PYDIFLUMETOFEN

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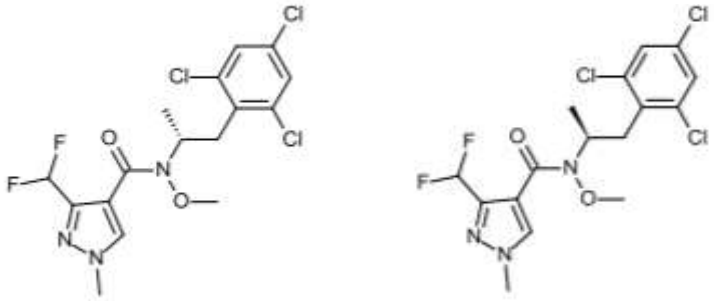
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Explanation

Pydiflumetofen (Fig. 1) is the International Organization for Standardization–approved common name for the 50:50 enantiomer ratio of (*S*)-3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-amide (SYN546968) and (*R*)-3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-amide (SYN546969) (International Union of Pure and Applied Chemistry), with the Chemical Abstracts Service number 1228284-64-7.

Fig. 1. Chemical structures of pydiflumetofen enantiomers



***S*-enantiomer** ***R*-enantiomer**

Source: European Commission (2018)

Both pydiflumetofen enantiomers are biologically active. Pydiflumetofen is a member of the *N*-methoxy-(phenyl-ethyl)-pyrazole-carboxamide group of fungicides. Its mode of action (MOA) is respiration inhibition at complex II (succinate dehydrogenase) in mitochondria of phytopathogenic fungi.

Pydiflumetofen has not been previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

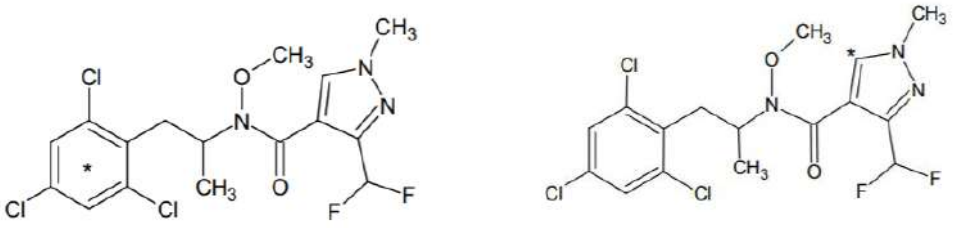
All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with relevant national or international test guidelines, unless otherwise indicated. No additional information from a literature search was identified that complemented the toxicological information submitted for the current assessment.

Evaluation for acceptable intake

1. Biochemical aspects

The positions of the radiolabels used in the absorption, distribution, metabolism and excretion studies with pydiflumetofen are shown in Fig. 2.

Fig. 2. Positions of radiolabels in the two pydiflumetofen enantiomers



Positions of the radiolabels: *: [pyrazole-5-^{14}C]pydiflumetofen (*left*); *: [phenyl-U-^{14}C]pydiflumetofen (*right*)
Source: MacDonald & Jewkes (2015)

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

In a pharmacokinetics study, CD-1 mice (eight of each sex per dose) were administered pydiflumetofen (lot/batch no. 2637-BA/110; purity 99.5%) by gavage in 0.5% weight per volume (w/v) carboxymethylcellulose containing 0.5% volume per volume (v/v) Tween 80 in distilled water for either a single day or daily for 7 days at 10, 30, 100, 200, 300, 500, 750 or 1000 mg/kg body weight (bw). An additional group of mice (12 of each sex) was administered pydiflumetofen intravenously in dimethyl sulfoxide (DMSO):10% w/v aqueous hydroxypropyl-β-cyclodextrin (5:95 v/v) at 1 mg/kg bw as a single dose. Blood was taken from four animals of each oral dose group at 1, 2, 4, 8, 12 and 24 hours after dosing on day 1 and day 7. Animals were terminated at the time of the third blood sample (12 or 24 hours). Animals retained for analysis on day 7 did not give blood on day 1. For the intravenously administered animals, blood was taken at 5 minutes and at 0.5, 1, 4, 6, 12 and 24 hours. The limit of quantification (LOQ) was 5 ng/mL blood.

The results of the pharmacokinetics study following intravenous or oral administration of pydiflumetofen are summarized in Table 1.

Intravenous administration of pydiflumetofen demonstrated rapid clearance and a high volume of distribution.

The total systemic exposure (area under the concentration–time curve [AUC]) to orally administered pydiflumetofen increased in an approximately dose-proportional manner in both sexes after a single dose (day 1), but sub-dose-proportionally after 7 days of repeated dosing, whereas the maximum concentration (C_{max}) increased sub-dose-proportionally at both times. The absolute oral bioavailability was low after 1 and 7 days of dosing (<10%) and lower after 7 days than after 1 day (<3%); it was also lower at higher doses.

After oral administration, the systemic exposure achieved on day 7 for each dose was markedly less than that on day 1, reflecting enzyme induction and increased first-pass clearance. Although it appeared that systemic exposure to pydiflumetofen was greater in females than in males at doses of 200–1000 mg/kg bw, no consistent sex-related trends were observed. The absolute oral bioavailability of pydiflumetofen was considered low and ranged from 3.6% to 10% in males and from 3.1% to 7.9% in females, across the respective doses on day 1 (Punler & Harris, 2014a).

The excretion of [phenyl-U-^{14}C]pydiflumetofen (lot/batch no. RDR-XIX-18; purity 98.9%) and [pyrazole-5-^{14}C]pydiflumetofen (lot/batch no. 5318IYC001-1; purity 98.5%) was investigated following the administration of a single oral dose of 10 or 300 mg/kg bw to groups of four male and four female CD-1 mice. Excretion samples were obtained over a 7-day period. After this period, the mice were humanely killed, and residual radioactivity was measured in the gastrointestinal tract and remaining carcass. The nature and identity of metabolites present in selected excreta and cage wash samples were also investigated (see section 1.2 below).

After the administration of [phenyl-U-^{14}C]pydiflumetofen or [pyrazole-5-^{14}C]pydiflumetofen at 10 mg/kg bw, a mean of 97–103% of the administered dose was eliminated in urine and faeces (including cage wash) over 7 days. The majority of administered radioactivity (87–97%) was excreted in the first 24 hours. The routes and rates of excretion were similar for both radiolabels and for both sexes. The majority of the dose was excreted in the faeces (63–79%), whereas urinary excretion accounted for 15–30% of the dose.

After the administration of [phenyl-U-^{14}C]pydiflumetofen or [pyrazole-5-^{14}C]pydiflumetofen at 300 mg/kg bw, a mean of 95–110% of the administered dose was eliminated in urine and faeces (including cage wash) over 7 days. The majority of administered radioactivity (88–103%) was excreted in the first 24 hours. The routes and rates of excretion were similar for both radiolabels and for both

Table 1. Pharmacokinetics (whole blood) of pydiflumetofen in mice following a single intravenous (1 mg/kg bw) or a single or multiple (10–1000 mg/kg bw) oral administration of radiolabelled pydiflumetofen

	Nominal dose (mg/kg bw)	**C_{max} (ng/mL)**	**T_{max} (h)**	**AUC_{0-t} (ng·h/mL)**	**$AUC_{0-\infty}$ (ng·h/mL)**	**$t_{½}$ (h)**	**F^a (%)**	**Rsq**	**MRT (h)**	**CL (mL/h·kg)**	**V_d (mL/kg)**
Intravenous											
Male	1	236	0.08	155	156	0.63	–	0.985	0.83	7 100	6 490
Female	1	214	0.10	154	167	1.40	–	0.842	1.39	6 750	13 500
Oral											
Male											
Day 1	10	47.9	1.00	88.0	104	1.25	6.60	0.990	–	–	–
	30	138	1.00	250	257	1.51	6.25	0.996	–	–	–
	100	601	1.00	1 560	1 590	1.22	9.51	1.00	–	–	–
	200	694	1.00	2 850	2 860	1.39	10.0	1.00	–	–	–
	300	598	1.00	3 490	3 630	2.28	7.54	0.998	–	–	–
	500	591	1.00	3 340	3 470	2.30	4.38	0.966	–	–	–
	750	798	0.667	5 710	6 040	2.55	5.79	0.918	–	–	–
	1 000	845	0.500	5 350	5 370	2.78	3.56	0.993	–	–	–
Day 7	10	14.7	1.00	20.7	NC	NC	NC	NC	–	–	–
	30	41.8	1.00	66.4	67.5	0.56	1.74	0.867	–	–	–
	100	80.8	0.500	345	358	2.33	2.18	0.968	–	–	–
	200	35.5	4.00	265	289	2.98	1.03	0.951	–	–	–
	300	54.7	2.00	370	384	2.19	0.818	0.971	–	–	–
	500	46.2	2.00	345	406	4.15	0.518	0.894	–	–	–
	750	47.9	8.00	487	NC	NC	NC	NC	–	–	–
	1 000	88.3	4.00	725	3 390[b]	25.9[b]	2.35[b]	0.187[b]	–	–	–

	Nominal dose (mg/kg bw)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng·h/mL)	$AUC_{0-\infty}$ (ng·h/mL)	$t_{½}$ (h)	F^a (%)	Rsq	MRT (h)	CL (mL/h·kg)	V_d (mL/kg)
Female											
Day 1	10	44.4	1.00	65.3	83.4[b]	1.44[b]	4.81[b]	0.686[b]	–	–	–
	30	113	1.00	136	138	0.916	3.27	0.970	–	–	–
	100	442	2.00	1 530	1 540	1.54	7.62	0.928	–	–	–
	200	577	2.00	2 100	2 100	1.41	7.87	0.878	–	–	–
	300	475	2.00	2 820	2 880	1.99	5.63	0.925	–	–	–
	500	447	1.00	2 450	2 570	2.47	3.07	0.950	–	–	–
	750	681	0.704	4 810	5 830	4.84	5.09	0.866	–	–	–
	1 000	809	0.500	4 150	4 390[b]	6.52[b]	2.79[b]	0.723[b]	–	–	–
Day 7	10	11.6	1.00	31.5	37.6[b]	2.43[b]	2.28[b]	0.680[b]	–	–	–
	30	28.1	1.00	56.0	57.4	0.604	1.34	0.970	–	–	–
	100	85.7	1.00	315	334[b]	2.31[b]	2.13[b]	0.775[b]	–	–	–
	200	50.2	1.00	390	415	2.85	1.37	0.800	–	–	–
	300	96.8	8.00	752	NC	NC	NC	NC	–	–	–
	500	68.8	4.00	601	789[b]	5.35[b]	0.946[b]	0.910[b]	–	–	–
	750	178	12.0	1 070	NC	NC	NC	NC	–	–	–
	1 000	108	12.0	1 140	NC	NC	NC	NC	–	–	–

AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration; *F*: bioavailability; CL: clearance; MRT: mean residence time; NC: not calculable, a terminal phase unable to be characterized; Rsq: square of the correlation coefficient for the terminal phase regression line; *t*: time; $t_{½}$: half-life; T_{max}: time to reach maximum concentration; V_d: volume of distribution

[a] Bioavailability on day 7 calculated using exposure estimates after single intravenous administration.

[b] Coefficient of determination was less than 0.800 and/or the extrapolation of the AUC to infinity represents more than 20% of the total.

Source: Punler & Harris (2014a)

sexes, with 76–94% of the dose excreted in the faeces and 7.2–15% in the urine. Nearly 50% of the 300 mg/kg bw dose was considered unabsorbed.

At both doses, excretion was essentially complete in all animals by 168 hours post-dosing, with 0.3% or less remaining in the carcass and gastrointestinal tract (Tomlinson et al., 2015).

Rats

In a preliminary pharmacokinetics study, Han Wistar rats were given a single dose of pydiflumetofen radiolabelled (^{14}C) in either the pyrazole position (lot/batch no. 5222MF-O00101, purity 97.8%; or lot/batch no. RDR-XIV-49, purity 97.2%) or the phenyl position (lot/batch no. DAD-XIII-38-5; purity 98.7%) intravenously at 1 mg/kg bw (one of each sex per radiolabel) or by gavage at 5 or 1000 mg/kg bw (six of each sex per radiolabel). The dosing vehicles were 5% w/v DMSO in 40% w/v aqueous hydroxypropyl-β-cyclodextrin (1 mg/kg bw); 5% v/v DMSO and 0.5% v/v Tween 80 in 0.5% w/v aqueous carboxymethylcellulose (5 mg/kg bw); and 20% v/v DMSO and 0.5% v/v Tween 80 in 0.5% w/v aqueous carboxymethylcellulose (1000 mg/kg bw). Blood was taken from 0.08 to 48 hours after dosing for intravenous administration and at the same time points and 72 hours after dosing for oral treatment. The following samples were collected from all animals to identify the biotransformation pathway (see section 1.2 below): urine and faeces (from predosing to 168 hours), cage wash (from 8 hours to termination of animals), expired air (predosing, 0–24 hours and 24–48 hours) and carcass (168 hours).

The pharmacokinetics of [^{14}C]pyrazole- or [^{14}C]phenyl-labelled pydiflumetofen in rats (whole blood) is summarized in Table 2. Mean recoveries of radioactivity were greater than 90% in all groups except for 1000 mg/kg bw females (recovery >85%). Blood and plasma radiolabel profiles were qualitatively similar for both radiolabels and both sexes, but significant differences were seen between the profiles for the 5 and 1000 mg/kg bw oral doses. Bioavailability was high at 5 mg/kg bw (63–87%), but much lower at 1000 mg/kg bw (<36%), resulting in an extended time to C_{max} (T_{max}) and a sub-dose-proportional AUC_{0-t}. At 5 mg/kg bw, absorption was rapid, with C_{max} reached in under an hour. Elimination of radiolabel was moderately slow, with the half-life between 20 and 51 hours, indicating that moderate accumulation of metabolites in plasma is likely to occur in repeated-dose studies.

Table 2. Pharmacokinetics (whole blood) of [^{14}C]pyrazole- or [^{14}C]phenyl-labelled pydiflumetofen following administration of a single intravenous (1 mg/kg bw) or a single oral (5 or 1000 mg/kg bw) dose to rats

	Males			**Females**		
	Intravenous	**Oral**		**Intravenous**	**Oral**	
Parameter	**1 mg/kg bw**	**5 mg/kg bw**	**1 000 mg/kg bw**	**1 mg/kg bw**	**5 mg/kg bw**	**1 000 mg/kg bw**
Pyrazole radiolabel						
C_{max} (ng equiv/g)	352	418	20 600	296	472	20 200
AUC_{0-t} (ng equiv·h/g)	2 150	6 800	724 000	2 020	7 800	724 000
T_{max} (h)	0.08	0.5	30	0.08	1	24
$t_{½}$ (h)	28	46	22	33	43	20
MRT (h)	35	–	–	38	–	–
F (%)	–	63.3	33.7	–	77.2	35.8
V_{ss} (g/kg)	12 100	–	–	13 600	–	–
Recovery (% of dose)						
Urine	18.67	20.83	21.93	17.23	26.01	27.16

	Males			Females		
	Intravenous	Oral		Intravenous	Oral	
Parameter	**1 mg/kg bw**	**5 mg/kg bw**	**1 000 mg/kg bw**	**1 mg/kg bw**	**5 mg/kg bw**	**1 000 mg/kg bw**
Faeces	74.29	73.54	66.64	78.24	70.38	54.90
Cage wash	1.11	2.14	1.90	1.84	1.89	3.58
Carcass	0.32	0.22	0.13	0.22	0.15	0.12
Total	94.39	96.73	90.60	97.53	98.43	85.76
Phenyl radiolabel						
C_{max} (ng equiv/g)	298	562	11 200	358	715	10 100
T_{max} (h)	0.08	1	24	0.08	1	24
AUC_{0-t} (ng equiv·h/g)	2 140	8 520	449 000	2 930	12 800	353 000
$t_{½}$ (h)	51	46	22	34	43	20
MRT (h)	59	–	–	41	–	–
F (%)	–	79.6	21	–	87.4	12.0
V_{ss} (g/kg)	16 200	–	–	9 650	–	–
Recovery (% of dose)						
Urine	23.7	23.49	13.19	20.55	19.02	15.07
Faeces	64.65	74.3	93.17	72.75	81.24	67.7
Cage wash	2.04	2.62	1.44	0.97	0.70	2.33
Carcass	0.31	0.32	0.11	0.24	0.19	0.14
Total	90.81	100.79	107.95	94.55	101.19	85.28

AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration; equiv: equivalents; *F*: bioavailability; MRT: mean residence time; *t*: time; $t_{½}$: half-life; T_{max}: time to reach maximum concentration; V_{ss}: volume of distribution at steady state

Source: Webbley & Williams (2015)

The predominant route of excretion in both sexes following oral administration was faecal, which accounted for between 55% and 93% of the administered dose, with urine accounting for less than 30%. At the low dose, the faecal content was due primarily to excretion in the bile, whereas at the high dose, unabsorbed pydiflumetofen contributed substantially to the faecal content. The observation of a peak in blood levels in females at 6–8 hours after intravenous administration was characteristic of enterohepatic recirculation; this peak was also seen at around 10 hours following administration of a 5 mg/kg bw oral dose in both sexes. Excretion was largely complete within the first 72 hours. Residual radiolabel in the carcass accounted for less than 0.5% of the administered dose, and negligible amounts were recovered in expired air (Webbley & Williams, 2015).

