**Rat**	OECD TG 416 (2001)	9	[redacted] (2017) Study No. [redacted] 15-0018

Assessment of the evidence

In this step, the information is assembled into lines of evidence, integrating information for both adversity and endocrine activity for the EATS modalities. The data were included in the Excel template provided as Appendix E of the EFSA/ECHA 2018 guidance (data not shown). According to this template each study was given a unique identification number (Study ID Matrix) that is important for its identification in the data-matrix and Lines of Evidence (LoE) spreadsheets of the Excel.

The assessment of the evidence provided was divided into the following categories:

i) a review of the Estrogen, Androgen and Steroidogenic (EAS) modalities and

ii) a review or the Thyroid (T) modality.

All endocrine relevant parameters for which adverse changes were identified in the different studies, were considered by HSE and are discussed below mainly to differentiate specific effects from those considered secondary to other toxic effects. Changes not considered being treatment-related and/or adverse in the previous sections of this B6 document have not been included in this assessment.

EAS modalities

Adversity and specificity

Several parameters relevant to assessing the endocrine disrupting potential of inpyrfluxam for the EAS modalities have been evaluated in a number of toxicology studies. These parameters include potential developmental effects, and potential effects on sexual/reproductive organs and reproductive performance in both Level 4 and Level 5 studies.

EAS-mediated parameters

Adrenal findings (weight changes, histopathology) in rats and dogs

Adrenal findings were seen in rats and dogs.

In the 28-day repeat dose study in rats, fine vacuolisation of cortical cells in the zona fasciculata of the adrenal was seen in males and females from 246.4/263 mg/kg bw/day ([redacted] (2014), Study ID matrix – 1). Significant systemic toxicity was observed at and above this dose (decreased body weights and body weight gains (-23,1% and -25.4% in males and females at 246.4/263 mg/kg bw/day), clinical-chemistry parameters indicative of liver damage and disruption in lipid metabolism, changes in liver weight, and histopathological findings of the liver and bone marrow) with the MTD (Maximum Tolerated Dose) being reached or exceeded at 246.4/263 mg/kg bw/day already.

In addition, a dose dependent increase in the fine vacuolization of cortical cells in the zona glomerulosa of the adrenal gland was seen from 85.9 mg/kg bw/day in males only ([redacted] (2014), Study ID matrix - 1). The findings observed at 85.9 mg/kg bw/day was not statistically significant when compared to the control group. However, the findings at doses of 246.4 mg/kg bw/day and higher were statistically significant. No similar effects were observed in the zona glomerulosa in females or other repeat-dose studies conducted in rats or dogs. In addition, males at this dose showed a 9.5% decrease in body weight gain. Therefore, this finding at 85.9 mg/kg bw/day in males appears to be an isolated occurrence of minimal toxicological significance.

In the 90-day repeat dose study in rats, a reduction in adrenal weights was noted at the top dose of 255/292 mg/kg bw/day in males and females and fine vacuolisation of cortical cells in the zona fasciculata of the adrenal gland was seen in males at the top

dose and in females from 144 mg/kg bw/day ([redacted] (2016), Study ID matrix – 2). However, significant systemic toxicity was observed at and above 144 mg/kg bw/day (decreased body weights and body weight gains (-21% in females at 144 mg/kg bw/day), clinical-chemistry parameters indicative of liver damage, changes in liver weight (with associated hypertrophy), and histopathological findings of the liver), with the MTD being reached or exceeded at 144 mg/kg bw/day already.

In the 90-day repeat dose study in dogs, zona fasciculata cell vacuolation of the cortical cells of the adrenal gland was seen from 160 mg/kg bw/day in males and at the top dose of 750/500 mg/kg bw/day in females ([redacted] (2016), study matrix ID-6). However, significant systemic toxicity was noted at and above this dose (vomiting, signs of anaemia, changes in clinical-chemistry parameters indicative of liver damage, increased liver weight and histopathological findings in the liver, gall bladder, kidney and optic nerve) with the MTD being reached or exceeded at 160 mg/kg bw/day already.

In the one-year repeat dose study in dogs, zona fasciculata cell vacuolation of the cortical cells of the adrenal gland was noted in males and females from 30 mg/kg bw/day ([redacted] (2017), study matrix ID-7). However, significant systemic toxicity was noted at and above this dose (vomiting, clinical chemistry parameters indicative of liver damage, increased liver weights, and histopathological findings of the liver) with the MTD being reached or exceeded at 30 mg/kg bw/day already.

Overall, the changes observed in the adrenal glands of both sexes in rats and dogs occurred above the MTD and therefore they do not raise concerns regarding endocrine disruption.

Uterus and ovary findings (weight changes, histopathology) in rats

Uterus and ovary findings were seen in rats

In the 28-day repeat dose study in rats, decreases in ovary weights and uterine weights (absolute and/or relative) were seen from 263 mg/kg bw/day. These changes were associated with vacuolation of the interstitial gland of the ovary and atrophy of the uterus. However, significant systemic toxicity was noted at and above this dose (decreased body weights and body weight gains (-25.4%), clinical-chemistry parameters indicative of liver damage and disruption in lipid metabolism, changes in liver weight and histopathological findings of the liver and bone marrow) with the MTD being reached or exceeded at 263 mg/kg bw/day already.

In the 90-day repeat dose study in rats, decrease in ovary weights at 292 mg/kg bw/day and vacuolation of the interstitial gland of the ovary from 144 mg/kg bw/day was noted in females ([redacted] (2016), Study ID matrix – 2). However, significant systemic toxicity was observed at and above 144 mg/kg bw/day (decreased body weights and body weight gains (-21%), clinical-chemistry parameters indicative of liver damage,

changes in liver weight (with associated hypertrophy), and histopathological findings of the liver) with the MTD being reached or exceeded at 144 mg/kg bw day.

Similar effects (and any possible association with effects on fertility) were not seen in the rat 2-generation study (2017; Study ID Matrix-9), possibly due to the lower dose levels used in this study. Overall, the changes observed in the ovary and uterus of female rats occurred above the MTD and therefore they do not raise concerns regarding endocrine disruption.

EAS-mediated reproductive and developmental parameters

No EAS-mediated reproductive and developmental effects were observed in either the F0 or F1 generations in the 2-generation rat study (2017; Study ID Matrix-9). Inpyrfluxam had no effect on male or female fertility or reproductive performance up to the top dose of 113/86 mg/kg bw/day; gestation duration, oestrus cycle and spermatogenic endpoints were also unaffected by treatment. There was also no effect on litter size, sex ratio, pup survival and developmental landmarks.

In the developmental toxicity studies in rats or rabbits, there were no effects on the mean number of live foetuses, percentage incidence of resorptions, foetal deaths, sex ratio, variations or malformations. ((2017b), study matrix ID-11; (2017c), study matrix ID-14).

Overall, there were no EAS-mediated reproductive and developmental adverse effects in the available studies in rats and rabbits.

<u>In vitro EAS- activity information</u>

Inpyrfluxam did not have any effect on androgen or estrogen production in a steroidogenesis assay ((2017b), study matrix ID-17a&b) or hERα or hAR -mediated transactivation activity ((2017a), study matrix ID- 18) in vitro.

Overall conclusion on adverse effects and endocrine activity related to the EAS-modalities

In all species investigated (rat, mouse, dog) there were no specific adverse effects on reproductive organs and other endocrine organs related to the EAS modalities following repeated exposure to inpyrfluxam. In addition, there were no specific adverse effects on reproduction in the rat and on development in the rat and rabbit. Overall, there was no clear and specific pattern of adversity for the EAS modalities. In addition, there was no evidence of EAS activity in a steroidogenesis assay and in a hERα or hAR transactivation test.

T- modality

Adversity and specificity

Several parameters relevant to assessing the endocrine disrupting potential of inpyrfluxam for the T-modality have been evaluated in a number of toxicology studies.

Thyroid weight and histopathology in rats, mice and dogs

In the 28-day repeat dose study in rats, a dose dependent increase in follicular epithelial cell hypertrophy of the thyroid was observed in males and females from 246.4/263 mg/kg bw/day ((2014), Study ID matrix-1). Significant systemic toxicity was observed at and above this dose (decreased body weights and body weight gains(-23,1% and -25.4% in males and females at 246.4/263 mg/kg bw/day), clinical-chemistry parameters indicative of liver damage and disruption in lipid metabolism, changes in liver weight, and histopathological findings of the liver and bone marrow) with the MTD being reached or exceeded at 246.4/263 mg/kg bw/day already.

In the 90-day repeat dose study in rats, an increase in follicular epithelial cell hypertrophy of the thyroid was observed in females from 144 mg/kg bw/day ((2016), Study ID matrix-2). However, significant systemic toxicity was observed at and above 144 mg/kg bw/day (decreased body weights and body weight gains (-21% at 144 mg/kg bw/day), clinical-chemistry parameters indicative of liver damage, changes in liver weight (with associated hypertrophy), and histopathological findings of the liver) with the MTD being reached or exceeded at 144 mg/kg bw/day already. It is noted that no effects on the thyroid were noted in the rat 2-year study ((2017), study matrix ID:3a&b) day up to doses causing significant toxicity.

In the two-generation reproductive study in rats, increased thyroid weights and thyroid follicular epithelial cell hypertrophy were seen in F0 and F1 parental females during the pre-mating and mating period at the top dose of 86 mg/kg bw/day ((2017), study matrix ID-9). However, systemic toxicity was observed at this dose (decreased body weights and body weight gains (-16% in F0 and -11% in F1), increase in liver weight (with associated hypertrophy) with the MTD being reached.

In the 90-day repeat dose study in mice, an increase in follicular epithelial cell hypertrophy of the thyroid was observed in males and females at the top dose of 973/1097 mg/kg bw/day ((2016a), study matrix ID-4). However, liver toxicity was observed at this dose (increased liver weight with associated hypertrophy and fatty change and changes in clinical-chemistry parameters indicative of liver damage) and it is noted that the dose at which these thyroid effects were seen are at or above the limit dose. It is also noted that no effects on the thyroid were noted in the mouse carcinogenicity study ((2017), study matrix ID-5) up to doses causing significant toxicity.

In the 90-day repeat dose study in dogs, thyroid follicular epithelial cell hypertrophy was seen from 160 mg/kg bw /day in females and 700/500 mg/kg bw/day in males ([redacted] (2016), study matrix ID-6). However, systemic toxicity was noted at and above 160 mg/kg bw/day (vomiting, signs of anaemia, changes in clinical-chemistry parameters indicative of liver damage, increased liver weight and histopathological findings in the liver, gall bladder, kidney and optic nerve) with the MTD being reached or exceeded at 160 mg/kg bw/day already. It is also noted that no effects on the thyroid were seen in dogs in the 52-week study ([redacted] (2017), study matrix ID-7) up to the top dose of 160 mg/kg bw/day.

T- activity information

Thyroid mechanistic information is available from in vivo and in vitro studies.

In the in vivo mechanistic study in rats, inpyrfluxam induced liver enzymes, including UGT enzyme ([redacted] (2017a), study ID matrix-.15a&15b). UGT induction may then be responsible for the thyroid follicular hypertrophy observed in the study. However, the study did not show a decrease in thyroid hormones and there was only a slight increase in TSH in males. It is possible the dose used in the study was not sufficiently high to affect thyroid hormone levels. However, as thyroid follicular hypertrophy, a sensitive parameter of thyroid homeostasis disruption, occurred, thyroid hormone perturbations must also have occurred.

In the in vivo mechanistic study in mice, inpyrfluxam induced liver enzymes, including UGT, at mRNA expression level ([redacted] (2017b), study ID matrix-.16). Thyroid hormones (serum T3 and T4) were decreased, but unexpectedly TSH levels were also decreased in females and no follicular hypertrophy was observed in either sex. The findings of this study are difficult to interpret due to the inconsistencies noted from the expected pattern of results. However, any thyroid hormone disruption caused by inpyrfluxam in the mouse may be the consequence of liver enzyme induction.

Inpyrfluxam did not have any effect on hTRα -mediated transactivation ([redacted] (2019), study matrix ID-23) and TPO activity ([redacted](2019), study matrix ID-25) in vitro.

Overall conclusion on adverse effects and endocrine activity related to the T-modality

Overall, the changes observed in the thyroid in rats, mice and dogs occurred at or above the MTD/limit dose and therefore they do not raise concerns regarding endocrine disruption. In addition, whilst such effects occurred in the short-term studies, they were not replicated in the long-term studies up to doses causing significant toxicity. Overall, therefore, inpyrfluxam does not present a clear and specific pattern of adversity for the T modality in relation to effects on the thyroid gland. In addition, mechanistic information suggests that the thyroid effects might be secondary to liver

effects and that inpyrfluxam does not have any effect on hTRα -mediated transactivation and TPO activity.

Conclusion

Conclusion and sufficiency of the data

The data package available to characterise the toxicity profile of inpyrfluxam is considered sufficient to determine whether EATS-mediated adversity is exerted following repeated exposure to inpyrfluxam. All studies from the data package presented in the DAR were conducted recently (2012 – 2017), followed the most current OECD guidelines available at the time of conduct and were compliant with GLP standards.

EAS modalities

EAS-mediated adversity has been sufficiently investigated, based on the available repeated dose toxicity studies and in particular a modern 2-generation study.

In all species investigated (rat, mouse, dog) there were no specific adverse effects on reproductive organs and other endocrine organs related to the EAS modalities following repeated exposure to inpyrfluxam. In addition, there were no specific adverse effects on reproduction in the rat and on development in the rat and rabbit. Overall, there was no clear and specific pattern of adversity for the EAS modalities. In addition, there was no evidence of EAS activity in a steroidogenesis assay and in a hERα or hAR transactivation test.

Conclusion of the assessment of the EAS-modalities

Based on scenario 1a of the ECHA/EFSA/JRC guidance (2018) for the identification of endocrine disruptors HSE concludes that inpyrfluxam does not meet the ED criteria for the EAS modalities in relation to human health and that these modalities have been sufficiently investigated for this compound.