In a study of the absorption and excretion of pydiflumetofen, Han Wistar rats were administered pydiflumetofen radiolabelled (^{14}C) in either the pyrazole position (lot/batch no. 5271GAR001-4, purity 99.2%; or lot/batch no. 5283RJP001-2, purity 98.8%) or the phenyl position (lot/batch no. RDR-X-94; purity 99.1%) as a single intravenous dose of 1 mg/kg bw (four rats of each sex per radiolabel) or a single oral dose (24 rats of each sex per dose for each radiolabel) of 5 mg/kg bw for both sexes, 100 mg/kg bw for females only or 300 mg/kg bw for males only. Blood samples were taken for analysis of pharmacokinetics over 4 days for the oral doses and 2 days for the intravenous dose. The dosing vehicles

were DMSO:10% aqueous hydroxypropyl-β-cyclodextrin (5:95 v/v) for intravenous dosing and 0.5% w/v aqueous carboxymethylcellulose containing 0.5% Tween 80 for oral dosing. After oral dosing, blood samples were taken from subgroups of four rats of each sex at 0.25 and 1 hour; 0.5 and 2 hours; 4 and 12 hours; 8 and 24 hours; 30 and 72 hours; or 48 and 96 hours. Each rat gave two non-sequential samples, with the second sample taken at termination; for example, four rats of each sex were sampled at 0.25 hour and then at termination at 1 hour, four rats of each sex were sampled at 0.5 and 2 hours, and so on. The intravenously dosed animals (four rats of each sex) were each sampled at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48 hours.

After intravenous administration of 1 mg/kg bw, the pharmacokinetics parameters were similar for both radiolabels and both sexes (Table 3). The long half-life in females for the phenyl label was attributable to a low coefficient of determination (i.e. broad scatter of data), preventing reliable estimation of the value. Overall, the half-life was of the order of 20–40 hours, and the volume of distribution (V_d) was large, at around 13–20 L/kg bw.

Following oral administration, there were no marked sex- or radiolabel-based differences in pharmacokinetics parameters (Table 4). The bioavailability was approximately 50% at 5 mg/kg bw, but closer to 30% at 100/300 mg/kg bw, and T_{max} was delayed at the higher doses. These observations, together with a sub-dose-proportional AUC at the higher doses, indicate a saturation of absorption at the higher doses (Hutton & O'Hagan, 2015).

Table 3. Pharmacokinetics (whole blood) of pydiflumetofen following a single intravenous (1 mg/kg bw) administration of radiolabelled pydiflumetofen to rats[a]

	Phenyl label		Pyrazole label	
Parameter	**Males**	**Females**	**Males**	**Females**
C_0 (µg equiv/g)	0.463	0.366	0.439	0.341
$t_{½}$ (h)	39.4[b]	182[b]	25.3	20.7
$AUC_{0–t}$ (µg equiv·h/g)	1.89	1.95	1.54	1.62
$AUC_{0–t}/D$	2.07	2.28	1.66	1.87
$AUC_{0–\infty}$ (µg equiv·h/g)	2.6	5.96	1.93	1.91
$AUC_{0–\infty}/D$	2.84[b]	6.90[b]	2.10	2.24
AUC_{extrap} (%)	26.8[b]	50.7[b]	18.9	15.8
$MRT_{0–\infty}$ (h)	37.3[b]	235[b]	38.7	32.7
CL (mL/h·kg)	355[b]	216[b]	475	447
V_d (mL/kg bw)	19 950[b]	27 300[b]	17 300	13 400

AUC: area under the concentration–time curve; AUC_{extrap}: percentage of AUC to infinity estimated by extrapolation of the apparent terminal phase; bw: body weight; C_0: initial concentration; CL: clearance; D: dose administered; equiv: equivalents; MRT: mean residence time; t: time; $t_{½}$: half-life; V_d: volume of distribution

[a] Pharmacokinetics parameters were derived using WinNonlin, version 5.2.1, Pharsight Corp., Mountain View, CA, USA.

[b] Coefficient of determination was less than 0.800 and/or the extrapolation of the AUC to infinity represents more than 20% of the total area – i.e. values are quantitatively unreliable.

Source: Hutton & O'Hagan (2015)

In a study on absorption, excretion and tissue distribution, Han Wistar rats (four of each sex per dose for each radiolabel) were administered a single dose of pydiflumetofen radiolabelled (^{14}C) in either the pyrazole position (lot/batch no. 5271GAR001-4, purity 99.2%; or lot/batch no. 5283RJP001-2, purity 98.8%) or the phenyl position (lot/batch no. RDR-XV-94; purity 99.1%) by gavage. The doses were 5 mg/kg bw for both sexes and 300 mg/kg bw for males only or 100 mg/kg bw for females only. The dosing vehicle was 0.5% w/v aqueous carboxymethylcellulose containing 0.5% Tween 80. The dosing regimen was repeated with equivalent groups of bile duct–cannulated rats to examine biliary

Table 4. Pharmacokinetics (whole blood) of pydiflumetofen following a single oral (5, 100 or 300 mg/kg bw) administration of radiolabelled pydiflumetofen to rats

	Phenyl label				Pyrazole label			
	5 mg/kg bw		300 mg/kg bw	100 mg/kg bw	5 mg/kg bw		300 mg/kg bw	100 mg/kg bw
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
C_{max} (µg equiv/g)	0.63	0.72	8.1	3.8	0.27	0.45	4.7	2.1
T_{max}[a] (h)	2	1	8	8	2	0.5	8	8
$t_{½}$ (h)	116[b]	82.1[b]	163[b]	160[b]	75.3[b]	68.5[b]	196[b]	NC
$AUC_{0–t}$ (µg equiv·h/g)	7.19	8.16	218	89.7	5.6	5.86	167	54.0
$AUC_{0–t}/D$	1.48	1.66	0.778	0.936	1.1	1.15	0.605	0.632
$AUC_{0–\infty}$ (µg equiv·h/g)	12.2[b]	11.7	488[b]	165[b]	8.05	7.84[b]	358[b]	NC
$AUC_{0–\infty}/D$	2.51[b]	2.38[b]	1.74[b]	1.72[b]	1.58[b]	1.53[b]	1.3[b]	NC
F (%)[c]	55	55	27.9	33.7	51.8	48.3	27.0	26.4

AUC: area under the concentration–time curve; bw: body weight: C_{max}: maximum concentration; D: dose administered; equiv: equivalents; F: bioavailability; NC: not calculable, a terminal phase unable to be characterized; t: time; $t_{½}$: half-life; T_{max}: time to reach maximum concentration

[a] Median reported for T_{max}.

[b] Coefficient of determination was less than 0.800 and/or the extrapolation of AUC to infinity represents more than 20% of the total area – i.e. values are quantitatively unreliable.

[c] F (%) was calculated using $AUC_{0–48\ h}$.

Source: Hutton & O'Hagan (2015)

excretion. Excretion samples were collected from non-cannulated animals for 7 days and from bile duct–cannulated animals over 3 days. At the end of this period, radiolabel was determined in blood, excreta, selected tissues, carcass and cage wash.

Following oral administration of phenyl- or pyrazole-labelled pydiflumetofen at 5 mg/kg bw to intact rats, 97–99% of the administered dose was eliminated in urine and faeces (including cage wash), with the majority (91–96%) recovered in the first 48 hours. The major route of elimination was in bile (67–76%), with urinary elimination accounting for 18–26%. Excretion was similar in both sexes and for both radiolabel positions.

In intact male rats given 300 mg/kg bw, 101–103% of the dose was recovered in excreta, with 98–100% recovered in the first 48 hours. Radiolabel recovery from the faeces accounted for 91–92% of the dose, and urine, 6.7–7.8%. For intact females given 100 mg/kg bw, 101–102% of the administered dose was eliminated in urine and faeces, 99% in the first 48 hours, with 84–85% in the faeces and 14–15% in the urine.

Absorption was limited by dose, from approximately 85–90% of the 5 mg/kg bw dose to 19–24% at 300 mg/kg bw in males and 50–55% at 100 mg/kg bw in females. The majority of the absorbed dose was excreted in faeces via bile elimination.

For each of these groups, excretion was essentially complete by 168 hours, with 0.1% or less remaining in the carcass and gastrointestinal tract. Radiolabel was undetectable in the majority of tissues. Low levels of radiolabel were found in the liver and kidney, consistent with the biliary and urinary elimination of absorbed pydiflumetofen (Table 5). Moreover, radioactive residues were not above the limit of detection (LOD) in the majority of tissues.

Table 5. Tissue levels[a] of radiolabelled pydiflumetofen in rats after a single oral dose

	Tissue levels (µg equivalents/g)							
	Males				Females			
	Phenyl label		Pyrazole label		Phenyl label		Pyrazole label	
Tissue	5 mg/kg bw	300 mg/kg bw	5 mg/kg bw	300 mg/kg bw	5 mg/kg bw	100 mg/kg bw	5 mg/kg bw	100 mg/kg bw
Lung	0.01	0.3	0.013	0.2	0.018	0.1	0.017	0.1
Liver	0.09	2.7	0.134	2.4	0.048	0.5	0.06	0.4
Kidney	0.035	0.9	0.041	0.6	0.024	0.2	0.02	0.1

bw: body weight; LOD: limit of detection for radioactivity

[a] All other tissues were at or close to the LOD: for 5 mg/kg bw: 0.03 µg equivalents/g; for 100 and 300 mg/kg bw: 0.2 and 0.07 µg equivalents/g for males and females, respectively.

Source: Hutton (2015a)

In bile duct–cannulated rats given 5 mg/kg bw, 97–99% of the administered dose was eliminated in urine, bile and faeces (including cage wash) over 72 hours. The routes and rates of excretion were similar for both radiolabels and both sexes, with the exception that urinary excretion for males was approximately twice that for females. The majority of the dose was excreted in the bile (67–81%), but radioactivity measured in the faeces accounted for 10–15% of the dose, and urinary excretion accounted for 6.3–13% of the dose.

In bile duct–cannulated males given 300 mg/kg bw, 98–100% of the administered dose was eliminated in urine, bile and faeces (including cage wash) over 72 hours. The routes and rates of excretion were similar for both radiolabels, with 76–79% of the dose excreted in the faeces, 15–18% in the bile and 2.5–4.4% in the urine. In bile duct–cannulated females given 100 mg/kg bw, 97–99% of the administered dose was eliminated in urine, bile and faeces (including cage wash) over 72 hours. The routes and rates of excretion were similar for both radiolabels, with 43–49% of the dose excreted in the faeces, 33–41% in the bile and 7–15% in the urine. Excretion was essentially complete in all bile duct–cannulated animals by 72 hours post-dosing, with 0.3% or less remaining in the carcass and gastrointestinal tract (including contents).

Absorption was approximately 85–90% at 5 mg/kg bw. The majority of the absorbed dose was excreted in faeces through bile elimination. Seven days after the administration, radioactive residues were not detectable in the majority of tissues. The highest mean concentrations were found in the liver and kidney, consistent with the biliary and urinary elimination of absorbed ^{14}C-labelled pydiflumetofen (Hutton, 2015a).

In a study on tissue depletion, Han Wistar rats (20 of each sex per dose) were administered a single oral (gavage) dose of pydiflumetofen radiolabelled (^{14}C) in either the pyrazole position (lot/batch no. 5283RJP001-2; purity 98.8%) or the phenyl position (lot/batch no. RDR-XV-94; purity 99.1%) at 5 mg/kg bw for both sexes and 300 mg/kg bw for males only or 100 mg/kg bw for females only. The dosing vehicle was 0.5% w/v aqueous carboxymethylcellulose containing 0.5% Tween 80. The tissue distribution and depletion of radiolabelled pydiflumetofen were ascertained at various time points following dosing. Groups of rats (four of each sex at each time point) were killed at sequential predetermined time points based on a previous pharmacokinetics study (Hutton & O'Hagan, 2015). The first termination occurred at around the T_{max}. For the 5 mg/kg bw dose groups, these were 1 hour (females only), 2 hours (males only), 12, 24, 72 and 120 hours for the phenyl label and 0.5, 12, 24, 48 and 96 hours for the pyrazole label. For the 100 and 300 mg/kg bw dose groups for both labels, these were 8, 24, 48, 72 and 96 hours. Residual radioactivity was measured in selected tissues/organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pancreas, spleen, testes, thymus, thyroid, uterus

and the remaining carcasses). Blood was taken at termination. Where appropriate, terminal-phase half-lives of depletion were calculated for individual tissues.

In all cases, the radiolabel was widely distributed, with peak levels in tissues obtained at the first sampling point (selected to correspond to C_{max}). At all doses, peak tissue levels were highest in the liver, kidneys and adrenals. At the time of the last sampling (96/120 hours), all tissue concentrations, with the exception of those in the liver and kidney, were below the blood concentration or were below the LOD of radioactivity (0.003 µg equivalents/g for the 5 mg/kg bw dose group; 0.2 and 0.07 µg equivalents/g for males and females, respectively, for the 100 and 300 mg/kg bw dose groups). By 96/120 hours following dosing, residual levels of radiolabel were less than or equal to 3% in both sexes, for both radiolabels and all doses. Throughout the study, the majority of tissue concentrations were generally slightly higher in females than in males at 5 mg/kg bw.

The depletion profile of radiolabel in tissues reflected that of the blood/plasma, with no evidence of a plateau in any tissue. The half-lives of the radiolabel in tissues were similar to or less than the half-life in blood/plasma (Hutton, 2015b).

Han Wistar rats (four of each sex per dose) were administered pydiflumetofen (purity 99.5%) orally (by gavage) in 0.5% Tween 80 with 0.5% w/v carboxymethylcellulose at a dose of 3, 10, 30, 100, 300, 500 or 1000 (males only) mg/kg bw per day for 7 days or as a single intravenous dose of 1 mg/kg bw. Blood was taken on day 1 (intravenous and oral) and day 7 (oral only) at 0.08 (intravenous only), 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours after dosing. The LOQ was 5.0 ng/mL of blood.

The results are summarized in Tables 6 and 7. The bioavailability of pydiflumetofen after a single oral dose was only 2–6% in males and 4.8–37% in females. In males, C_{max} and AUC were less than dose proportional above 300 mg/kg bw after a single dose; after repeated dosing for 7 days, the achieved C_{max} was approximately the same for doses of 100–1000 mg/kg bw per day, and AUC was unchanged between 300 and 1000 mg/kg bw per day. In females, the C_{max} and AUC were essentially unchanged between 100 and 500 mg/kg bw after single and repeated administration. In both sexes, the AUC and C_{max} after 7 days of dosing were substantially lower than after a single dose.

The T_{max} tended to increase with increasing dose in both sexes. There was negligible accumulation of pydiflumetofen between day 1 (single administration) and day 7 (repeated administration) at the 3 and 10 mg/kg bw per day doses in both sexes, with systemic exposure appreciably reduced at doses greater than 10 mg/kg bw per day with repeated oral administration. The volume of distribution (V_d) largely reflected extensive distribution beyond the central circulation. Systemic exposure was consistently greater in females than in males throughout the study (Punler & Harris, 2014b).