Table 6.8.4-5 Analysis of the evidence and identification of relevant scenario for the ED assessment of EATS-modality - Selection of relevant scenario

Adversity based on EAS-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/**No**	**1a**	Conclude: ED criteria not met because there is no **'EAS-mediated'** adversity	**X**
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	

No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing “EATS-mediated” parameters. Depending on the outcome move to corresponding scenario	
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

T- modality

T-mediated adversity (thyroid weight and histopathology) has been sufficiently investigated, based on the following studies in which thyroid effects were investigated:

- 28-day oral toxicity studies in the rat (Not GLP, but based on OECD TG 407)
- 90-day oral toxicity studies in the rat, mouse, dog (OECD TG 408)
- 1-year oral toxicity study in dogs (OECD TG 452 (2009)
- Chronic toxicity / carcinogenicity studies in the rat and mouse (OECD TG 453)
- 2-generation reproduction toxicity study in the rat (OECD TG 416).
- pre-natal developmental toxicity studies in rat and rabbit (OECD TG 414)

All were modern OECD compliant studies; although they were completed before the requirement to investigate thyroid hormones was added to OECD Guideline 408 (2018) (90-day study) and 414 (2018) (pre-natal developmental study in the rat), this is not considered to be a major deficiency (especially in view of the fact that thyroid hormones and TSH were measured in two mechanistic studies, one in rats and one in mice).

Overall, the changes observed in the thyroid gland in rats, mice and dogs occurred at or above the MTD/limit dose and therefore they do not raise concerns regarding endocrine disruption. In addition, whilst such effects occurred in the short-term studies, they were not replicated in the long-term studies up to doses causing significant toxicity. Overall, therefore, inpyrfluxam does not present a clear and specific pattern of adversity for the T modality in relation to effects on the thyroid gland. In addition,

mechanistic information suggests that the thyroid effects might be secondary to liver effects and that inpyrfluxam does not have any effect on hTRα -mediated transactivation and TPO activity.

Conclusion of the assessment of T-modality

Based on scenario 1a of the ECHA/EFSA/JRC guidance (2018) for the identification of endocrine disruptors, it is possible to conclude that inpyrfluxam does not meet the ED criteria for the T modality in relation to human health and that this modality has been sufficiently investigated.

Table 6.8.4-5: Analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality - Selection of relevant scenario

Adversity based on T-mediated parameters	Positive mechanistic OECD CF level 2/3 Test	Scenario	Next step of the assessment	Scenario selected
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no 'T-mediated' adversity	X
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	-
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	-
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no EAS-mediated endocrine activity observed	-
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "EATS-mediated" parameters. Depending on the outcome move to corresponding scenario	-
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	-

Overall conclusion on the ED assessment for humans

Overall, based on this analysis, inpyrfluxam does not meet the ED criteria for human health of Regulation (EC) No 2018/605 of 19 April 2018, amending Annex II to Regulation (EC) No 1107/2009, as it applies in GB.

HSE concludes that for the EATS-modalities, inpyrfluxam is not an ED and its ED potential has been sufficiently investigated, with no further information required.

B.6.8.5. Immunotoxicity

No specific immunotoxicity studies conducted with inpyrfluxam are available. However, an assessment of the immunotoxicity potential of inpyrfluxam has been performed by considering the available repeated dose toxicity, carcinogenicity and reproductive toxicity studies. These standard regulatory studies conducted with inpyrfluxam have assessed its potential impact on several immune-related endpoints, including haematological parameters such as total and differential white blood cell counts, spleen and thymus weights, and histopathology of the spleen, thymus, lymph nodes and bone marrow.

The results of the investigations on all immune-related endpoints are summarised below (Table 6.8.5 -1).

In the 90-day study in mice, there were dose dependent increases in globulin levels (by 10% and 12% in M ; by 10% and 14% in F) and A/G ratio (by 9% and 13% in M respectively and by 15% and 20% in F respectively) from 491/559 mg/kg bw/day. In the 90-day study in dogs, there were dose dependent decreases in globulin levels (by>10% in M) and A/G ratio (by >7% in M and by ≥20% in F) from 160 mg/kg bw/day. The histopathology findings in spleen noted at 700/500 mg/kg bw/day in both sexes in the 90-day dog study are not considered as an immune specific change due to the excessive toxicity (eg., mortality, vomiting, histopathological findings in various organs etc) at this dose. In the 1-year repeat dose study in dogs, a significant decrease in the A/G ratio was observed in males from 30 mg/kg bw/day.

In the combined chronic toxicity and carcinogenicity study in rats, a decrease in globulin level (by 13%in M) and an increase in A/G ratio (by 16% in M) were noted (from 95.9/86.4 mg/kg bw/day in M/F) in the chronic toxicity phase. These changes were accompanied by marked increases in neutrophil (by 41%) and monocyte (by 40%) counts. In the carcinogenicity phase, there were significant decreases in neutrophil (by 20% in males and 35% in females), monocytes (by 19% in M and by 28% in F) and white blood cell count (by 23% in F) at the high dose (78.4/65.8 mg/kg bw/day in M/F). In addition, there were increases in relative spleen weight (by 6% in F) and decreases in absolute spleen weight (by 16% in F) at the high dose (78.4/65.8 mg/kg bw/day in M/F). All these changes in differential white blood cells, globulin levels, A/G ratio and changes in spleen weight were considered to be treatment related and adverse. However, in the absence of histopathological findings, the changes in spleen weights were not considered to be adverse.

Although consistent haematological and biochemical changes were observed across three species (rats, mice, and dogs), the absence of alterations in immune-related

organs such as the spleen and thymus, combined with the presence of general systemic toxicity at the doses where these changes occurred, does not support an immunotoxic potential of inpyrfluxam.

In the one generation range finding study in rats, there were decreases in absolute (by 64% in M and 69% in F) and relative (by 30% in M and 29% in F) spleen weights at 254 mg/kg bw/ day; and absolute (by 61% in M and 72% in F) and relative (by 25% in M and 38% in F) thymus weight from 132 mg/kg bw/day in male pups and from 68 mg/kg bw/ day in female pups. There were no microscopic correlates for these organ weight changes; however, they were considered treatment-related and adverse since the relative weight change exceeded 15%.

In the two-generation study in rats, increases in both absolute and relative spleen weights were observed in F1 parental animals at 27.8/35.5 mg/kg bw (M/F). Additionally, increased absolute and relative spleen weights were noted in both F1 and F2 pups at 113/86 mg/kg bw/day (M/F). However, due to the low magnitude of change (<15%) and the lack of dose response, these changes were not considered to be treatment related.

No adverse changes in spleen and thymus weights or gross/histopathology findings in these organs were observed in rats or mice or dogs after short- or long-term repeated dose. Therefore, the treatment-related changes in spleen and/or thymus weight observed in rats in one and two generation studies, in the absence of associated histopathology, were not considered to be immune-specific effects.

Overall, the investigations on all immune-related endpoints in repeated dose studies (in the rat, mouse, dog and rabbit) and generational studies (in rats) did not find evidence of any consistent immune-specific effects caused by inpyrfluxam. Therefore, it can be concluded that inpyrfluxam does not show immunotoxic potential.

Table 6.8.5-1: List of effects on immune related parameters investigated in the studies conducted with inpyrfluxam

Species, duration of treatment, route of administration	Rat, 28d - oral	Rat, 90d- oral	Mouse, 90d- oral	Dog, 90d- oral	Dog, 1-year - oral	Rat, 28 d - dermal	Rat combined chronic & carc.	Mouse, carc.	Rat, One gen	Rat, Two gen
OECD TG No.	Similar to 407 ♂ / ♀	408 ♂ / ♀	408 ♂ / ♀	409 ♂ / ♀	452 ♂ / ♀	452 ♂ / ♀	453 ♂ / ♀	451 ♂ / ♀	451 ♂ / ♀	416 ♀
Total leukocyte count (WBC)	-	↑/-	-	-	-	-	-/↓	-	-	-
Differential blood count	-	↑/-	-	↑/-	-	-	-	-	-	-
Lymphocytes										
Neutrophils	-	↑/-	-	-	-	-	↓/↓	-	-	-
Eosinophils	-	-	-	-	-	-	-	-	-	-
Basophils	-	-	-	-	-	-	-	-	-	-
Monocytes	-	-	-	-	-	-	↓/↓	-	-	-
Globulin	-	-	↑/↑	↓/↓	↓/↓	-	↓/-	-	-	-
A/G ratio	-	-	↓/↓	↓/↓	↓/↓	-	↓/-	-	-	-
Necropsy										
Lymph node-enlargement	-	-	-	-	-	-	-	↑/↓	-	-
Thymus -enlargement	-	-	-	-	-	-	-	↑/↓	-	-
Spleen weight										
Abs.	-	↓/↓	-	-	-	-	-/↓	-	↓/↓	-/↑ ($F1^P$) or ↓/↓
Rel.	-	-	-	-	-	-	-/↑	-	↓/↓	-/↑ ($F1^P$) or ↓/↓
Spleen histopathology	-	-	-	↑ / ↑	-	-	-	-	-	-
Thymus weight										
Abs.	↓/-	-	-	-	-	-	-	-	↓/↓	-
Rel.	↓/-	-	-	-	-	-	-	-	↓/↓	-
Thymus histopathology	↑/↑	-	-	-	-	-	-	-	-	-
Lymph node histopathology	-	-	-	-	-	-	-	-	-	-

Amyloid lesions										
Lymph node	-	-	-	-	-	-	-	↑/↑	-	-
Forestomach	-	-	-	-	-	-	-	↑/-	-	-
Glandular stomach	-	-	-	-	-	-	-	-/↑	-	-
Duodenum	-	-	-	-	-	-	-	↑/-	-	-
Bone marrow histopathology	↑/↑	-	-		-	-	-	-	-	-

-: no effect;
↑/↓: adverse increased/decreased;
↑/↓: treatment-related increased/decreased;
↑/↓: changes observed were not treatment-related
p = parental animals; d = day; carc. = carcinogenicity; EOGRTS = extended one generation reproductive toxicity study

B.6.9. Medical Data and Information

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

According to the statement from the manufacturer, Sumitomo Chemical Co., Ltd. inpyrfluxam has not been commercially manufactured or marketed. It has only been synthesized in the laboratory and/or at a pilot plant at the facility of Sumitomo Chemical Co. Ltd for research and development purposes.

Health checks are routinely conducted on staff involved in the synthesis or research and development of inpyrfluxam. Sumitomo has confirmed that no inpyrfluxam-related health issues have been identified by or reported to medical personnel.

B.6.9.2. Data collected on humans

Inpyrfluxam has not been introduced on the market yet, therefore there is no information on record.

B.6.9.3. Direct observation

Inpyrfluxam has not been introduced on the market yet, therefore there is no information on record.

B.6.9.4. Epidemiological studies

Inpyrfluxam has not been introduced on the market yet, therefore there is no information on record.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

Inpyrfluxam is not considered to be acutely toxic via the dermal and inhalation route but is classified for acute oral toxicity Category 3 based on two acute oral toxicity studies in rats. It is not a skin or eye irritant, or a skin sensitiser.

In acute toxicity studies in animals, mortality and clinical signs of toxicity (reduction in spontaneous activity and ataxic gait) were evident at approximately 180 mg/kg bw/day and above. The same would be expected to occur in humans if equivalent dose levels were consumed. However, no cases of intoxication with inpyrfluxam have yet been observed.

No specific clinical tests have been performed in humans.

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

There is no known antidote for inpyrfluxam and the following first aid measures should be observed:

General	Terminate exposure, remove person from scene of spillage or other contamination

In case of skin contact:	Remove contaminated clothing and thoroughly wash the affected parts of the body with soap and water
In case of eye contact:	Rinse eyes with clean water for several minutes. Obtain medical advice
In case of ingestion:	Do not induce vomiting. Obtain medical advice

B.6.9.7. Expected effects of poisoning

There are no known effects of poisoning from inpyrfluxam.

B.6.10. References Relied On

Literature review

This literature review was conducted by the applicant following the current EFSA Guidance (EFSA Journal 2011;9(2):2092) for identifying scientific peer-reviewed open literature on the active substance inpyrfluxam and its relevant metabolites, as required by the data requirements of assimilated Regulation (EC) No 1107/2009.

Study	Updated LITERATURE REVIEW REPORT
Reference	Exponent International Ltd
Date performed	14 January 2025
Test facility	Exponent International Ltd – U.K.
Report reference	1403863.UK0 – 4472
Guideline(s)	EFSA Journal 2011;9(2):2092. Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009.
Deviations from the guideline	No
GLP	N/A
Date of the search:	14 January 2025
Date span of the search:	July 2013 to July 2023
Study acceptable	Yes

Methods

Input parameters

Inpyrfluxam and its metabolites were considered in the literature search, and the input parameters were as below:

Common name	Synonyms	Search engine	Fields searched
Inpyrfluxam	(*R*)-3-(difluoromethyl)-1-methyl-*N*-(1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-yl)-1*H*-pyrazole-4-carboxamide 1352994-67-2 S-2399	CAS (Sci-finder n) Proquest Dialogue	Full Text
3'-OH-S-2840	3-(difluoromethyl)-*N*-(3-hydroxy-1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-yl)-1-methyl-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-CH_2OH-S-2840	3-(difluoromethyl)-N-((1R,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide 3-(difluoromethyl)-N-((1S,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-CH_2OH-S-2840	3-(difluoromethyl)-N-((1R,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide 3-(difluoromethyl)-N-((1S,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1-methyl-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-S-2840	3-(difluoromethyl)-*N*-(1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-yl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-CH_2OH-S-2840	3-(difluoromethyl)-N-((1R,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide 3-(difluoromethyl)-N-((1S,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-CH_2OH-S-2840	3-(difluoromethyl)-N-((1R,3R)-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide 3-(difluoromethyl)-N-((1S,3S)-1-(hydroxymethyl)-1,3-dimethyl-2,3-	CAS (Sci-finder n) Proquest Dialogue	Full Text

	dihydro-1H-inden-4-yl)-1H-pyrazole-4-carboxamide		
7'-OH-S-2399	(*R*)-3-(difluoromethyl)-*N*-(7-hydroxy-1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-yl)-1-methyl-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-CH_2OH-3'-OH-S-2840	*N*-[(1*RS*,3*RS*;1*RS*,3*SR*)-2,3-dihydro-1,3-dimethyl-3-hydroxy-1-(hydroxymethyl)-1*H*-inden-4-yl)]-1-methyl-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
glucuronide of 1'-CH_2OH-3'-OH-S-2840	Glucuronide of *N*-[(1*RS*,3*RS*;1*RS*,3*SR*)-2,3-dihydro-1,3-dimethyl-3-hydroxy-1-(hydroxymethyl)-1*H*-inden-4-yl)]-1-methyl-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
ATMI	(*R*)-1,1,3-trimethyl-2,3-dihydro-1*H*-inden-4-amine 125349-37-3	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-COOH-S-2840	(1S,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid (1R,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-COOH-S-2840	(1R,3R)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid (1S,3S)-4-(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1H-indene-1-carboxylic acid	CAS (Sci-finder n) Proquest Dialogue	Full Text
1',1'-bis(CH_2OH)-S-2840	*N*-(1,1-bis(hydroxymethyl)-3-methyl-2,3-dihydro-1*H*-inden-4-yl)-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text

N-des-Me-1',1'-bis(CH_2OH)-S-2840	*N*-(1,1-bis(hydroxymethyl)-3-methyl-2,3-dihydro-1*H*-inden-4-yl)-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-CH_2OH-3'-OH-S-2840	3-(difluoromethyl)-*N*-(3-hydroxy-1-(hydroxymethyl)-1,3-dimethyl-2,3-dihydro-1*H*-inden-4-yl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-1'-COOH-S-2840 NDM-1'-COOH-S-2840	4-(3-(difluoromethyl)-1*H*-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1*H*-indene-1-carboxylic acid	CAS (Sci-finder n) Proquest Dialogue	Full Text
DFPA	3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxylic acid 176969-34-9	CAS (Sci-finder n) Proquest Dialogue	Full Text
N-des-Me-DFPA	3-(difluoromethyl)-1*H*-pyrazole-4-carboxylic acid 151734-02-0	CAS (Sci-finder n) Proquest Dialogue	Full Text
DFPA-$CONH_2$	3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide 925689-10-7	CAS (Sci-finder n) Proquest Dialogue	Full Text
1'-CH_2OH-S-2840-sulfate	(4-(3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1*H*-inden-1-yl)methyl hydrogen sulfate	CAS (Sci-finder n) Proquest Dialogue	Full Text
Glu-1'-CH_2OH-S-2840	6-((4-(3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamido)-1,3-dimethyl-2,3-dihydro-1*H*-inden-1-yl)methoxy)-3,4,5-trihydroxytetrahydro-2*H*-pyran-2-carboxylic acid	CAS (Sci-finder n) Proquest Dialogue	Full Text
3'-OH-S-2840-dehydrate	3-(difluoromethyl)-1-methyl-*N*-(1,1,3-trimethyl-1*H*-inden-4-yl)-1*H*-pyrazole-4-carboxamide	CAS (Sci-finder n) Proquest Dialogue	Full Text

Inpyrfluxam is a new active substance, and no formulations have yet been commercialised, therefore, the search did not include any products.

Search strategy

The search strategy was based on a single concept search in CAS (Sci-finder n), Proquest and Dialogue databases.

Databases searched

CAS DATABASES

SciFinder-n, a resource from the Chemical Abstracts Service (CAS), a division of the American Chemical Society (ACS), is a curated database of chemical and bibliographic information. It is a core research tool for chemistry, biochemistry, chemical engineering, materials science, nanotechnology, physics, environmental science and other science and engineering disciplines.

CAS DATABASES:	FREQUENCY OF UPDATES
SciFinder-n	Updated daily

DIALOG DATABASES

Dialog is the premier online retrieval service with the most comprehensive content collection and most powerful search language available. Dialog is the worldwide leader in providing online-based information in science. The database holds data from more than 800 million unique records of key information, accessible via the Internet. Content areas include, but are not limited to, biomedical research, biotechnology, chemicals, environment, food and agriculture, medicine and science and technology.

DIALOG DATABASES:	FREQUENCY OF UPDATES
AGRICOLA	All PROQUEST databases are current and updated regularly, except as noted
AGRIS	
Analytical Abstracts	
Aqualine	
Aquatic Science & Fisheries Abstracts (ASFA)	
BIOSIS Toxicology	
CAB ABSTRACTS	
Ecology Abstracts	
Embase	
Endocrinology Abstracts	
Environment Abstracts	
FSTA	
GEOBASE	
GeoRef	
MEDLINE	
Meteorological & Geoastrophysical Abstracts	
Oceanic Abstracts	
Pollution Abstracts	

DIALOG DATABASES:	FREQUENCY OF UPDATES
ToxFile	
Toxicology Abstracts	
TOXLINE	
Water Resources Abstracts	

Time period

The literature search has been performed to cover the 10 years (from July 2013 to July 2023) prior to the expected submission of the dossier.

Selection process

The selection process resulted in two categories of publication:

1. Studies considered being non-relevant after initial (rapid) review.
2. Potentially relevant articles requiring more detailed consideration of abstracts and / or full-text documents to assess relevance.

Criteria of relevance applied

Studies relevant to the dossier are those that inform one or more data requirement(s), including hazard identification, hazard characterisation and exposure assessment, for the active substance under assessment, its relevant metabolites, or plant protection products.

As part of the determination of relevancy for toxicology, the following criteria are considered to be fundamental when considering the relevance of an open-literature study (EFSA Journal 2011;9(2):2092):

- the test species,
- the test material,
- the use of different doses,
- the specific endpoints of interest.

Studies that are relevant to the data requirements are studies that appropriately address these components, i.e. studies which present a well-identified test material (including purity and impurity profile); a test relevant to the mammalian toxicological assessment (preferred species are rats and mice; the dog is the preferred non-rodent species); a number of animals per group sufficient to establish a statistical significance; several dose levels tested (at least 3), preferably including a negative control, to establish a dose response; relevant route of administration in terms of risk assessment (oral, dermal or inhalation); and a description of the observations, examinations and analysis or necropsy performed.