Rabbits

In a toxicokinetics study, pregnant New Zealand white rabbits (four per dose) were administered pydiflumetofen (batch/lot no. SMU2EP12007; purity 98.5%) as a suspension in 1% w/v aqueous carboxymethylcellulose by gavage as a single dose (on gestation day [GD] 6) or repeated daily doses (GDs 6–27) of 100, 300, 750 or 1000 mg/kg bw. Animals were monitored for clinical signs and mortality daily, and feed intake and body weight were measured daily. At termination, a gross pathology examination was conducted, but no examination of the uterine contents was performed. Blood for analysis of toxicokinetics was taken immediately before dosing, at 2, 4, 6, 8, 12 and 24 hours after dosing and on days 6, 13 and 27. The LOQ was 5 ng/mL.

There were no treatment-related deaths or effects on clinical signs, gross pathology, feed intake or (in general) body weight. One female at 1000 mg/kg bw per day lost body weight between days 24 and 28 and had a very small overall body weight gain of only 0.5 kg. This same animal showed laboured breathing on day 25 of gestation, reduced faecal output on days 25, 27 and 28 and a reduced feed intake.

Table 6. Pharmacokinetics (whole blood) of pydiflumetofen after a single or repeated (7 days) oral administration to rats

	Single administration (mg/kg bw)							Repeated administration (mg/kg bw per day)						
	3	**10**	**30**	**100**	**300**	**500**	**1 000**	**3**	**10**	**30**	**100**	**300**	**500**	**1 000**
Males														
C_{max} (ng/mL)	7.86	12.4	38.9	242	602	380	612	8.5	14.9	17.2	34.3	63.3	41.9	64.5
T_{max} (h)	2.0	2.0	2.0	4.0	6.0	6.0	7.0	2.3	2.0	2.0	6.0	10.0	7.0	9.0
$t_{½}$ (h)	NC	NC	2.76	3.17	3.53	3.76	4.08	NC	NC	NC	3.57	6.34	3.37	5.85
$AUC_{0–t}$ (ng·h/mL)	11.22	41.65	227.4	1 613	6 096	3 644	7 546	13.5	57.8	109	291	666	448	832
$AUC_{0–\infty}$ (ng·h/mL)	NC	NC	232.6	1 496	6 358	3 737	7 855	NC	NC	NC	391	2 725	429	1 096
R_{ac}	–	–	–	–	–	–	–	NC	NC	NC	0.2	0.2	0.1	0.1
F (%)	NC	2.8[a]	3.0	6.0	6.3	2.3	2.6	NC	2	1	1	0.9	0.3	0.3
Females														
C_{max} (ng/mL)	76	178	527	674	639	640	–	76.5	146	272	259	252	286	–
T_{max} (h)	1.0	2.0	3.0	5.0	7.0	8.0	–	1.0	2.0	3.0	4.0	9.0	10.0	–
$t_{½}$ (h)	2.74	2.96	3.0	3.15	5.69	7.02	–	2.19	2.99	4.43	2.89	3.46	3.24	–
$AUC_{0–t}$ (ng·h/mL)	273	774	4 410	8 124	9 827	10 170	–	244	612	1 674	1 946	2 471	3 035	–
$AUC_{0–\infty}$ (ng·h/mL)	296	820	4 489	8 266	10 740	11 060	–	264	768	1 868	2 045	2 544	3 438	–
R_{ac}	–	–	–	–	–	–	–	0.9	1.1	0.4	0.3	0.3	0.3	–
F (%)[b]	23.0	21.0	36.8	20.8	7.6	4.8	–	24	18	16	6	2	2	–

AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration; F: bioavailability; NC: not calculable; R_{ac}: accumulation ratio compared with single dose; t: time; $t_{½}$: half-life; T_{max}: time to reach maximum concentration

[a] Value unreliable. Coefficient of determination was less than 0.800 and/or the extrapolation of AUC to infinity represents more than 20% of the total area.

[b] Approximated from dose-normalized AUC day 7 / single-dose intravenous AUC (see Table 7).

Source: Punler & Harris (2014b)

Table 7. Pharmacokinetics (whole blood) of pydiflumetofen after a single intravenous administration (1 mg/kg bw) to rats

Parameter	Males	Females
C_0 (ng/mL)	727	411
$t_{½}$ (h)	1.26	1.75
AUC_{0-t} (ng·h/mL)	253	344
$AUC_{0-\infty}$ (ng·h/mL)	266	361
CL (mL/h·kg)	3 414	2 644
V_d (mL/kg)	6 134	6 632
MRT (h)	1.1	2.0

AUC: area under the concentration–time curve; bw: body weight; C_0: initial concentration; CL: clearance; MRT: mean residence time; t: time; $t_{½}$: half-life; V_d: volume of distribution
Source: Punler & Harris (2014b)

The toxicokinetics parameters on day 6 and day 27 are shown in Table 8. Blood levels increased sub-proportionally to dose, with the AUC and C_{max} at 750 and 1000 mg/kg bw essentially similar at all time points. The T_{max} was extended at 750 and 1000 mg/kg bw. These observations reflect extended and incomplete absorption at the higher doses. Where calculable, the elimination half-life was approximately 6 hours.

Table 8. Toxicokinetics (whole blood) of pydiflumetofen following repeated oral administration to pregnant rabbits

	Day 6				Day 27			
Parameter	**100 mg/kg bw**	**300 mg/kg bw**	**750 mg/kg bw**	**1 000 mg/kg bw**	**100 mg/kg bw**	**300 mg/kg bw**	**750 mg/kg bw**	**1 000 mg/kg bw**
C_{max} (ng/mL)	26.4	44.1	71.2	79.3	87.5	118	102	116
T_{max} (h)	2–8	2–24	4–8	4–12	4–12	4–8	8	4–12
$t_{½}$ (h)	NC	NC	NC	NC	5.4	6.4	NC	NC
$AUC_{0-t\ last}$ (ng·h/mL)	254	722	1 010	1 140	1 110	1 560	1 850	2 050
$AUC_{0-24\ h}$ (ng·h/mL)	344	722	1 010	1 140	1 110	1 560	1 850	2 050
AUC_{∞} (ng·h/mL)	NC	NC	NC	NC	758	2 300	–	–
Dose-proportionality ratio versus lowest dose	–	3.0	7.5	10.0	–	3.0	7.5	10.0
Dose-proportionality ratio (C_{max})	–	1.7	2.7	3.0	–	1.3	1.2	1.2
Dose-proportionality ratio ($AUC_{0-24\ h}$)	–	2.1	2.9	3.3	–	1.4	1.7	1.8

–: not relevant; AUC: area under the concentration–time curve; bw: body weight; C_{max}: maximum concentration; NC: not calculable; t: time; $t_{½}$: half-life; T_{max}: time to reach maximum concentration
Source: Penn (2015a)

Overall, the toxicokinetics of pydiflumetofen in pregnant rabbits indicated a subproportional increase in systemic exposure with dose, with no apparent increase in systemic exposure between 750 and 1000 mg/kg bw per day (Penn, 2015a).

(b) Dermal route

No studies were submitted.

1.2 Biotransformation

(a) Mice

The biotransformation study in mice was conducted as part of the absorption and excretion study described in section 1.1 above (Tomlinson et al., 2015).

Up to 86% of the administered radioactivity was structurally identified following administration of a single oral dose of pydiflumetofen (SYN545974) to mice. The major metabolites present in urine and faeces were qualitatively and quantitatively similar between males and females and across doses. Some quantitative differences were observed in faeces from male and female mice, but these were not considered biologically relevant. Unchanged pydiflumetofen was not detected in urine. Although unchanged pydiflumetofen accounted for less than 4.4% of the dose in the faeces at 10 mg/kg bw, the unchanged parent was the major component recovered at up to 48.8% of the administered dose at 300 mg/kg bw.

Metabolites detected in urine were numerous and generally comprised a low percentage of the administered dose. The major components detected in urine were those formed following the cleavage of the parent compound and included the phenyl-derived metabolites 2,4,6-trichlorophenol (2,4,6-TCP) sulfate, 3-hydroxy-2,4,6-TCP sulfate and 2,4,6-TCP glucuronide. The major pyrazole-derived metabolite was identified as SYN548263. Other components identified included demethylated and/or hydroxylated pydiflumetofen and their corresponding glucuronide and sulfate conjugates.

Metabolites detected in faeces were mostly identified as demethylated and/or hydroxylated and/or dechlorinated pydiflumetofen. Only one label-specific metabolite was observed, and this was the pyrazole-derived metabolite SYN548263, at up to 4.7% of the dose. Desmethyl-hydroxy pydiflumetofen isomers and didesmethyl hydroxy pydiflumetofen isomers accounted for a large percentage of the administered dose in faeces, at up to 14.0% and 13.5% of the dose, respectively. SYN547897 was detected in nearly all samples and accounted for 2.9–11.4% of the dose. Only two other metabolites accounted for more than 5% of the dose: SYN547890 (6.0%) and dihydroxy SYN545974 (5.8%).

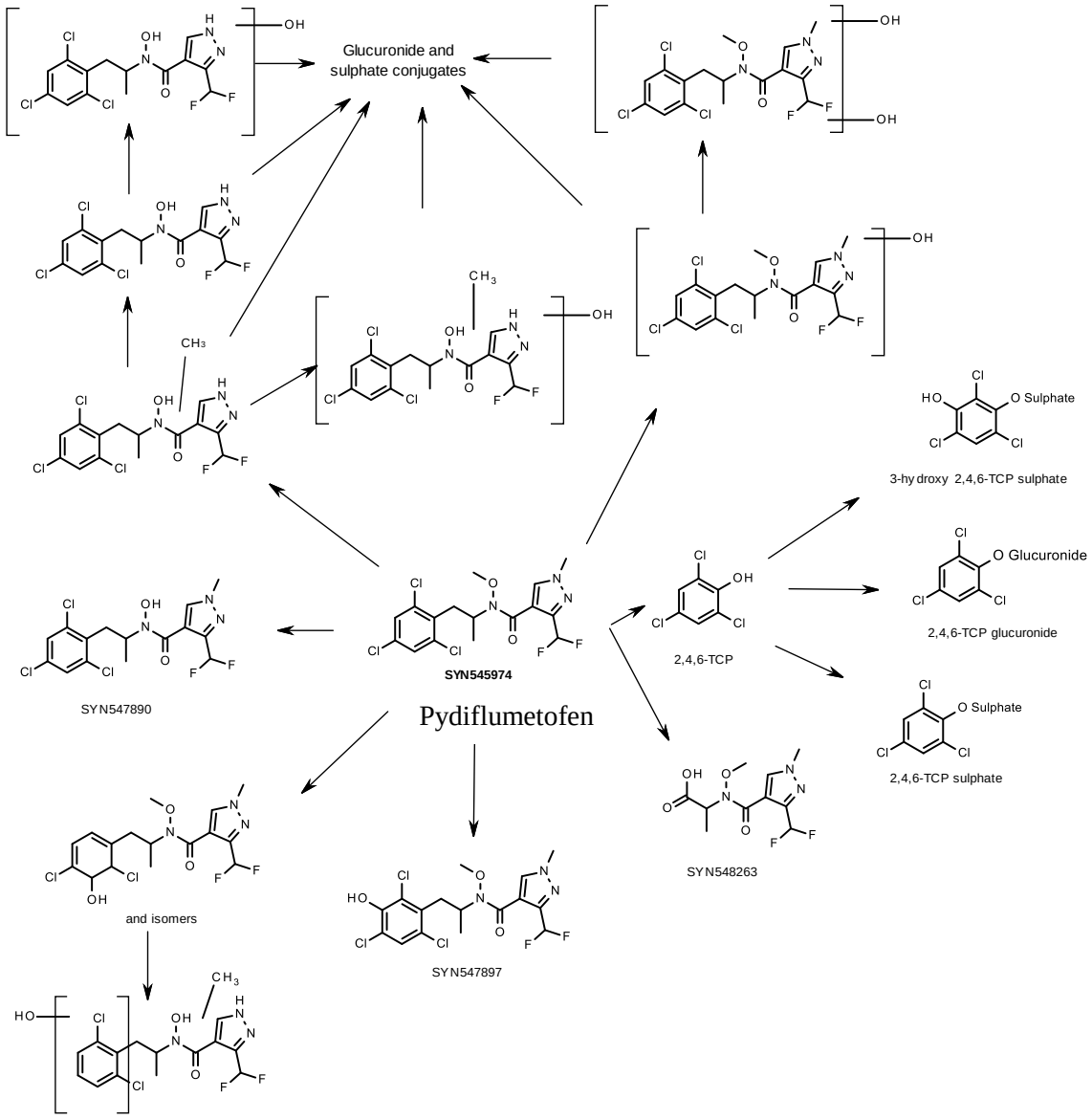
These results indicate that pydiflumetofen was extensively metabolized in the mouse via demethylation, hydroxylation and dechlorination, and subsequently by glucuronidation and sulfation. The molecule also cleaved at the benzylic carbon to yield the phenyl-derived metabolite 2,4,6-TCP and the pyrazole-derived metabolite SYN548263. These cleavage products were further metabolized via direct glucuronidation and sulfation and also following hydroxylation and sulfation to 3-hydroxy-2,4,6-TCP sulfate.

The biotransformation proceeded by:

- hydroxylation to give hydroxylated and dihydroxylated metabolite isomers;
- demethylation to SYN547890;
- demethoxylation to SYN545547, accompanied by hydroxylation;
- hydroxylation and demethylation to give desmethyl hydroxy metabolites and didesmethyl hydroxy metabolites;
- cleavage of pydiflumetofen to give the pyrazole-derived metabolite SYN548263 and the phenyl-derived metabolite 2,4,6-TCP;
- dechlorination and hydroxylation to SYN547894 and other dechlorinated hydroxy and dechlorinated dihydroxy metabolites; and
- glucuronic acid conjugation and sulfate conjugation.

A proposed metabolic pathway for pydiflumetofen in mice is shown in Fig. 3.

Fig. 3. The proposed metabolic pathway for pydiflumetofen in mice following oral administration



Source: Tomlinson et al. (2015)

(b) Rats

In the preliminary pharmacokinetics study described above in section 1.1, metabolism involved both phase I and phase II pathways, including oxidation, glucuronidation and sulfation, plus cleavage at the amide bond to form pyrazole-related metabolites and cleavage of the parent molecule to form 2,4,6-TCP-related metabolites (Webbley & Williams, 2015).

The nature and identity of pydiflumetofen metabolites in faeces, urine, bile, plasma and cage wash were ascertained from intact and bile duct–cannulated Han Wistar rats following administration

of a single oral (gavage) dose of pydiflumetofen radiolabelled in either the pyrazole or phenyl position at 5 mg/kg bw for both sexes, 100 mg/kg bw for females or 300 mg/kg bw for males. Samples were derived from two pharmacokinetics studies (Hutton, 2015a; Hutton & O'Hagan, 2015) whose materials and methods were described above in section 1.1.

Analyses of the radiolabelled moieties in excreta identified up to 84.8% of the administered dose. Pydiflumetofen was extensively metabolized, and all metabolites composing greater than 4.8% of the dose were identified. There were 35 unknown metabolites observed, each accounting for less than 4.8% of the dose. In general, the major metabolites present were qualitatively and quantitatively similar, irrespective of dose and sex.

At 5 mg/kg bw, unchanged parent compound accounted for less than 3.9% of the dose in excreta. At higher doses (100 or 300 mg/kg bw), unchanged pydiflumetofen was the major component recovered, at up to 48.2% of the administered dose, almost all of which was in the faeces (reflecting the dose-limited bioavailability at higher doses). Based on the high bioavailability of radiolabel and the low proportion of circulating radiolabel identified as parent pydiflumetofen, a high level of first-pass metabolism was considered likely.

Only two metabolites (2,4,6-TCP sulfate and SYN548263) were present at more than 10% of the administered dose. Numerous other cleavage products, and others that retained both the phenyl and pyrazole ring moieties, were identified. Cleavage of pydiflumetofen occurred following hydroxylation of the carbon adjacent to the trichlorophenyl ring to give SYN547948, which was then cleaved to yield 2,4,6-TCP and SYN548263. Subsequently, 2,4,6-TCP was sulfated, and this moiety accounted for the largest proportion of the administered dose, at up to 14.9%.