The criteria considered for relevancy of studies relating to individual toxicology data requirements are detailed in the table below:

Data requirement (data point)	Relevancy criteria considered
Active substance	
Studies on absorption, distribution, metabolism and excretion in mammals (KCA 5.1)	1. Well-defined test material. 2. *In vivo* tests in relevant test species. 3. *In vitro* tests. 4. PBPK modelling. 5. Specific endpoint can be clearly related to this data requirement.
Acute toxicity (KCA 5.2)	1. Well-defined test material. 2. Relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement.
Short-term toxicity (KCA 5.3)	1. Well-defined test material. 2. Relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement.
Genotoxicity (KCA 5.4)	1. Well-defined test material. 2. *In vitro* tests. 3. *In vivo* tests in relevant test species. 4. Specific endpoint can be clearly related to this data requirement.
Long-term toxicity and carcinogenicity (KCA 5.5)	1. Well-defined test material. 2. Relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement
Reproductive toxicity (KCA 5.6)	1. Well-defined test material. 2. Relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement.
Neurotoxicity studies (KCA 5.7)	1. Well-defined test material. 2. *In vivo* tests in relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement.

Data requirement (data point)	Relevancy criteria considered
Other toxicological studies (KCA 5.8)	1. Well-defined test material. 2. *In vitro* tests. 3. *In vivo* tests in relevant test species. 4. Relevant route of exposure. 5. Specific endpoint can be clearly related to this data requirement.
Medical data (KCA 5.9)	1. Well-defined test material. 2. Epidemiological studies. 3. Poisonings, clinical cases. 4. Relevant route of exposure.
Plant protection products	
Acute toxicity (KCP 7.1)	1. Well-defined test material. 2. Relevant test species. 3. Relevant route of exposure. 4. Specific endpoint can be clearly related to this data requirement.
Data on exposure (KCP 7.2)	1. Well-defined test material. 2. Field studies. 3. Calculations. 4. Specific endpoint can be clearly related to this data requirement.
Dermal absorption (KCP 7.3)	1. Well-defined test material. 2. *In vitro* tests. 3. *In vivo* tests in relevant test species. 4. Specific endpoint can be clearly related to this data requirement.

Studies identified as relevant to the risk assessment were considered for reliability assessment. Full-text articles were assessed in order to further determine whether the information contained in the study could impact on the endpoints and risk assessment parameters related to the active substance. Reviews of the relevance of the articles brought up in the literature search were carried out by experts in the relevant technical disciplines.

The reliability assessment for any relevant studies was carried out based on general principles informed by Klimisch et al. (1997)[11], Schneider et al., (2009) (ToxRTool)[12], and Kaltenhäuser et al. (2017)[13]. On the basis of these principles the following categories of reliability were assigned:

Code	Category
1	Reliable without restriction
2	Reliable with restriction
3	Not reliable
4	Not assignable

Results

Findings of the literature review

Summary of the review	n
Total number of summary records retrieved from search	352
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	349
Number of summary records of potential/unclear relevance assessed in further detail (by abstract/full-text)	3
Number of studies excluded from the risk assessment after detailed assessment of full-text documents (i.e. not relevant)	2
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	1
Number of studies included in the dossier as supporting information (reliability criteria 1-2)	1

Three hundred and fifty-two articles were retrieved from all database searched. After a rapid assessment for relevance using the title or abstract, 349 articles were removed from the selected articles and the 3 remaining articles of potential relevance to the regulatory data package for the active substance were assessed in further detail by examining the abstracts and full texts. Only one article was found to be relevant after detailed assessment; however, it is not considered relevant to toxicology.

[11] Klimisch, H-J., Andreae, M. & Tillmann, U. (1997) A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology 25 pp 1-5

[12] Schneider, K, Schwarz, M, Burkholder, I, Kopp-Schneider, A, Edler, L, Kinsner-Ovaskainen, A, et al., 2009. “ToxRTool”, a new tool to assess the reliability of toxicological data. Toxicol. Lett. 189, 138e144.

[13] Kaltenhäuser et al., 2017. Relevance and reliability of experimental data in human health risk assessment of pesticides. Regulatory Toxicology and Pharmacology Aug 2017;88:227-237. doi:10.1016/j.yrtph.2017.06.01

References relied on:

KCA Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Owner
KCA 5.1.1/01	[redacted]	2016a	Metabolism of S-2399 in Rats [redacted], Study No. 4315, Sumitomo Chemical Co., Ltd. Report No: TPM-0026 GLP, unpublished	Y	Y	SUM
KCA 5.1.1/02	[redacted]	2016b	Metabolism of S-2399 in Rats (Repeated oral Administration) [redacted], Study No. 4338. Sumitomo Chemical Co., Ltd. Report No: TPM-0027 GLP, unpublished	Y	Y	SUM
KCA 5.1.1/03	[redacted]	2017	Comparative *in vitro* metabolism study of [pyrazolyl-4-^{14}C]S-2399 in rat and human liver microsomes Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. 4370. Sumitomo Chemical Co., Ltd. Report No: TPM-0052 GLP, unpublished	N	Y	SUM
KCA 5.1.1/04	[redacted]	2018	*In vitro* metabolism of [pyrazolyl-4-^{14}C]S-2399 in dog liver microsomes Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. 4391. Sumitomo Chemical Co., Ltd. Report No: TPM-0056 GLP, unpublished	N	Y	SUM
KCA 5.2.1/01	[redacted]	2015a	Acute Oral Toxicity Study of S-2399 Technical Grade in Rats [redacted] Study No. 4310 Sumitomo Chemical Co., Ltd. Report No: TPT-0018 GLP, unpublished	Y	Y	SUM

KCA 5.2.1/02	[redacted]	2017a	Acute Oral Toxicity Study of S-2399 Technical Grade in Rats (Up-and-Down-Procedure) [redacted], Study No. 4374 Sumitomo Chemical Co., Ltd. Report No: TPT-0109 GLP, unpublished	Y	Y	SUM
KCA 5.2.2/01	[redacted]	2015b	Acute Dermal Toxicity Study of S-2399 Technical Grade in Rats [redacted] Study No. 4306 Sumitomo Chemical Co., Ltd. Report No: TPT-0013 GLP, unpublished	Y	Y	SUM
KCA 5.2.3/01	[redacted]	2015c	Acute Inhalation Toxicity Study of S-2399 Technical Grade in Rats [redacted], Study No. 4309 Sumitomo Chemical Co., Ltd. Report No: TPT-0015 GLP, unpublished	Y	Y	SUM
KCA 5.2.4/02	[redacted]	2025a	In vitro Skin Irritation Test of S-2399 TG using EpiOcularTM EIT (OCL-200) Chemicals Evaluation and Research Institute, Hita (CERI Hita), Japan, Study No. H21-0082 Sumitomo Chemical Co., Ltd. Report No: TPT-0211 GLP, unpublished	Y	Y	SUM
KCA 5.2.5/02	[redacted]	2025a	In vitro Skin Irritation Test of S-2399 TG using EpiDermTM SIT (EPI-200) Chemicals Evaluation and Research Institute, Hita (CERI Hita), Japan, Study No. F41-0045 Sumitomo Chemical Co., Ltd. Report No: TPT-0212 GLP, unpublished	Y	Y	SUM
KCA 5.2.6/01	[redacted]	2015c	Skin sensitization test of S-2399 Technical Grade in guinea pigs (Maximization Test) [redacted], Study No. 4303 Sumitomo Chemical Co., Ltd. Report No: TPT-0011 GLP, unpublished	Y	Y	SUM
~~KCA 5.2.7/01~~	[redacted]	~~2016~~	~~*In vitro* 3T3 NRU Phototoxicity Study of S-2399 Technical Grade in Cultured Mammalian Cells (Amended Final Report)~~ ~~LSI Medience Corporation, Japan, Study No. B150567.~~ ~~Sumitomo Chemical Co., Ltd. Report No TPT-0037~~ ~~GLP, unpublished~~	~~N~~	~~Y~~	~~SUM~~