The metabolites identified in the urine and faeces of rats are shown in Tables 9 and 10.

Table 9. Metabolite profile in urine and faeces after administration of a single oral dose of 5 mg/kg bw [phenyl-U-^{14}C]pydiflumetofen or [pyrazole-U-^{14}C]pydiflumetofen to rats

	% of administered dose					
	Males			Females		
Compounds	**Urine[a]**	**Faeces[b]**	**Total excreta**	**Urine[a]**	**Faeces[b]**	**Total excreta**
[phenyl-U-^{14}C]Pydiflumetofen						
Pydiflumetofen	ND	2.2	2.2	ND	3.9	3.9
TCPM glucuronide	1.7	ND	1.7	ND	ND	ND
Dihydroxy pydiflumetofen	ND	4.4	4.4	0.2	2.0	2.2
Dechlorinated dihydroxy thiomethyl pydiflumetofen	ND	ND	ND	ND	1.9	1.9
Desmethyl hydroxy pydiflumetofen[c]	ND	8.1	8.1	ND	5.0	5.0
Desmethyl hydroxy pydiflumetofen sulfate	0.1	ND	0.1	ND	ND	ND
2,4,6-TCP sulfate	14.9	ND	14.9	7.8	ND	7.8
SYN547948	ND	2.7	2.7	ND	ND	ND
Hydroxy pydiflumetofen[d]	ND	ND	ND	ND	4.6	4.6
SYN547891 glucuronide	ND	ND	ND	0.4	ND	0.4
Hydroxy SYN545547	ND	ND	ND	ND	2.1	2.1
2,4,6-TCP	4.0	ND	4.0	6.6	ND	6.6
SYN547894	ND	2.1	2.1	ND	3.4	3.4

Compounds	% of administered dose					
	Males			Females		
	Urine[a]	Faeces[b]	Total excreta	Urine[a]	Faeces[b]	Total excreta
SYN547897	ND	5.2	5.2	0.1	8.2	8.3
Hydroxy pydiflumetofen sulfate	ND	ND	ND	0.2	ND	0.2
Dechlorinated hydroxy thiomethyl pydiflumetofen	ND	1.9	1.9	ND	6.3	6.3
SYN547890	ND	2.3	2.3	ND	5.9	5.9
SYN545547	ND	1.3	1.3	ND	0.9	0.9
Unidentified components	0.8[e]	27.4[f]	28.2	3.2[g]	17.0[h]	20.2
Post-extraction solids	NA	13.1	13.1	NA	11.0	11.0
Total identified	20.7	30.2	50.9	15.3	44.0	59.3
Total unidentified	0.8	27.4	28.2	3.2	17.0	20.2
Total accounted for	21.5	70.7	92.2	18.5	72.0	90.5
Losses/gains	0.0	2.6	2.6	-0.1	3.8	3.7
Total	21.5	73.3	94.8	18.4	75.8	94.2
LOQ	0.3	1.8	NA	0.3	1.8	NA
[pyrazole-U-^{14}C]Pydiflumetofen						
Pydiflumetofen	ND	2.6	2.6	ND	3.1	3.1
SYN548264	2.2	ND	2.2	0.7	ND	0.7
Desmethyl SYN548265 glucuronide	0.4	ND	0.4	0.5	ND	0.5
Desmethyl SYN548263	2.0	ND	2.0	1.3	ND	1.3
SYN548265 glucuronide	1.4	ND	1.4	1.4	ND	1.4
SYN548263	8.9	5.1	14.0	4.3	ND	4.3
SYN548265	0.2	ND	0.2	0.1	ND	0.1
Dihydroxy pydiflumetofen	ND	6.0	6.0	ND	1.4	1.4
Dechlorinated dihydroxy thiomethyl pydiflumetofen	ND	ND	ND	ND	1.0	1.0
Desmethyl hydroxy pydiflumetofen[i]	ND	2.1	2.1	ND	2.7	2.7
Desmethyl hydroxy pydiflumetofen sulfate	0.5	ND	0.5	ND	ND	ND
Dechlorinated dihydroxy pydiflumetofen	ND	ND	ND	ND	1.9	1.9
SYN547948	ND	ND	ND	ND	2.5	2.5
Hydroxy SYN547891	ND	2.6	2.6	ND	ND	ND
SYN547891 glucuronide	0.1	ND	0.1	ND	ND	ND
Hydroxy pydiflumetofen	ND	1.6	1.6	ND	0.6	0.6
SYN547894	ND	1.4	1.4	ND	3.9	3.9
SYN547897	ND	3.0	3.0	ND	4.8	4.8
Dechlorinated hydroxy thiomethyl pydiflumetofen	ND	1.4	1.4	ND	5.9	5.9

	% of administered dose					
	Males			Females		
Compounds	Urine[a]	Faeces[b]	Total excreta	Urine[a]	Faeces[b]	Total excreta
SYN547890	ND	2.5	2.5	ND	4.4	4.4
SYN545547	ND	0.7	0.7	ND	ND	ND
Unidentified components	10.3[j]	21.7[k]	32.0	10.0[l]	24.8[m]	34.8
Post-extraction solids	NA	11.8	11.8	NA	10.2	10.2
Total identified	15.7	29.0	44.7	8.3	32.2	40.5
Total unidentified	10.3	21.7	32.0	10.0	24.8	34.8
Total accounted for	26.0	62.5	88.5	18.3	67.2	85.5
Losses/gains	0.1	4.6	4.7	0.0	2.7	2.7
Total	26.1	67.1	93.2	18.3	69.9	88.2
LOQ	0.2	1.4	NA	0.3	1.7	NA

bw: body weight; LOQ: limit of quantification; NA: not applicable; ND: not detected; TCP: trichlorophenol; TCPM: trichlorophenol methanol

[a] 0–72 hours.

[b] 0–96 hours.

[c] Aggregate of two retention times for desmethyl hydroxy pydiflumetofen.

[d] Aggregate of two retention times for hydroxy pydiflumetofen.

[e] A total of six unidentified components, with no individual component accounting for greater than 0.3% of the dose.

[f] A total of 19 unidentified components, one of which accounted for 3.2% of the dose and another for 2.7% of the dose, with no other individual component accounting for greater than 2.0% of the dose.

[g] A total of 27 unidentified components, with no individual component accounting for greater than 0.4% of the dose.

[h] A total of 14 unidentified components, one of which accounted for 2.7% of the dose and another for 2.5% of the dose, with no other individual component accounting for greater than 1.9% of the dose.

[i] Aggregate of two retention times for desmethyl hydroxy pydiflumetofen.

[j] A total of 24 unidentified components, one of which accounted for 4.4% of the dose and another for 1.1% of the dose, with no other individual component accounting for greater than 0.5% of the dose.

[k] A total of 23 unidentified components, with no individual component accounting for greater than 1.8% of the dose.

[l] A total of 35 unidentified components, one of which accounted for 2.8%, with no other individual component accounting for greater than 0.6% of the dose.

[m] A total of 31 unidentified components, one of which accounted for 2.3% of the dose and another two for 1.8% of the dose, with no other individual component accounting for greater than 1.3% of the dose.

Source: MacDonald & Jewkes (2015)

Table 10. Metabolite profile in plasma after administration of a single oral dose of [phenyl-U-^{14}C]pydiflumetofen to rats

	% of AUC[a]			
	Males		Females	
Compound	5 mg/kg bw	300 mg/kg bw	5 mg/kg bw	100 mg/kg bw
Pydiflumetofen	1.9	1.3	2.8	5.0
Dihydroxy pydiflumetofen glucuronide	ND	0.9	1.4	1.0
HTCP sulfate	6.1	4.8	9.3	9.2
TCPM glucuronide	3.4	3.4	0.9	2.4
Dechlorinated dihydroxy pydiflumetofen glucuronide[b]	2.2	1.9	1.2	0.9

Compound	% of AUC[a]			
	Males		Females	
	5 mg/kg bw	300 mg/kg bw	5 mg/kg bw	100 mg/kg bw
Hydroxy pydiflumetofen glucuronide	1.0	3.1	1.6	1.0
2,4,6-TCP sulfate	41.1	44.1	41.0	32.2
SYN547948	ND	ND	2.5	3.6
Desmethyl hydroxy pydiflumetofen sulfate	ND	1.8	ND	ND
SYN547891 glucuronide	2.7	1.8	3.6	1.8
2,4,6-TCP	4.3	2.4	5.2	5.3
SYN547897	0.8	1.5	4.3	2.5
Unidentified components	11.3[c]	4.7[d]	10.9[e]	8.3[f]
Additional extracts	NA	<LOQ	NA	<LOQ
Post-extraction solids	18.8	18.3	13.1	12.5
Total identified	63.5	67.0	73.8	64.9
Total unidentified	11.3	4.7	10.9	8.3
Total accounted for	93.6	90.0	97.8	85.7
Losses/gains	6.4	10.0	2.2	14.3
Total	100.0	100.0	100.0	100.0
LOQ	0.8	1.2	0.9	1.2

AUC: area under the concentration–time curve; bw: body weight; HTCP: hydroxylated 2,4,6-trichlorophenol; LOQ: limit of quantification; NA: not applicable; ND: not detected; TCP: trichlorophenol

[a] 0–96 hours.

[b] Values aggregated for dechlorinated dihydroxy pydiflumetofen glucuronide from different retention times.

[c] A total of eight unidentified components, with no individual component accounting for greater than 2.5% of the AUC.

[d] A total of five unidentified components, with no individual component accounting for greater than 1.7% of the AUC.

[e] A total of 11 unidentified components, with no individual component accounting for greater than 2.0% of the AUC.

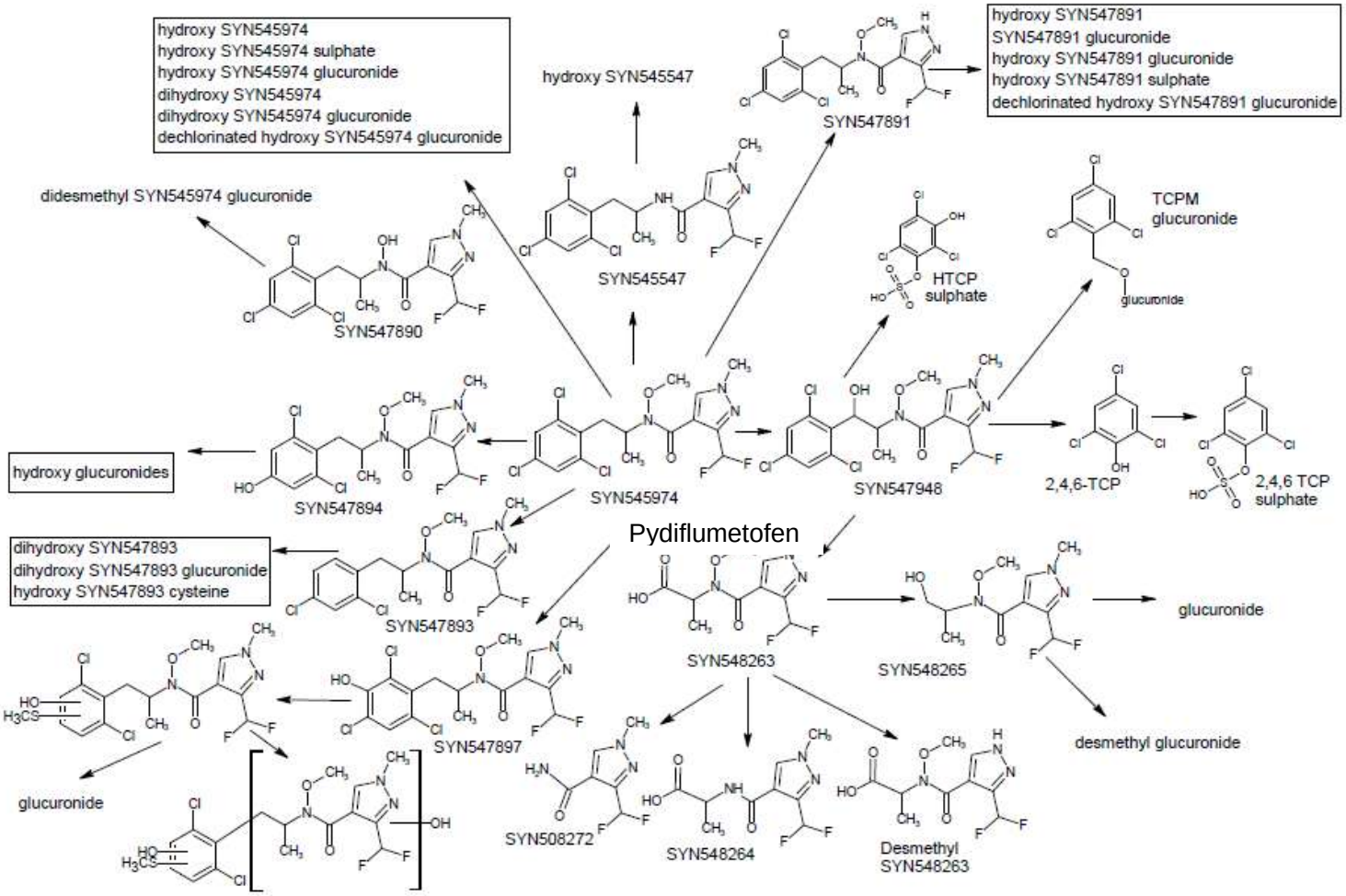
[f] A total of 10 unidentified components, with no individual component accounting for greater than 1.9% of the AUC.

Source: MacDonald & Jewkes (2015)

The proposed metabolic pathway of pydiflumetofen in rats is shown in Fig. 4.

Overall, the biotransformation proceeded by hydroxylation to SYN547897, SYN547948 and other hydroxylated and dihydroxylated isomers, demethylation to SYN547890 and *N*-desmethyl SYN547890, demethoxylation to SYN545547, followed by subsequent hydroxylation to hydroxy SYN545547, hydroxylation and demethylation to hydroxy SYN547891 and other desmethyl hydroxy metabolites, cleavage of pydiflumetofen to give the pyrazole-derived metabolites SYN548265, SYN548263, SYN548264, desmethyl SYN548263 and SYN508272 and the phenyl-derived metabolite 2,4,6-TCP, dechlorination to SYN547893, dechlorination and hydroxylation to SYN547894 and other dechlorinated hydroxyl and dechlorinated dihydroxy metabolites, glutathione conjugation followed by metabolism of the conjugate to give dechlorinated hydroxy thiomethyl SYN545974 and dechlorinated dihydroxy thiomethyl SYN545974, and glucuronic acid conjugation and some sulfate conjugation (MacDonald & Jewkes, 2015).

Fig. 4. Biotransformation pathway for pydiflumetofen in rats



Source: MacDonald & Jewkes (2015)

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of pydiflumetofen is summarized in Table 11.

Table 11. Summary of acute toxicity of pydiflumetofen

Study (species)	Purity (%); lot/batch no.	Result	Reference
Oral (rats)	98.5; SMU2EP12007	LD_{50} > 5 000 mg/kg bw	Petus-Árpásy (2012)
Dermal (rats)	98.5; SMU2EP12007	LD_{50} > 5 000 mg/kg bw	Petus-Árpásy (2013)
Inhalation (rats)	98.5; SMU2EP12007	LC_{50} > 5.11 mg/L	Nagy (2013)
Skin irritation (rabbits)	98.5; SMU2EP12007	Not irritating	Török-Bathó (2012a)
Eye irritation (rabbits)	98.5; SMU2EP12007	Mildly irritating	Török-Bathó (2012b)
Skin sensitization (LLNA) (mice)	98.5; SMU2EP12007	Not sensitizing	Hargitai (2013)

bw: body weight; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; LLNA: local lymph node assay

(a) Lethal doses

The oral median lethal dose (LD_{50}) for pydiflumetofen (purity 98.5%) using carboxymethylcellulose (0.5% v/v) as the vehicle in female CRL:(WI) Wistar rats was greater than 5000 mg/kg bw (limit dose). No deaths occurred at 5000 mg/kg bw, and no significant clinical observations or macroscopic findings were noted (Petus-Árpásy, 2012).

The dermal LD_{50} for pydiflumetofen (purity 98.5%) using tap water as the vehicle in male and female CRL:(WI) Wistar rats was studied by application of pydiflumetofen to a shaved area representing 10% of the body surface. The shaved area was then covered with a gauze pad, and the entire trunk was wrapped in a semi-occlusive plastic wrap for 24 hours. No deaths occurred at 5000 mg/kg bw (limit dose). Clinical signs were limited to decreased activity in all animals on day 1 after treatment. There were no treatment-related effects on body weight or body weight gain (Petus-Árpásy, 2013).

The inhalation median lethal concentration (LC_{50}) for pydiflumetofen (purity 98.5%) was greater than 5.11 mg/L (mass median aerodynamic diameter 3.54 µm ± 2.32 geometric standard deviation) in air for male and female CRL:(WI) Wistar rats after a 4-hour nose-only exposure. One death occurred in a group of five female rats after exposure on day 0. Clinical signs included wet fur, puffed coat and fur staining on the day of treatment and for several days after treatment. In addition, animals showed signs of laboured, gasping and noisy respiration, sneezing, decreased activity, prostration and ataxia on the day of exposure. Signs of noisy respiration or weakened condition were noted in some animals in the first 2 days after exposure. There were no significant effects on body weight during the 14-day observation period (Nagy, 2013).

(b) Dermal irritation

The skin irritating potential of pydiflumetofen (purity 98.5%) was investigated in three male New Zealand white rabbits. Pydiflumetofen was applied as an undiluted powder to intact clipped skin on the back and flanks of each rabbit. Test substance (0.5 mg) was applied to the skin under a patch of gauze (2.5 cm × 2.5 cm) that was held in place with adhesive bandage, and the entire trunk was wrapped in plastic wrap and elastic stocking for 4 hours. Treated areas were washed with water and scored at 24,

48 and 72 hours after removal of the patch. Under the conditions of the test, there was no evidence of skin irritation by pydiflumetofen (Török-Bathó, 2012a).

(c) Ocular irritation

The eye irritating potential of pydiflumetofen (purity 98.5%) was investigated in three male New Zealand white rabbits. Pydiflumetofen (0.1 g) was applied as an undiluted powder to one eye of each rabbit, while the other eye served as the control. Eyes were rinsed with saline and scored at 1, 24, 48 and 72 hours post-treatment. All animals demonstrated a slight initial pain reaction on instillation of pydiflumetofen. There was a slight discharge in one rabbit and conjunctival redness in all rabbits at 1 hour. At 24 and 48 hours, conjunctival redness was noted in only one rabbit. Fluorescein staining was negative in all animals at all time points. There were no additional clinical signs or effects on body weight. As all clinical signs had reversed by 72 hours, the study was subsequently terminated. Under the conditions of the test, pydiflumetofen was considered mildly irritating to the eyes of rabbits (Török-Bathó, 2012b).

(d) Dermal sensitization

Pydiflumetofen (purity 98.5%) was tested for dermal sensitization potential using the local lymph node assay in female CBA/JRj mice. Pydiflumetofen (50%, 25% or 25% w/v) was applied to the dorsal surface of the animals' ears (25 µL/ear) for 3 consecutive days using an acetone:olive oil (4:1 v/v) vehicle. Five hours prior to termination on day 6, tritiated methyl thymidine was injected via the tail vein. Cell proliferation in the local lymph nodes was measured by incorporation of the tritiated methyl thymidine. There were no deaths, signs of systemic toxicity or effects on body weight during the study. In addition, there were no signs of irritation at the site of application. Precipitate of the test substance was noted on the ears of animals treated with either 50% or 25%. The appearance of the lymph nodes was normal in all pydiflumetofen-treated animals compared with positive controls (25% w/v α-hexylcinnamaldehyde). There were no significant increases in the stimulation index for pydiflumetofen in this study. Under the conditions of the test, pydiflumetofen was not considered to cause skin sensitization (Hargitai, 2013).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 28-day toxicity study, CD-1 mice (six of each sex per group) were given pydiflumetofen (lot/batch no. LOT2491-DC/110; purity 98.6%) in the diet at a concentration of 0, 500, 1500, 4000 or 7000 parts per million (ppm) (equal to 0, 76, 213, 612 and 1115 mg/kg bw per day for males and 0, 96, 266, 701 and 1312 mg/kg bw per day for females, respectively). Satellite groups (six of each sex) that received 0 or 7000 ppm (equal to 925 and 1132 mg/kg bw per day for males and females, respectively, euthanized on day 4; and 1053 and 1595 mg/kg bw per day for males and females, respectively, euthanized on day 8) were euthanized on days 4 and 8 for the analysis of plasma levels of pydiflumetofen and cell proliferation activity in the liver. The results of the latter analysis (Haines, 2012) are described in section 2.6(d) below.

There were no treatment-related effects on mortality, clinical signs, feed consumption, haematology, gross pathology or histopathology. Body weight gains were lower in all treated male groups over the first 11 days, resulting in lower terminal body weights. Feed consumption values, however, were highly variable and were not a clear indication as to the cause of the lower body weight gains. The relationship between dose and terminal body weight was weak, however, except at the highest dose, and the relationship with treatment at 500 and 1500 ppm is questionable, with the terminal body weight at 1500 ppm comparable to control values. In the absence of any other toxicologically relevant findings at the lower doses and the absence of similar effects in females, the apparent reduction

in body weight gains at doses less than 4000 ppm was considered to be of uncertain relationship to treatment. Triglycerides were higher (2.54 mmol/L vs 1.18 mmol/L in controls) and phosphates were lower (1.56 mmol/L vs 2.31 mmol/L in controls) in males at 7000 ppm. Although there were only three animals per group used for clinical chemistry, all values for triglycerides at 7000 ppm were higher than the highest control value. Absolute liver weights were increased in both sexes at 7000 ppm on days 4 and 8 and in all treated groups at termination. Toxicokinetic analysis was unreliable and unusable, with blood levels of pydiflumetofen in some control animals similar to or higher than those in animals dosed at 500 or 1500 ppm.

The no-observed-adverse-effect level (NOAEL) was 4000 ppm (equal to 612 mg/kg bw per day), based on reduced body weight gain, an increase in absolute liver weights and an increase in serum triglyceride levels at 7000 ppm (equal to 1115 mg/kg bw per day) (Strepka, 2012a).

In a 91-day toxicity study, 10 Crl:CD-1(ICR) mice of each sex per group were administered pydiflumetofen (lot/batch no. 2637-BA/110; purity 99.5%) in the diet at a concentration of 0, 100, 500, 4000 or 7000 ppm (equal to 0, 17.5, 81.6, 630 and 1158 mg/kg bw per day for males and 0, 20.4, 106, 846 and 1483 mg/kg bw per day for females, respectively). In addition to test guideline parameters, a detailed functional observational battery (FOB) study (including motor activity) was undertaken pretrial and at week 12. Whole blood concentrations of pydiflumetofen were analysed from samples taken at various time points on days 2, 16, 30 and 91. Histopathological examinations were conducted only for mice in the 0 and 7000 ppm groups, except for the liver, which was also examined in the 100, 500 and 4000 ppm groups.

There were no effects of treatment on mortality, clinical signs, feed consumption and feed utilization, water intake, haematology or gross pathology. Dose-related and consistent changes in body weight gain were not observed in either sex. In the blood, levels of cholesterol in males at 4000 ppm and in both sexes at 7000 ppm and levels of triglyceride in both sexes at 7000 ppm were statistically significantly increased. Slight (approximately 10%) increases in globulin levels in males of all treated groups were not considered treatment related due to a lack of dose–response relationship. A slight (approximately 15%) decrease in glucose levels in females in the higher dose groups was not considered toxicologically relevant, as the decreases were within the historical control range (11.09 ± 0.62 mmol/L, mean ± standard deviation) for the testing laboratory (Shearer & Robertson, 2016). Absolute and relative (to body weight) liver weights were increased at 500 ppm and above in males and at 4000 ppm and above in females. These treatment-related changes in the liver correlated with an increased incidence of hepatocyte hypertrophy (minimal) in males and females. Liver hypertrophy in males at 500 ppm was considered adaptive, as there were no findings indicating liver damage in this group.

The key results are summarized in Table 12.

Table 12. Summary of findings from the 91-day toxicity study of pydiflumetofen in mice

	Males					**Females**				
Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**4 000 ppm**	**7 000 ppm**	**0 ppm**	**100 ppm**	**500 ppm**	**4 000 ppm**	**7 000 ppm**
Blood biochemistry										
Cholesterol	3.9	4.1	4.8	4.9*	5.9**	2.8	2.6	2.7	3.6	3.8**
Triglycerides	1.50	1.26	1.46	1.70	2.79**	1.13	1.14	0.90	1.72	1.77*
Globulin	19	21*	22**	21*	22**	17	17	17	18	18
Glucose	12.3	12.33	12.29	11.88	11.30	12.86	12.30	11.93	10.89*	10.88*
Liver weight										
Absolute (g)	1.94	1.96	2.29*	2.76**	3.24**	1.63	1.64	1.66	2.60**	2.51**

Parameter	Males					Females				
	0 ppm	100 ppm	500 ppm	4 000 ppm	7 000 ppm	0 ppm	100 ppm	500 ppm	4 000 ppm	7 000 ppm
Covariate (g)	1.90	1.99	2.18*	2.82**	3.32**	1.58	1.61	1.71	2.56**	2.59**
Relative (%)[a]	4.7	4.9	5.3	7.0	8.3	5.0	5.1	5.4	8.0	8.4
Histopathology										
No. of mice examined	10	10	10	10	10	10	10	10	10	10
Hepatocellular hypertrophy	0	0	2	4	5*	0	0	0	6*	7**

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Relative to body weight. No statistical analyses performed on relative liver weights.

Source: Shearer (2015)

Concentrations of pydiflumetofen in blood were generally below the LOQ (5 ng/mL) in control animals, but values were above the LOQ in 18/160 samples taken from control animals. In general, females had substantially higher levels in blood than males at each dose (see Appendix 3).

The NOAEL was 500 ppm (equal to 81.6 mg/kg bw per day), based on increased cholesterol concentrations associated with liver hypertrophy at 4000 ppm (equal to 630 mg/kg bw per day) (Shearer, 2015).

Rats

In a 28-day toxicity study, six Han Wistar rats of each sex per group were administered pydiflumetofen (lot/batch no. 2491-DC/110; purity 98.6%) in the diet at a concentration of 0, 500, 4000, 8000 or 16 000 ppm (equal to 0, 43, 343, 677 and 1322 mg/kg bw per day for males and 0, 40, 322, 619 and 1174 mg/kg bw per day for females, respectively). An additional six rats of each sex for the control and high-dose groups provided satellite groups for termination on days 3 and 7. Satellite groups were subjected to necropsy, gross pathology and organ weight measurement, but not histology. Toxicokinetics was investigated using blood samples taken from the main group on days 4 and 28 of the study, and cell proliferation studies were conducted on satellite animals but not reported. At necropsy, tissues from animals in the control and high-dose groups were examined histologically, and the liver was examined in all groups except females at 500 ppm. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as the covariate, but group mean organ to body weight ratios (relative organ weights) were reported without any statistical analysis.

There were no treatment-related effects on mortality, clinical signs, water consumption (visual estimation only) or gross pathology. Body weight was lower in both sexes at 16 000 ppm. For females, the lower body weight was due to an initial body weight loss on the first day, which took until day 7 to return to pretreatment levels. The reduced body weight gain was associated with a marked reduction in feed consumption in both sexes over the first 1 or 2 days (in males, approximately 50% reduction at 8000 and 16 000 ppm; in females, approximately 40–75% reduction without dose dependency at 4000 ppm and above on day 1, and 50% reduction at 16 000 ppm on day 2). The lower feed consumption in this early phase was suggested by the study author to be related to lower palatability and may have caused the lower body weight gain, rather than systemic toxicity per se. However, the slight (approximately 10%), but consistent, reduction in body weight gain throughout the study period in males at 16 000 ppm was considered adverse.

Lower white blood cell counts (5.95×10^9/L, 8.32×10^9/L, 7.06×10^9/L and 7.54×10^9/L vs 9.2×10^9/L in controls) and lymphocyte counts (4.62×10^9/L, 6.94×10^9/L, 5.52×10^9/L and 6.24×10^9/L vs 7.65×10^9/L in controls) in all treated groups of males were not considered treatment related owing to a lack of dose dependency and reproducibility in 90-day and long-term studies in rats. In

females, a substantially lower serum glutamate dehydrogenase level in all treated groups (4.0, 3.9, 3.0 and 3.0 IU/L vs 9.6 IU/L in controls) was not considered toxicologically relevant owing to the decrease, not increase, in magnitude and lack of change in related parameters. A lower glucose level was observed in all treated female groups, which was statistically significant but without a dose–response relationship across a 32-fold dose range, and which was within the historical control range for the testing laboratory. Slightly, but statistically significantly, lower alanine aminotransferase (ALT) activity in females at 8000 and 16 000 ppm (29 and 28 IU/mL vs 40 IU/mL in controls) was unlikely to be adverse in isolation. Higher urinary volumes in females at 4000 ppm and higher were observed, with a correspondingly slightly lower specific gravity. There was no real dose–response relationship for specific gravity, and the increase in volume was not statistically significant at any dose, owing to high variability in individual values.

Covariate liver weights (liver weight adjusted for covariance to terminal body weight) were increased in males at 4000 ppm and higher and in females at 500 ppm and higher (10.89, 11.47, 13.31, 14.25 and 14.64 g for males; 6.50, 7.36, 8.34, 8.68 and 9.160 g for females; at 0, 500, 4000, 8000 and 16 000 ppm, respectively). In the satellite group (0 and 16 000 ppm) for cell proliferation activity, covariate liver weights were increased statistically significantly on both examined days (approximately 20% and 30% increases on days 4 and 8, respectively).

Treatment-related findings were confined to minimal centrilobular hypertrophy in males at 4000 ppm and above and in females at 8000 ppm and above (0, 0, 4, 5 and 6 in males at 0, 500, 4000, 8000 and 16 000 ppm, respectively; and 0, 0, 3 and 5 in females at 0, 4000, 8000 and 16 000 ppm, respectively). Minimal inflammatory cell foci were scattered in all dose groups including controls in both sexes. Their incidence increased in males at 8000 and 16 000 ppm (incidence: 1, 1, 1, 3 and 3 at 0, 500, 4000, 8000 and 16 000 ppm, respectively). However, this finding was not related to hepatocellular hypertrophy except at 16 000 ppm, and inflammatory changes in the liver were not noted in either the 91-day or chronic toxicity study in rats. Therefore, this finding was not considered treatment related. As no findings indicated liver damage in the treated groups, the liver hypertrophy observed at 500 ppm and above was considered adaptive.

The toxicokinetics data appear to be unreliable. Pydiflumetofen was detected in both control and treated groups, values were highly variable across individuals in each group and there was not a relationship between levels in blood and dose. The study report does not discuss the source of the errors/inconsistencies. Regardless of the source of the errors in this study, the toxicokinetics from this study are unusable.

The NOAEL was 8000 ppm (equal to 619 mg/kg bw per day), based on reduction of body weight gain at 16 000 ppm (equal to 1174 mg/kg bw per day) (Strepka, 2012b).

In a 91-day toxicity study, Crl:WI(Han) rats (10 of each sex per dose) were administered pydiflumetofen (lot/batch no. 2637-BA/110; purity 99.5%) in the diet at a concentration of 0, 250, 1500, 8000 or 16 000 ppm (equal to 0, 18.6, 111, 587 and 1187 mg/kg bw per day for males and 0, 21.6, 127, 727 and 1325 mg/kg bw per day for females, respectively). In addition to test guideline parameters, a detailed FOB, including motor activity and body temperature, was conducted pretest and at week 12. Blood samples (50 µL) were collected on days 2, 9, 28 and 91 from all animals to determine concentrations of pydiflumetofen in whole blood. Histopathological examination was conducted on only the control and high-dose animals, except for the liver and thyroid glands, which were also examined at 250 and 1500 ppm. The left lateral lobes of the liver were placed in separate RNA-free tubes for analysis of microsomal protein and uridine diphosphate (UDP) glucuronosyltransferase (UGT) activity, which was conducted as a separate study from the present study and is described in section 2.6(d).