KCA 5.3.1/01	[redacted]	2014	One-month Oral Toxicity Study of -2399 in Rats [redacted], Study No. S1554 Sumitomo Chemical Co., Ltd. Report No: TPT-0100 GLP, unpublished	Y	Y	SUM
KCA 5.3.2/01	[redacted]	2016	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in Rats. Final report amendment. [redacted], Study No. [redacted] 13-0069 Sumitomo Chemical Co., Ltd. Report No: TPT-0048 GLP, unpublished	Y	Y	SUM
KCA 5.3.2/02	[redacted]	2016a	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in Mice. Final report Amendment. [redacted] Study No. [redacted] 13-0068 Sumitomo Chemical Co., Ltd. Report No: TPT-0050 GLP, unpublished	Y	Y	SUM
KCA 5.3.2/03	[redacted]	2016	S-2399 Technical Grade: Repeated Dose 90-Day Oral Toxicity Study in Dogs [redacted], Study No. [redacted] 13-0106 Sumitomo Chemical Co., Ltd. Report No: TPT-0057 GLP, unpublished	Y	Y	SUM
KCA 5.3.2/04	[redacted]	2017	S-2399 Technical Grade: Repeated Dose 1-Year Oral Toxicity Study in Dogs [redacted] Study No. [redacted] 14-0096 Sumitomo Chemical Co., Ltd. Report No: TPT-0076. GLP, unpublished	Y	Y	SUM
KCA 5.3.3/01	[redacted]	2015	A 28-Day Repeated Dose Dermal Toxicity Study of S-2399 Technical Grade in Rats [redacted] Study No. P140742 Sumitomo Chemical Co., Ltd. Report No: TPT-0022 GLP, unpublished	Y	Y	SUM

KCA 5.4.1/01	[redacted]	2014a/2017	Reverse mutation test of S-2399 Technical Grade in bacterial systems. Amendment of final report Sumitomo Chemical Co. Ltd, Japan, Study No. 4289 Sumitomo Chemical Co., Ltd. Report No: TPT-0004 GLP, unpublished	N	Y	SUM
KCA 5.4.1/02	[redacted]	2014b	*In Vitro* Chromosomal Aberration Test on S-2399 Technical Grade in Chinese Hamster Lung Cells (CHL/IU) Sumitomo Chemical Co. Ltd, Japan, Study No. 4288 Sumitomo Chemical Co., Ltd. Report No: TPT-0005 GLP, unpublished	N	Y	SUM
KCA 5.4.1/03	[redacted]	2014	S-2399 TG: Gene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* (V79/HPRT) Harlan CCR, Germany, Study No. 1601100 Sumitomo Chemical Co., Ltd. Report No: TPT-0002 GLP, unpublished	N	Y	SUM
KCA 5.4.2/01	[redacted]	2015	Micronucleus Test on S-2399 Technical Grade in CD-1 Mice [redacted] Study No. 4290 Sumitomo Chemical Co., Ltd. Report No: TPT-0021 GLP, unpublished	Y	Y	SUM
KCA 5.5/01, KCA 5.5/02	[redacted]	2017	S-2399 Technical Grade: Combined Chronic Toxicity and Carcinogenicity study in Rat [redacted] Study No. [redacted] 14-0046 Sumitomo Chemical Co., Ltd. Report No: TPT-0090 GLP, unpublished	Y	Y	SUM
KCA 5.5/03	[redacted]	2017	S-2399 Technical Grade: Carcinogenicity study in Mice [redacted], Study No. [redacted] 14-0047 Sumitomo Chemical Co., Ltd. Report No: TPT-0089. GLP, unpublished	Y	Y	SUM

KCA 5.5/04	[redacted]	2017a	Study for Mode of Action Analysis for Rat Liver and Thyroid findings by S-2399 Technical Grade [redacted], Study No. S1782 Sumitomo Chemical Co., Ltd. Report No: TPT-0092 Non-GLP, unpublished	Y	Y	SUM
KCA 5.5/05	[redacted]	2017b	Study for Mode of Action Analysis for Mouse Liver and Thyroid findings by S-2399 Technical Grade [redacted] Study No. S1767 Sumitomo Chemical Co., Ltd Report No: TPT-0099 Non-GLP, unpublished	Y	Y	SUM
KCA 5.6.1/01	[redacted]	2015a	S-2399 Technical Grade: Dose Range-Finding Reproduction Toxicity Study in Rats [redacted], Study No. [redacted] 13-0080 Sumitomo Chemical Co., Ltd. Report No: TPT-0007 Non-GLP, unpublished	Y	Y	SUM
KCA 5.6.1/02	[redacted]	2017	S-2399 Technical Grade: Reproduction toxicity study in Rats [redacted], Study No. [redacted]-15-0018 Sumitomo Chemical Co., Ltd. Report No: TPT-0088 GLP, unpublished	Y	Y	SUM
KCA 5.6.2/01	[redacted]	2015b	S-2399 Technical Grade: Dose Range-Finding Teratogenicity Study in Rats [redacted], Study No. [redacted] 13-0087 Sumitomo Chemical Co., Ltd. Report No: TPT-0009 Non-GLP, unpublished	Y	Y	SUM

KCA 5.6.2/02	[redacted]	2017a	S-2399 Technical Grade: Teratogenicity study in Rats [redacted], Study No. [redacted] 14-0071 Sumitomo Chemical Co., Ltd. Report No: TPT-0084 GLP, unpublished	Y	Y	SUM
KCA 5.6.2/03	[redacted]	2017b	S-2399 Technical Grade: Additional Teratogenicity Study in Rats [redacted], Study No. [redacted] 16-0018 Sumitomo Chemical Co., Ltd. Report No: TPT-0073 GLP, unpublished	Y	Y	SUM
KCA 5.6.2/04	[redacted]	2015c	S-2399 Technical Grade: Dose Range-Finding Teratogenicity Study in Rabbits [redacted], Study No. IET 13-0088 Sumitomo Chemical Co., Ltd. Report No: TPT-0012 Non-GLP, unpublished	Y	Y	SUM
KCA 5.6.2/05	[redacted]	2015d	S-2399 Technical Grade: Additional Dose Range-Finding Teratogenicity Study in Rabbits [redacted], Study No. IET 14-0031 Sumitomo Chemical Co., Ltd. Report No: TPT-0014 Non-GLP, unpublished	Y	Y	SUM
KCA 5.6.2/06	[redacted]	2017c	S-2399 Technical Grade: Teratogenicity study in Rabbits [redacted], Study No. [redacted] 15-0017 Sumitomo Chemical Co., Ltd. Report No: TPT-0082. GLP, unpublished	Y	Y	SUM
KCA 5.6.2/07	[redacted]	2012	Preliminary Prenatal Developmental Toxicity Study in rats with S-2399 [redacted] Study No. S1767 Sumitomo Chemical Co., Ltd. Report No: TPT-0103. GLP, unpublished	Y	Y	SUM