The results are summarized in Table 13. There were no treatment-related effects on mortality, clinical signs, ophthalmoscopy, FOB, body temperature, pathology, haematology, coagulation rate or urine analysis. Body weights in males at 16 000 ppm were statistically significantly and consistently

Table 13. Summary of findings from the 91-day toxicity study of pydiflumetofen in rats

	Males					Females				
Parameter	**0 ppm**	**250 ppm**	**1 500 ppm**	**8 000 ppm**	**16 000 ppm**	**0 ppm**	**250 ppm**	**1 500 ppm**	**8 000 ppm**	**16 000 ppm**
Body weight (g)										
Day 0	246	242	248	244	242	161	160	160	163	172
Day 1	249	247	249	234	232*	165	163	161	156	161
Day 4	263	261	265	250	246*	170	171	169	166	172
Day 7	279	278	279	264	259*	181	182	177	176	181
Day 14	304	300	301	286	280*	194	193	189	187	192
Day 28	344	337	336	313	302**	207	206	203	199	207
Day 56	393	380	386	359	347*	229	227	223	216	228
Day 91	434	418	419	382*	368**	237	238	230	223	229
Feed consumption (g/day)										
Day 0	22.4	22.4	23.4	20.4	22.6	15.7	13.7	15.6	19.1	16.7
Day 1	23.3	22.9	18.5*	11.0**	11.3**	16.2	14.2	15.8	9.9*	7.2**
Day 2	20.8	19.9	20.5	13.1**	12.6**	14.6	14.8	11.9	15.3	9.0
Day 3	22.9	23.0	23.4	22.7	22.1	17.7	15.5	14.5	10.5*	11.1*
Day 91	23.6	23.9	23.0	21.3	22.0	17.7	16.9	16.9	18.1	17.3
Blood biochemistry										
ALP (U/L)	93	89	65**	64**	57**	59	45	35**	34**	36**
Chol (mmol/L)	2.0	2.1	2.3	1.9	1.9	1.7	1.7	2.0	2.3**	2.3**
Liver weight (g)										
Absolute	14.51	14.39	17.57*	17.80**	18.30**	7.63	8.19	8.85**	10.38**	10.71**
Covariate	13.07	13.79	16.85**	18.81**	19.90**	7.52	8.04	8.87**	10.58**	10.75**
Histopathology										
No. of rats examined	10	10	10	10	10	10	10	10	10	10
Hepatocellular hypertrophy minimal	0	0	5*	7**	10**	0	0	0	6*	9**
Thyroid, follicular cell hypertrophy	0	0	4	6*	7**	0	0	0	4*	8**

ALP: alkaline phosphatase; Chol: cholesterol; ppm: parts per million; U: units; *: $P < 0.05$; **: $P < 0.01$
Source: Shearer & Robertson (2015)

lower than control values throughout the study period. Body weights in males at 8000 ppm were slightly (within approximately 10%), but consistently, lower after 1 month of treatment. Body weight gain was reduced in both sexes at 8000 and 16 000 ppm, associated with reduced feed consumption over the first 2–3 days of treatment. Body weight gain was also lower in males at 1500 ppm, but did not reach statistical significance. Examination of the individual animal data indicates that four of the 10 animals at 1500 ppm had body weight gains lower than or equal to the lowest body weight gain in control

animals, and there was a clear shift in values downwards. The lower body weight gain was associated with reduced feed utilization and consistent with the overall dose–response relationship. Transient reduced body weight gains on the first 2 days in males at 1500 ppm and higher and in females at 8000 ppm and higher were related to lower feed consumption, suggesting lower palatability in this period. No statistically significant changes in body weight were found in males at 1500 ppm or in females in all treated groups. Therefore, the transient changes in body weight gains in males at 1500 ppm were not considered treatment related.

Compared with controls, alkaline phosphatase (ALP) activity was reduced in both sexes at 1500, 8000 and 16 000 ppm, and cholesterol was higher in females at 8000 and 16 000 ppm. A decrease in the magnitude of ALP is generally accepted as having no toxicological relevance. In addition, there were no corresponding findings in the hepatobiliary system, gastrointestinal tract or bone marrow. Therefore, the lower ALP levels in both sexes at 1500 ppm and above were not considered adverse. In urine analysis, increases in volume accompanied by lower pH were observed in both sexes at 8000 ppm and above. As no other change indicating renal toxicity was observed in these dose groups, these changes were not considered to be toxicologically relevant.

Treatment-related changes in liver and thyroid weights and histopathological observations were found. A dose-related increase in liver weight was observed in both sexes at 1500 ppm and above. Incidences of minimal hepatocyte hypertrophy and minimal to mild follicular cell hypertrophy in thyroid were increased in males at 1500 ppm and above and in females at 8000 ppm and above. The liver findings were associated with a corresponding increase in liver weight, except in females at 1500 ppm. The increase in liver weight in females at 1500 ppm was considered adaptive, as there were no findings related to hepatotoxicity or thyroid effects.

With the exception of some isolated observations of low levels of pydiflumetofen in blood from control animals, these values were generally below the LOQ (5.0 ng/mL). A saturation of absorption appears to occur above 1500 ppm in both sexes, with no or only slight increases in levels in blood at higher doses. In general, females had substantially higher levels in blood than males at each dose (see Appendix 3).

The NOAEL was 250 ppm (equal to 18.6 mg/kg bw per day), based on follicular cell hypertrophy in the thyroid at 1500 ppm (equal to 111 mg/kg bw per day) (Shearer & Robertson, 2015).

Dogs

In a 28-day toxicity study, a single beagle dog of each sex per dose was administered pydiflumetofen (lot/batch no. 2637AA/110; purity 99.3%) by oral capsule at a dose of 0, 100, 300 or 1000 mg/kg bw per day for 28 days. Animals were monitored daily for mortality, clinical signs and feed consumption. Ophthalmoscopy, clinical chemistry, haematology and urine analysis were conducted. Concentrations of pydiflumetofen in blood were determined on days 1 and 28 (LOQ < 5.0 ng/mL). All animals were necropsied after 28 days of treatment, selected organs were weighed, and the liver and any gross lesions were examined for histopathology.

There were no effects of treatment on mortality, clinical signs, body weight gain, feed consumption, ophthalmoscopy, gross pathology, haematology, coagulation, urine analysis parameters or liver histopathology. A clear trend for increased ALP was observed in both sexes at all doses. Relative liver weight showed a clear increase at 300 mg/kg bw per day and above, although no liver histopathology was observed. Pydiflumetofen concentrations in blood increased with increasing dose, although peak levels were slightly less than dose proportional (Strepka & Shearer, 2015).

In a 13-week toxicity study, beagle dogs (four of each sex per dose) were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in capsules at a dose of 0, 30, 300 or 1000 mg/kg bw per day. In addition to test guideline parameters, a weekly FOB was conducted. Toxicokinetics was investigated on days 1 and 28 and during week 13, with blood samples collected

predosing and 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours after dosing in all animals, except in control animals, from which only predosing samples were taken.

There were no treatment-related effects on mortality, clinical signs, ophthalmoscopy, organ weights, gross pathology or histopathology (except liver), haematology or urine analysis. At 1000 mg/kg bw per day, two of the four males and all females lost body weight during the initial weeks of dosing, with some taking several weeks to regain this lost body weight and others, most notably females, continuing to show erratic body weight change. Body weight gain was lower for the female group at this dose compared with controls, and their final body weights were notably lower than control values. There was no significant difference in overall body weight gain or final body weight for the male group. There was a corresponding effect on feed consumption in males and females at 1000 mg/kg bw per day. At 300 mg/kg bw per day, one male and two females showed slight body weight losses during the first 2 weeks of the treatment period, but thereafter gained body weight normally. In the absence of a consistent and overall effect on body weight and body weight gain, this was considered not adverse.

Plasma ALP and triglycerides were increased at 300 mg/kg bw per day and above in both sexes, associated with increased liver weights. However, ALT, aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) were unaffected. At 300 mg/kg bw per day, there were no indications of liver damage in the histopathology that might correlate with the increased ALP. In addition, no changes were found in bone marrow or adrenals, organs with the potential to affect plasma ALP in dogs. The slight, but continuous, increase in triglycerides in males at 1000 mg/kg bw per day was treatment related. Bilirubin was decreased in males at 300 mg/kg bw per day and higher and in females at 1000 mg/kg bw per day in week 13; however, individual values overlapped with those in the controls, and the relationship was inconsistent across the time points. In week 8, for example, the bilirubin levels were lower in males at 300 and 1000 mg/kg bw per day than in controls. Consequently, the apparent effect on bilirubin was concluded to be incidental.

The only treatment-related histopathological finding was minimal hepatocyte hypertrophy observed at 1000 mg/kg bw per day.

No adverse observations were made in the animals treated with 30 mg/kg bw per day.

Pydiflumetofen levels in blood (LOQ: 5.00 ng/mL) showed a supraproportional increase relative to the dose increments for both the AUC and C_{max}, but without a prolongation of half-life. In males, for example, the C_{max} on day 1 was 24.6, 832 and 3510 ng/mL when animals were given pydiflumetofen at a dose of 30, 300 or 1000 mg/kg bw per day, respectively; and on day 91, the C_{max} was 38.3, 638 and 2070 ng/mL, respectively. Similarly, in males, the $AUC_{0–24\ h}$ on day 1 was 85.3, 6710 and 31 400 ng·h/mL when animals were given pydiflumetofen at a dose of 30, 300 or 1000 mg/kg bw per day, respectively; and on day 91, the $AUC_{0–24\ h}$ was 191, 6270 and 17 400 ng·h/mL, respectively.

Animals that received 300 or 1000 mg/kg bw per day were continuously exposed over the 24-hour period after dosing, but at 30 mg/kg bw per day, exposure was limited to up to 8 hours after dosing. Exposure tended to be higher in males, most notably at 300 mg/kg bw per day. There was no evidence of accumulation in plasma. Absorption was relatively slow, with a T_{max} of the order of 4–12 hours at 300 and 1000 mg/kg bw per day, and the half-life was varied, between 2.6 and 4.9 hours in males and between 2.4 and 4.5 hours in females, with some (equivocal) indication of a shorter half-life after 90 days of treatment.

The results observed in the 13-week toxicity study in dogs are summarized in Table 14.

Although ALP was increased at 300 mg/kg bw per day, there was no alteration of liver histology at 300 mg/kg bw per day and only minimal hepatocyte hypertrophy at 1000 mg/kg bw per day, with no other compelling evidence of pathology in liver. On this basis, although the increase in ALP is clearly treatment related at 300 and 1000 mg/kg bw per day, it is concluded to be non-adverse.

The NOAEL was 300 mg/kg bw per day, based on reduced body weight gain and increased triglycerides at 1000 mg/kg bw per day (Blunt, 2015a).

Table 14. Summary of findings from the 13-week toxicity study of pydiflumetofen in dogs

	Males				Females			
Parameters	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Body weight (kg)								
Day −1	11.45	11.73	11.55	11.78	10.48	10.08	10.53	10.85
Day 7	11.50	11.93	11.55	11.68	10.55	10.20	10.45	10.48
Day 35	11.53	12.05	11.63	11.73	10.95	10.58	10.50	10.35
Day 63	11.78	12.43	11.85	12.03	11.43	11.18	11.10	10.60
Day 91	11.68	12.58	12.03	11.90	11.63	11.43	11.10	10.83
Weight gain (kg)								
Days −1 to 7	0.05	0.20	0.00	−0.10	0.08	0.13	−0.08	−0.38*
Days −1 to 14	0.15	0.20	−0.05	−0.10	0.18	0.23	−0.15**	−0.38*
Days −1 to 21	0.20	0.43	0.00	0.00	0.28	0.38	−0.13	−0.33*
Days −1 to 28	0.18	0.28	0.00	−0.05	0.40	0.45	−0.03	−0.45*
Days −1 to 35	0.08	0.33	0.08	−0.05	0.48	0.50	−0.03	−0.50*
Days −1 to 49	0.18	0.80	0.63	0.85	0.38	0.18	0.13	−0.28*
Days −1 to 56	0.23	0.83	0.38	0.85	0.05	0.30	−0.20	−0.33*
Days −1 to 63	0.33	0.95	0.70	1.08	0.30	0.45	0.25	−0.13*
Days −1 to 77	0.33	1.05	0.73	1.13	0.35	0.60	0.25	−0.55*
Days −1 to 91	0.23	1.15	0.85	1.35	0.48	0.58	0.13	−0.03**
ALP (U/L)								
−1 week								
Mean	73.5	81.5	89.3	71.5	100.5	83.8	94.5	88.5
SD	19.8	10.4	16.2	23.9	32.3	12.8	35.9	23.0
4 weeks								
Mean	71.5	85.8	251.8	349.0**	94.0	91.0	214.3	328.3*
SD	25.6	5.2	86.6	167.8	25.9	23.1	79.8	149.3
8 weeks								
Mean	74.0	86.0	286.8	379.0*	86.0	87.3	247.5	374.3*
SD	28.1	8.7	134.7	198.1	31.4	20.0	89.0	195.7
13 weeks								
Mean	68.5	70.0	252.3**	384.3**	77.8	76.8	236.5	327.8**
SD	24.0	6.3	132.9	203.8	26.7	17.0	99.7	193.9
Triglycerides (mg/dL)								
−1 week								
Mean	28.5	34.8	36.5	33.5	38.8	42.0	38.8	32.3

Parameters	Males				Females			
	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day
SD	3.1	8.6	9.5	8.8	7.1	1.4	5.0	2.9
4 weeks								
Mean	28.3	35.8	68.3*	96.8*	50.3	54.8	51.8	79.3
SD	1.7	5.7	20.0	23.5	12.3	14.9	7.7	28.3
8 weeks								
Mean	26.3	31.0	41.3	67.3*	44.5	58.0	54.3	71.0
SD	2.1	4.8	9.9	29.7	6.1	13.3	9.8	29.8
13 weeks								
Mean	30.3	29.0	51.0	74.0**	44.8	52.8	47.5	61.5
SD	6.2	5.2	10.9	37.4	9.4	21.9	8.1	3.1
Liver weight, covariate (g)								
Mean	375.7	382.7	492.9*	529.9**	356.6	355.9	414.3	522.0**
Histopathology								
Hypertrophy, hepatocyte, minimal	0/4[a]	0/4	0/4	4/4	0/4[a]	0/4	0/4	4/4

ALP: alkaline phosphatase; bw: body weight; SD: standard deviation; U: units; *: $P < 0.05$; **: $P < 0.01$

[a] The number of animals with the finding/the number of dogs examined.

Source: Blunt (2015a)

In a 1-year toxicity study, beagle dogs (four of each sex per dose) were administered pydiflumetofen (lot/batch no. SMU2EFP12007; purity 98.5%) in capsules at a dose of 0, 30, 100 or 300 mg/kg bw per day. In addition to test guideline parameters, a weekly FOB was conducted.

Although group mean body weights and body weight gains for the treated groups were generally lower than those in the concurrent controls, there was no dose–response relationship, and the greatest difference was in the 30 mg/kg bw per day group. Body weight gains in this group were substantially below control values, particularly over the first 3 weeks. Body weights were highly variable between individuals within groups, with the body weights of the control males at the end of the study ranging from 11.4 kg to 16.0 kg, for example. The body weight of the single heavy animal in the male controls (16 kg) was 3 kg higher than that of the next heaviest dog (13 kg) and significantly influenced the apparent between-group differences. Consequently, intermittent achievement of statistical significance between groups was concluded to reflect the large variation and small group sizes and was not an adverse effect of treatment.

Plasma ALP was increased in both sexes at 300 mg/kg bw per day at all time points throughout the study (e.g. males: 146.5 U/L vs 38.3 U/L in controls; females: 125.5 U/L vs 40.3 U/L in controls, at week 52). The ALP increases were dose related, but the levels fell with increased age. The increased ALP levels at 300 mg/kg bw per day, although potentially reflecting liver pathology, were not associated with increases in other liver enzymes, such as AST, ALT or GGT.

No statistically significant change was observed in organ weights except for an increased trend in liver weight at 300 mg/kg bw per day. The mean liver weights were increased at 300 mg/kg bw per day due primarily to substantially higher liver weights in one male and two females at this dose. Increased liver weights at 300 mg/kg bw per day were not associated with histological alterations.

Increases in absolute (with or without covariate) and relative thyroid weights were observed in both sexes at 300 mg/kg bw per day, with statistical significance in males only, and only the covariate thyroid weight was statistically significantly increased in males at 100 mg/kg bw per day. There were no corresponding histopathological findings in the thyroid in either sex in any treated dose groups. In the absence of any histological alterations, the toxicological relevance of this observation is doubtful.

The results are summarized in Table 15.

Table 15. Summary of findings from the 1-year toxicity study of pydiflumetofen in dogs

	Males				Females			
Parameters	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**100 mg/kg bw per day**	**300 mg/kg bw per day**	**0 mg/kg bw per day**	**30 mg/kg bw per day**	**100 mg/kg bw per day**	**300 mg/kg bw per day**
ALP (U/L)								
Week −1								
Mean	103.8	114.0	105.0	100.0	81.8	101.5	78.8	98.3
SD	17.0	40.9	15.4	12.8	4.6	40.8	23.6	18.9
Week 13								
Mean	56.8	98.8	104.8	200.5**	52.8	71.8	58.5	144.3*
SD	8.8	23.5	51.8	77.2	16.7	11.5	16.0	69.0
Week 26								
Mean	45.3	83.5	109.0	173.5*	43.8	63.8	55.5	133.5*
SD	11.3	19.9	67.8	64.5	18.2	15.4	22.9	70.7
Week 52								
Mean	38.3	66.5	92.3	146.5 **	40.3	65.0	61.3	125.5*
SD	11.2	20.7	54.6	64.5	27.2	9.6	26.6	75.2
Body weight (kg)								
Mean	13.3	10.9	11.8	12.2	10.4	8.6	10.0	9.4
SD	2.0	0.5	2.3	1.1	1.2	0.8	1.2	0.7
Liver weight								
Absolute (g)								
Mean	376.8	369.9	371.7	468.3	313.5	324.2	339.6	376.7
SD	70.4	37.8	54.0	98.7	30.0	92.8	47.0	48.6
Covariate (g)								
Mean	346.1	398.3	377.2	465.0*	297.8	345.2	330.7	380.4
Relative (%)[a]								
Mean	2.848	3.380	3.174	3.843	3.061	3.732	3.421	4.001
SD	0.433	0.202	0.280	0.689	0.456	0.763	0.559	0.471
Thyroid weight								
Absolute (g)								

Parameters	Males				Females			
	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day	0 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day	300 mg/kg bw per day
Mean	0.988	1.063	1.215	1.385*	0.795	0.660	1.038	1.138
SD	0.158	0.154	0.231	0.174	0.061	0.139	0.471	0.349
Covariate (g)								
Mean	0.897	1.146	1.231*	1.375**	0.863	0.570	1.076	1.122
Relative (%)[a]								
Mean	0.007 5	0.009 7	0.010 3	0.011 4	0.007 8	0.007 7	0.010 9	0.012 0
SD	0.000 9	0.001 5	0.001 2	0.000 8	0.001 2	0.001 5	0.006 6	0.003 4

ALP: alkaline phosphatase; bw: body weight; SD: standard deviation; U: units; *: $P < 0.05$; **: $P < 0.01$
[a] Relative to body weight.
Source: Blunt (2015b)

Consequently, the increased liver weights at 300 mg/kg bw per day are concluded on balance to be adaptive and non-adverse. Increased thyroid weights in the absence of histological alterations are likely to reflect increased turnover of thyroid hormones secondary to induction of metabolism enzymes, reflected in the increased liver weight.

The NOAEL was 300 mg/kg bw per day, the highest dose tested (Blunt, 2015b).

(b) Dermal application

Rats

In a dermal toxicity study, Han Wistar rats (10 of each sex per dose) were clipped across the back; 24 hours later, pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) was applied at a dose of 0, 100, 300 or 1000 mg/kg bw per day (5 days per week for 28 days) under a surgical gauze pad (5 cm × 5 cm) moistened with 0.5 mL of purified water and held in place with a semi-occlusive dressing and restrainer bandage for 6 hours. After 6 hours, the dressing was removed, and the skin was washed with lukewarm water and dried with disposable paper towels. The fur of the animals was clipped as needed, but at least once weekly. In addition to test guideline parameters, a weekly FOB was conducted. Histopathological analysis was conducted in control and high-dose rats.

There were no effects of treatment on mortality, clinical signs, dermal signs, FOB, feed consumption, body weight, urine analysis, organ weights, gross pathology or histopathology.

A minor increase in the incidence of alveolitis was observed in high-dose males (5/10 vs 3/10 in controls), but not females. This was attributed by the study report's author to be due to accidental inhalation of dust and/or test item, which is not unusual in a dermal study. This conclusion would appear to be reasonable, and the report notes that similar lesions, although lower in incidence, were recorded in control animals.

A statistically significantly lower grip strength of the hind legs in females at 1000 mg/kg bw per day was seen in week 4. The finding is considered incidental, as no effect was observed in the forelegs, and the finding was limited to females only. Some differences in locomotor activity were noted at one interval (20–30 minutes in males at 100 mg/kg bw per day and 30–40 minutes in females at 300 or 1000 mg/kg bw per day) in week 4, but there was no dose dependency, the pattern was opposite in males and females, and total locomotor activity over the whole 1-hour interval was not affected.

Potential pydiflumetofen-attributable effects on clinical chemistry parameters were limited to small changes in values, including higher calcium (2.84 mmol/L vs 2.74 mmol/L in controls),

phospholipid (2.06 mmol/L vs 1.65 mmol/L in controls) and total cholesterol (1.00 mmol/L vs 1.56 mmol/L in controls) levels in females at 1000 mg/kg bw per day, and higher globulin (23.36 g/L vs 21.91 g/L in controls) and total protein (69.01 g/L vs 66.76 g/L in controls) levels in males at 1000 mg/kg bw per day. However, as the values were in general within historical control ranges and/or were not associated with any other pathology, these findings were considered incidental to treatment. Similarly, increased relative monocyte counts in females in low-dose (0.019), mid-dose (0.021) and high-dose (0.019) animals compared with controls (0.013) and absolute monocyte counts in high-dose (0.11×10^9/L) females compared with controls (0.06×10^9/L) were within historical control (95% tolerance) ranges (relative monocytes: 0.011–0.024; absolute monocytes: 0.05–0.14×10^9/L) and therefore were not considered to be toxicologically relevant.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Sieber, 2013).

(c) Exposure by inhalation

No studies were submitted.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

A carcinogenicity study was conducted in which 50 male and 50 female CD-1 (ICR) mice per group were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in the diet at a concentration of 0, 75, 375 or 2250 ppm (equal to 0, 9.2, 45.4 and 287.9 mg/kg bw per day for males and 0, 9.7, 48.4 and 306.2 mg/kg bw per day for females, respectively) for a period of at least 80 weeks.

Animals were monitored for mortality and clinical signs regularly. Body weights and feed consumption were measured and recorded throughout the study. At week 80, prior to terminal kill, blood samples of all surviving animals were withdrawn for haematological examination. Blood smears were obtained from all surviving animals in weeks 52/53 and week 80. However, analysis of blood cell morphology was not performed, because no treatment-related changes were observed in total and differential white blood cell counts or haematological parameters at termination.

The summary of results is shown in Table 16. Mortality in males and females at 375 and 2250 ppm was lower than that of their respective controls, but this was not statistically significant. There were no treatment-related effects on clinical observations or haematological parameters in males or females in any treated groups. Slight (approximately within 10%), but consistent, depression of body weight and reductions of body weight gain were observed in both sexes at 2250 ppm, although only statistically significantly for females at 2250 ppm for the overall treatment period. The reductions were statistically significant in males throughout the study, and the depressions were in males during 5–11 months and in females from 8 months up to termination. These slight reductions of body weight gain were considered treatment related. Feed intakes were comparable in both sexes throughout the study, but feed efficiency was slightly lowered in males at 2250 ppm. Treatment-related increases in absolute liver weight in males and covariate liver weight in both sexes were observed at 2250 ppm. Macroscopically, an increased incidence of liver mass was observed in males at 2250 ppm. Most of the increased mass corresponded to liver tumours in this group, as described below.

In the microscopic examination, a statistically significantly increased incidence of centrilobular hepatocellular hypertrophy was observed in males at 375 ppm and above. The intensity of hypertrophy was enhanced in a dose-dependent manner. Various types of foci of hepatocellular alteration were observed in mice, including controls. Although the focus is known to be a common change in aged mice, the incidence of eosinophilic foci of cellular alteration was increased in males at 2250 ppm, and the increase was considered to be treatment related. The incidences in males at 375 ppm and lower and in females of all treated groups showed no statistically significant differences from those in the control group.

Table 16. Summary of findings from the carcinogenicity study in mice treated with pydiflumetofen

	Males				Females			
Parameters	**0 ppm**	**75 ppm**	**375 ppm**	**2 250 ppm**	**0 ppm**	**75 ppm**	**375 ppm**	**2 250 ppm**
Mortality[a]	13/50	12/50	10/50	5/50	24/50	18/50	10/50	15/50
(%)	(26)	(24)	(20)	(10)	(48)	(36)	(20)	(30)
Body weight (g)								
Day 1	34.5	34.4	34.4	33.6	25.0	25.0	24.7	24.7
Day 8	36.0	35.5	35.5	34.4**	26.6	26.8	26.2	26.0
Day 15	37.0	36.7	36.6	35.9	27.3	28.1	27.3	27.0
Day 85	43.1	43.6	43.8	41.2	33.2	34.1	32.9	32.1
Day 169	47.3	47.8	47.0	44.3*	36.3	37.4	37.5	35.3
Day 239	50.4	51.2	50.1	46.7*	40.5	41.2	41.4	37.5*
Day 323	52.0	52.8	51.9	47.9*	42.1	44.2	43.5	39.8
Day 407	52.6	54.5	52.8	49.1	45.1	46.6	44.0	40.9*
Day 561	53.6	54.1	52.8	50.2	46.7	46.8	44.0	41.3*
Body weight gain (g)								
Days 1–92	9.1	10.0	9.5	8.1	7.8	9.4*	9.0	7.5
Days 92–183	3.5	3.4	3.2	2.4*	4.0	2.7*	3.6	2.6*
Days 183–281	4.0	4.0	3.5	3.6	4.9	5.4	5.2	4.3
Days 281–379	1.0	2.0	1.5	1.2	1.8	4.5**	1.8	1.5
Days 379–463	0.9	0.2	0.9	1.6	0.3	−0.3	−0.5	−1.1
Days 463–561	1.6	0.4	0.2*	−0.3**	0.4	−0.2	−0.4	−0.2
Days 1–561	19.6	19.6	18.8	16.8	21.4	22.1	19.5	16.2**
Liver weights								
Absolute (g)	2.943	2.974	3.189	4.034**	2.356	2.275	2.337	2.562
Covariate (g)	2.803	2.877	3.144	4.269**	2.272	2.227	2.344	2.660**
Relative (% of body weight)[b]	5.412	5.541	5.925	7.953	5.019	4.924	5.347	6.331
Microscopic findings								
No. of mice examined	50	50	49[c]	50	48[c]	50	50	48[c]
Hepatocellular hypertrophy, centrilobular	0	0	6*	18**	0	0	0	0
Focus of cellular alteration								
Basophilic	0	2	1	0	0	0	0	0
Clear cell	3	2	2	0	0	0	0	0
Eosinophilic	1	4	6	10*	1	0	1	2
Hepatocellular adenoma, including multiple	4	6	9*	22**	0	0	0	1
Hepatocellular adenoma[d]	4	6	2	8	0	0	0	1

Parameters	Males				Females			
	0 ppm	75 ppm	375 ppm	2 250 ppm	0 ppm	75 ppm	375 ppm	2 250 ppm
Hepatocellular adenoma, multiple[e]	0	0	7**	14**	0	0	0	0
Hepatocellular carcinoma[f]	2	3	4	10*	0	0	0	0
Hepatocellular carcinoma, multiple[g]	0	0	0	2	0	0	0	0

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] No. of animals dead or killed when moribund/no. of animals examined.

[b] No statistical analysis was conducted for relative weights of organs.

[c] A reduced number of livers was examined, as postmortem changes precluded examination.

[d] Historical control data on adenoma in males were 45/250 (18.0%), minimum to maximum range 10–28%.

[e] Minimum to maximum range of historical control data on multiple adenoma in males was 5–14 for 250 mice.

[f] Historical control data on carcinoma in males were 7/250 (2.8%), minimum to maximum range 0–6%.

[g] Minimum to maximum range of historical control data on multiple carcinoma in males was 3–5 for 250 mice.

Source: Robertson (2015)

As for neoplastic changes, the incidences of hepatocellular adenomas and carcinomas were statistically significantly (Fisher's exact test) increased in males at 2250 ppm. Multiple hepatocellular adenomas and carcinomas were also statistically significantly increased in this group. Peto analysis showed a significant dose-related trend in the incidence of hepatocellular adenomas and carcinomas when all treated groups were analysed. However, the trend analysis was not statistically significant when the incidence at 2250 ppm was excluded from the analysis. The increased incidences of altered foci, adenomas and carcinomas in the liver in males at 2250 ppm were considered to be treatment related. No statistically significant differences in liver tumours or foci of hepatocellular alteration, which are accepted as a precancerous lesion of hepatocellular adenoma in rodents, were observed in males at 375 ppm, but the incidence of multiple adenomas was statistically significantly increased at this dose, although the combined incidence of single and multiple adenomas was the average (18%) of historical control data. Increased multiplicity of tumours is accepted as one of the promoting effects for carcinogenesis. Therefore, the marginal increase in hepatocellular adenomas and multiple adenomas at 375 ppm was considered to be treatment related. There were no differences in these lesions in females. Other tumour incidences were not affected by the treatment in either sex.

The NOAEL for toxicity was 375 ppm (equal to 45.4 mg/kg bw per day), based on a reduction of body weight gain in males at 2250 ppm (equal to 287.9 mg/kg bw per day). The NOAEL for carcinogenicity was 75 ppm (equal to 9.2 mg/kg bw per day), based on an increased incidence of liver tumours in male mice at 375 ppm (equal to 45.4 mg/kg bw per day) (Robertson, 2015).

Rats

In a combined chronic toxicity and carcinogenicity study, groups of 52 male and 52 female Han Wistar Crl: WI (Han) rats were treated with pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in the diet at a concentration of 0, 200, 1000 or 6000 ppm (equal to 0, 9.9, 51.0 and 319 mg/kg bw per day, respectively) for males and 0, 150, 450 or 1500 ppm (equal to 0, 10.2, 31.0 and 102 mg/kg bw per day, respectively) for females for 104 weeks for the carcinogenicity study. For the chronic toxicity study, a further four groups of 12 males and 12 females were included and dosed for 52 weeks at the same dietary concentrations as for the carcinogenicity study.

Animals were monitored regularly for viability and for clinical signs. Body weights and feed consumption were measured and recorded at predetermined intervals from pretrial up until the completion of treatment. Prior to the terminal kill, blood samples were collected from all surviving animals for haematological analysis. Blood smears and clinical pathology samples were obtained from animals in the chronic toxicity study group at weeks 14, 27 and 52. Blood films were made from all

survivors in the carcinogenicity study group; however, blood cell morphology was not performed, because no treatment-related effects on haematological parameters were found at termination. All animals were necropsied and subjected to histopathological examination. Qualitative or quantitative functional observations and motor activity were examined as part of the neurological examination in rats of the chronic toxicity group.

The summary of findings from the combined chronic toxicity and carcinogenicity study in rats is shown in Table 17.