KCA 5.7.1/01	[redacted]	2015	S-2399 Technical Grade: Dose Range-Finding Study for Acute Neurotoxicity Study in Rats [redacted], Study No. [redacted] 14-0107 Sumitomo Chemical Co., Ltd. Report No: TPT-0036 GLP, unpublished	Y	Y	SUM
KCA 5.7.1/02	[redacted]	2016b	S-2399 Technical Grade: Acute Oral Neurotoxicity Study in Rats [redacted], Study No. [redacted] 14-0108 Sumitomo Chemical Co., Ltd. Report No: TPT-0044 GLP, unpublished	Y	Y	SUM
KCA 5.7.1/03	[redacted]	2016	S-2399 Technical Grade: Repeated Dose 90-Day Oral Neurotoxicity Study in Rats [redacted], Study No. [redacted] 15-0037 Sumitomo Chemical Co., Ltd. Report No: TPT-0058. GLP, unpublished	Y	Y	SUM
KCA 5.7.1/04	[redacted] & [redacted]	2014	Annex: Positive control data of neurotoxicity study [redacted], Sumitomo Chemical Co., Ltd. Report No: TPT-0045 Non-GLP, unpublished	Y	Y	SUM
KCA 5.8.1.1/01	[redacted]	2017b	Acute Oral Toxicity Study of 3'-OH-S-2840 in Rats [redacted] Study No. 4361 Sumitomo Chemical Co., Ltd. Report No: TPT-0074 GLP, unpublished	Y	Y	SUM
KCA 5.8.1.1/02	[redacted]	2018	3'-OH-S-2840: Repeated Dose 90-Day Oral Toxicity Study in Rats [redacted], Study No. [redacted] 17-0024 Sumitomo Chemical Co., Ltd. Report No: TPT-0127 GLP, unpublished	Y	Y	SUM

KCA 5.8.1.1/03	[redacted]	2017a	3'OH-S-2840: Bacterial Reverse Mutation Test The Institute of Environmental Toxicology, Japan, Study No. IET 16-0064 Sumitomo Chemical Co., Ltd. Report No: TPT-0070 GLP, unpublished	N	Y	SUM
KCA 5.8.1.1/04	[redacted]	2017a	3'-OH-S-2840: Gene Mutation Assay in Chinese Hamster V79 Cells *In Vitro* (V79/HPRT) Envigo CRS GmbH, Germany, Study No. 1813701 Sumitomo Chemical Co., Ltd. Report No: TPT-0105 GLP, unpublished	N	Y	SUM
KCA 5.8.1.1/05	[redacted]	2017b	3'-OH-S-2840: Chromosome Aberration Test in Cultured Mammalian Cells The Institute of Environmental Toxicology, Japan, Study No. IET16-0065 Sumitomo Chemical Co., Ltd. Report No: TPT-0081 GLP, unpublished	N	Y	SUM
KCA 5.8.1.1/06	[redacted]	2020	*In Vitro* Micronucleus (MNvit) Test of 3'-OH-S-2840 in Human Lymphoblast Cell Line (TK6) Biosafety Research Centre Inc, Japan. Study No.: J352 (028-225) Sumitomo Chemical Co., Ltd. Report No: TPT-0166 GLP, unpublished	N	Y	SUM
KCA 5.8.1.2/01	[redacted]	2017c	Acute Oral Toxicity Study of 1'-COOH-S-2840 in Rats [redacted] Study No. 4369 Sumitomo Chemical Co., Ltd. Report No: TPT-0085 GLP, unpublished	Y	Y	SUM
KCA 5.8.1.2/02	[redacted]	2017	Reverse Mutation test 1'COOH-S-2840 in bacterial systems Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. 4366 Sumitomo Chemical Co., Ltd. Report No: TPT-0086 GLP, unpublished	N	Y	SUM

KCA 5.8.1.2/03	[redacted]	2017b	1'-COOH-S-2840: Gene Mutation Assay In Chinese Hamster V79 Cells *In Vitro* (V79/HPRT) Envigo CRS GmbH, Germany, Study No. 1813801 Sumitomo Chemical Co., Ltd. Report No: TPT-0104 GLP, unpublished	N	Y	SUM
KCA 5.8.1.2/04	[redacted]	2017a	*In Vitro* Chromosomal Aberration test on 1-'COOH-S-2840 in Chinese hamster lung cells (CHL/IU) Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. 4363 Sumitomo Chemical Co., Ltd. Report No: TPT-0107 GLP, unpublished	N	Y	SUM
KCA 5.8.1.2/05	[redacted]	2020	*In Vitro* Micronucleus (MNvit) Test of 1'-COOH-S-2840 in Human Lymphoblast Cell Line (TK6) Biosafety Research Centre Inc, Japan. Study No.: J351 (028-224) Sumitomo Chemical Co., Ltd. Report No: TPT-0165 GLP, unpublished	N	Y	SUM
KCA 5.8.3/01	[redacted]	2017b	Evaluation of effects of S-2399 on human estrogen receptor alpha and human androgen receptor using *in vitro* reporter gene assay Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. RGA-124, Sumitomo Chemical Co., Ltd. Report No: TPT-0071 Non-GLP, unpublished	N	Y	SUM
KCA 5.8.3/02	[redacted]	2017c	*In vitro* steroidogenesis assay of S-2399 with H295R cell line Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. HK002 Sumitomo Chemical Co., Ltd. Report No: TPT-0075 Non-GLP, unpublished	N	Y	SUM
KCA 5.8.3/04	[redacted]	2019	Evaluation of the effect of S-2399 Technical Grade on human thyroid hormone receptor alpha using *in vitro* reporter gene assay Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. RGA-147, Sumitomo Chemical Co., Ltd. Report No: TPT-0156 Non-GLP, unpublished	N	Y	SUM

KCA 5.8.3/05	[redacted]	2020	*In vitro* inhibition assay of sodium/iodide symporter with S-2399 TG Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. S2096, Sumitomo Chemical Co., Ltd. Report No: TPT-0167 Non-GLP, unpublished	N	Y	SUM
KCA 5.8.3/06	[redacted]	2019	*In vitro* thyroperoxidase inhibition assay with S-2399TG Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., Japan, Study No. S2017, Sumitomo Chemical Co., Ltd. Report No: TPT-0168 Non-GLP, unpublished	N	Y	SUM
KCA 5.8.3/07	[redacted] t al.,	2020	Updated Assessment of Endocrine Disrupting potential of Inpyrfluxam (S-2399). Exponent International Ltd., UK Report No.: 2006010.UK0-4943 Sumitomo Chemical Co., Ltd. Report No: TPT-0172 Non-GLP, unpublished	N	Y	SUM

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