Table 17. Summary of findings in the chronic toxicity and carcinogenicity study of pydiflumetofen in rats

	Males				Females			
Parameter	**0 ppm**	**200 ppm**	**1 000 ppm**	**6 000 ppm**	**0 ppm**	**150 ppm**	**450 ppm**	**1 500 ppm**
Mortality								
Toxicity study	1/12	0/12	0/12	0/12	1/12	0/12	1/12	2/12
Carcinogenicity study	14/52	23/52	7/52	10/52	23/52	13/52	10/52	16/52
Combined (%)	23	36	11	16	38	20	17	28
Body weight gain (g) (combined[a])								
Days 1–92	266	268	245**	213**	117.4	116.5	111.5	106.5**
Days 92–190	73	75	63**	62**	24.1	21.8	21.0	18.5**
Days 190–274	44	41	41	43	13.6	16.2	12.6	11.7
Days 274–358	41	44	36	30**	22.7	20.8	15.9*	11.0**
Days 358–442	36	35	30	28**	26.1	19.9	22.2	18.5
Days 442–526	28	20	23	19	20.6	26.2	17.2	17.9
Days 526–610	23	11	16	11	23.5	27.2	23.7	14.2
Days 610–722	32	10*	23	22	25.4	28.2	22.8	34.1
Days 1–722	550	513	476**	429**	267.5	273.8	241.2	230.8*
Body weight (g) (combined[a])								
Day 1	153	152	154	148*	129	128	125*	128
Day 29	295	299	282**	256**	194	195	188*	186**
Day 64	382	381	362**	324**	230	231	223*	220**
Day 120	443	448	420*	383**	252	254	243*	240**
Day 232	513	517	484**	448**	277	275	263**	259**
Day 358	573	578	538**	496**	307	303	285**	276**
Day 484	633	628	576**	535**	345	339	314**	305**
Day 610	671	645	600**	554**	372	375	341*	324**
Day 722	704	664	628**	576**	395	402	365	359*
Feed utilization (g body weight gain/100 g feed consumed) (combined)								
Weeks 1–4	22.3	22.9	20.7**	18.8**	14.2	14.6	14.4	13.7
Weeks 5–8	11.6	11.0	11.6	10.5**	6.1	6.0	6.4	6.3
Weeks 1–13	13.0	13.2	12.4*	11.6**	7.3	7.2	7.2	6.9

Parameter	Males				Females			
	0 ppm	200 ppm	1 000 ppm	6 000 ppm	0 ppm	150 ppm	450 ppm	1 500 ppm
Blood biochemistry								
GGT (µmol/L)								
Week 14	2.00	2.00	2.00	7.08**	2.18	2.25	2.09	2.17
Week 27	2.00	2.00	2.00	8.08**	2.00	2.00	2.00	2.09
Week 52	2	2	2	11**	2	2	2	2
Liver weight (g, absolute, covariance analysis)								
Chronic toxicity study	15.90	15.28	18.40	21.56**	9.011	9.141	9.785	10.38*
Carcinogenicity study	17.93	19.53	20.01*	22.17**	10.78	11.02	11.59	11.87*
Histopathology								
Liver								
Hepatocellular hypertrophy								
Chronic toxicity study	0/11[b]	0/12	5/12*	11/12**	0/11	0/12	0/11	4/10
Carcinogenicity study	0/52	0/51	3/52	39/52**	0/52	0/52	0/51	3/52
Eosinophilic inclusions	0/52	0/52	0/52	19/52	0/52	0/52	0/52	0/52
Thyroid								
Hyperplasia								
Total	4/52	3/50	3/50	7/52	1/51	3/52	0/51	3/51
Follicular cell, focal	2/52	1/50	1/50	4/52	0/51	1/52	0/51	0/51
Follicular cell, cystic focal	2/52	2/50	2/50	3/52	1/51	2/52	0/51	3/51
Follicular cell adenoma	8/52	3/50	4/50	5/52	1/51	0/52	0/51	3/51
Lymph node								
Erythrophagocytosis, mesenteric								
Chronic toxicity study	1/12	0/12	1/12	7/12*	1/12	1/12	0/12	0/12
Carcinogenicity study	7/35	11/30	16/30	8/37	8/41	7/41	7/34	5/39

GGT: gamma-glutamyl transferase; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Twelve and 52 animals were allocated for the chronic toxicity study and the carcinogenicity study, respectively.

[b] Indicating an incidence of finding (X) per the number of animals examined (Y) as X/Y.

Source: Robertson (2016)

No treatment-related changes in clinical observations or increased mortality were observed during the treatment period. Lower body weights and reduced body weight gains were consistently noted throughout the study in both sexes at the two highest doses. These changes were dose dependent, and body weight gains were reduced by more than 10% and 20% at 1000 and 6000 ppm in males, respectively, compared with control animals. In females, the reductions in body weight gain were less than 10% for the 450 ppm treatment group and greater than 10% for the 1500 ppm group, compared with control animals. Slight, but consistent, decreases in feed consumption were observed in males (within a 10% decrease) and females (within a 5% decrease) at the highest doses (males: 6000 ppm;

females: 1500 ppm). Treatment-related lower feed consumption was observed in males at 1000 ppm and above in a dose-dependent manner.

There were no consistent treatment-related findings associated with ophthalmoscopy, neurotoxicity, urine analysis or haematology in either sex. In blood biochemistry, increases in GGT in males at weeks 14, 27 and 52, decreases in glucose and triglycerides in males at week 52, and decreases in glucose in females at week 14 were found at the high doses. The individual GGT values in males at 6000 ppm were more than 3 µmol/L, the upper LOQ at all time points, whereas the GGT values in males of the other treated groups and the control group were less than 3 µmol/L. Most of the GGT values were 2 µmol/L, the lower LOQ in the GGT assay. No other hepatotoxicity was observed at 6000 ppm in either the chronic toxicity or the carcinogenicity study. However, the consistent increase in GGT was considered to be an adverse effect on the liver. The decreases in glucose or triglycerides in males or females at the high doses were considered to reflect the consistently lower body weights at these doses.

The pathological analysis identified increased liver weights and an increased incidence of hepatocellular hypertrophy in males at 1000 ppm and above and in females at 1500 ppm (the highest dose tested in females) in both the chronic toxicity and carcinogenicity studies. The incidence and severity of the hypertrophy increased with dose and in males at 6000 ppm were associated with cytoplasmic eosinophilic inclusions. Although liver hypertrophy was consistently observed with treatment, precancerous lesions or tumours in the liver were not increased by the treatment. The toxicological relevance of eosinophilic inclusions in hepatocytes was not determined, but the change was considered to be treatment related. In the thyroid, there was a slight numerical increase in the incidence of follicular cell adenomas in females at 1500 ppm, which did not reach statistical significance on pairwise comparison or show a dose-related trend (Peto $P = 0.16$). The incidence of preneoplastic or related findings, focal follicular cell hyperplasia or follicular cell hypertrophy, was not increased in this group. In addition, the incidence at 1500 ppm was within the historical control range (mean 2.2%, range 0–10% in 1276 rats). Therefore, the increase in follicular cell adenoma in females was not considered to be treatment related. The increased incidence of erythrophagocytosis in mesenteric lymph nodes in males at 6000 ppm in the chronic toxicity group was considered incidental, as there was no increase in this lesion in the carcinogenicity study. Although various histopathological findings were found in this study, there were no treatment-related changes other than those described above.

The liver hypertrophy found in males at 1000 ppm was considered to be adaptive but not adverse because there was no indication of hepatotoxicity.

The NOAEL for toxicity was 200 ppm (equal to 9.9 mg/kg bw per day), based on lower body weight and reduced body weight gain in males at 1000 ppm (equal to 51.0 mg/kg bw per day). No carcinogenicity was observed in rats in this study (Robertson, 2016).

2.4 Genotoxicity

The genotoxicity of pydiflumetofen and its individual isomers was examined in an adequate range of in vitro and in vivo studies (Table 18). Select studies are described briefly below.

(a) In vitro studies

Chromosomal aberration test using human lymphocytes

Three independent experiments were performed. In experiment I, the exposure was 4 hours with and without metabolic activation (S9 mix). In experiment IIA, the exposures were 4 hours with S9 mix and 22 hours without S9 mix. In experiment IIB, the exposure period was 22 hours without S9 mix. The chromosomes were prepared 22 hours after the start of treatment with the test substance.

Precipitation of the test material was noted at the end of treatment at and above 28.1 µg/mL without S9 mix, at and above 49 µg/mL with S9 mix (experiment I), at and above 16.1 µg/mL with and without S9 mix (experiment IIA) and at and above 20.0 µg/mL without S9 mix (experiment IIB).

Table 18. Results of genotoxicity assays on pydiflumetofen

Test system	Test object	Concentration/ dose	Purity	Results		Reference
				Without S9	With S9	
In vitro						
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2*uvr*A/pKM101, WP2pKM101	5 000 µg/plate	98.5	Negative[a]	Negative[b]	Sokolowski (2012)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvr*A/pKM101, WP2pKM101	5 000 µg/plate	96.7	Negative[a]	Negative[a]	Sokolowski (2014a)
Chromosomal aberration, mammalian	Human lymphocytes	150.8 µg/mL −S9 4 332 µg/mL +S9	98.5	Positive[c]	Negative[d]	Bohnenberger (2013a)
Point mutation (*tk* locus) assay	Mouse lymphoma L5178Y cells	60 µg/mL −S9 110 µg/mL +S9	98.5	Negative	Negative	Wollny (2013a)
In vivo						
Micronucleus induction	Mouse bone marrow	2 000 mg/kg bw (ip)	98.5	Negative	NA	Roth (2012)
Micronucleus induction	Mouse bone marrow	2000 mg/kg bw (ip)	96.7	Negative	NA	Dony (2014a)

bw: body weight; ip: intraperitoneal; NA: not applicable; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *tk*: thymidine kinase

[a] Precipitation observed at and above 1000 µg/plate.

[b] Precipitation observed at and above 333 µg/plate.

[c] Precipitation observed at and above 20.0 µg/mL (22-hour exposure) and at and above 28.1 µg/mL (4-hour exposure).

[d] Precipitation observed at and above 16.1 µg/mL (4-hour exposure).

No clastogenicity was observed in the presence of S9 mix at concentrations up to 4332 µg/mL. In the absence of S9 mix, clastogenicity was not observed after a 4-hour exposure, but was observed after a 22-hour exposure. In experiment IIA in the absence of S9 mix, one statistically significant increase (6.5% aberrant cells, excluding gaps) above the range of the historical solvent control data (0.0–3.0% aberrant cells, excluding gaps) was observed after treatment with 5.3 µg/mL, but without a dose–response relationship. In the confirmatory experiment IIB, statistically significant increases occurred after treatment with 20.0 and 40.0 mg/mL (7.5% and 9.5% aberrant cells, excluding gaps), clearly exceeding the laboratory historical solvent control range of 0.0–3.0% and showing a dose–response relationship. Concordant with the test facility and applicant analysis, pydiflumetofen was found to induce structural chromosomal aberrations in human lymphocytes in vitro in the absence of metabolic activation.

(b) In vivo studies

Two mouse bone marrow micronucleus tests were conducted, one of which was on the same batch of material (lot/batch no. SMU2EP12007) that gave a positive response in the in vitro chromosomal aberration test. The second study was conducted on a batch of material spiked with potential impurities (lot/batch no. SMU4FL762) to support the technical specification. Both in vivo micronucleus tests gave negative results. The in vivo mouse bone marrow micronucleus tests did not include an assessment of bone marrow exposure to pydiflumetofen; however, clinical signs observed in both in vivo studies were indicative of systemic absorption of the test material. In addition, a high volume of distribution was observed in pharmacokinetics of a single intravenous treatment with pydiflumetofen in mice (Punler & Harris, 2014a). Therefore, bone marrow will have been exposed to pydiflumetofen in the in vivo mouse micronucleus studies.

Overall, the positive in vitro clastogenicity finding for pydiflumetofen did not correspond to genotoxicity in vivo.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

A two-generation reproductive toxicity study was conducted in which Han Wistar rats (24 of each sex per dose) were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in the diet at a concentration of 0, 150, 750 or 4500 ppm for males and 0, 150, 450 or 1500 ppm for females (in the F_0 generation: pre-pairing, equal to 0, 9.1, 46.1 and 277 mg/kg bw per day for males and 0, 11.9, 36.1 and 116 mg/kg bw per day for females, respectively; during gestation, equal to 0, 12.6, 33.7 and 115 mg/kg bw per day for females, respectively; during lactation, equal to 0, 30.0, 87.0 and 291 mg/kg bw per day for females, respectively; in the F_1 generation: pre-pairing, equal to 0, 11.9, 59.1 and 364 mg/kg bw per day for males and 0, 14.1, 42.4 and 141 mg/kg bw per day for females, respectively; during gestation, equal to 0, 15.1, 39.5 and 141 mg/kg bw per day for females, respectively; and during lactation, equal to 0, 26.3, 77.8 and 267 mg/kg bw per day for females, respectively). Doses were selected based on the results of toxicokinetics studies, to be within the linear range for the dose–blood level relationship. Animals were treated for at least 10 weeks prior to pairing for the parental (F_0) generation from birth until pairing for mating for the F_1 generation. In addition to the test guideline parameters, the number of follicles in the ovaries was counted in the control and high-dose females, and sperm motility and concentration were assessed for all males killed at terminal necropsy.

In the F_0 generation, there were no treatment-related effects on mortality, clinical signs, gross pathology, reproductive performance, mating behaviour, conception, sperm parameters, pup survival or pup development. In the male parents at 4500 ppm, body weight gains were reduced during the first 5 weeks. Females at 1500 ppm had slightly longer estrous cycles (3.93 ± 0.10 days at 0 ppm vs 4.05 ± 0.24 days at 1500 ppm); however, the finding was not repeated in the F_1 generation and was within the historical control range (in four studies during 2009–2014, ranges of estrous cycles were 4, 3.8–4.5, 4.0–6.0 and 4.0–6.3 days per 23–25 females), so the observations were considered likely to be incidental.

Females at 1500 ppm had a slightly and statistically significantly shorter gestation length than controls (22.04 days compared with 22.29 days in controls; $P < 0.05$). However, this was incidental owing to a single animal delivering at 21.0 days, with all others delivering between 22.0 and 22.5 days, which is within the range of the concurrent control animals (days 21.5–23 of gestation). All pregnant females gave birth to live litters. Absolute and relative (to body weight) liver weights were increased in males at the intermediate and high doses, and the incidence of hepatocellular hypertrophy was increased in both sexes at the high dose. A statistically significant increase in thyroid weight and a corresponding increased incidence of minimal thyroid follicular cell hypertrophy were observed in males at 4500 ppm. The liver hypertrophy without findings related to liver damage or thyroid effects was considered adaptive.

In the F_1 generation, there were no effects of treatment on mortality, clinical signs, estrous cycle, fertility, mating performance, gestation length, postnatal survival, live births, sex ratio, sperm parameters or ovarian follicle counts. At weaning, the mean body weight of males at 4500 ppm was lower than the control value; body weight gains remained below control values until necropsy, and feed consumption was commensurately lower. At weaning, females at 1500 ppm also had lower body weights than controls, but body weight gains and feed consumption were comparable to those of controls thereafter. Although all animals delivered litters between days 21.0 and 23.5 of gestation, at 1500 ppm, mean gestation length was shorter; this was considered to be an artefact of comparison with a concurrent control range that was unusually narrow (22.0–23.0 days). Historical control data provided showed a gestation length range of 22–24 days. Absolute and relative liver weights were increased in males at 750 ppm and above and were associated with hepatocellular hypertrophy at 4500 ppm. Relative liver weights were elevated in females at 450 and 1500 ppm, but there were no histological correlates. Absolute thyroid weights were higher in all treated male groups; however, thyroid weights were within the normal range (0.012–0.032 g), with the exception of one male at 750 ppm. The observation was therefore concluded to be potentially incidental, but is consistent with the observations in the F_0 generation. There were no treatment-related effects on mortality, clinical signs, anogenital distance, body weights or gross pathology in litters from the F_1 generation.

There were no treatment-related changes in F_2 generation pups.

The results of the multigeneration study of pydiflumetofen in rats are summarized in Table 19.

Table 19. Summary of findings from the multigeneration reproductive toxicity study of pydiflumetofen in rats

	Males				Females			
Parameters / generation	**0 ppm**	**150 ppm**	**750 ppm**	**4 500 ppm**	**0 ppm**	**150 ppm**	**450 ppm**	**1 500 ppm**
Body weight gain (g)								
F_0 generation								
0–17 weeks	214.0	223.2	215.0	192.7*	–	–	–	–
0–10 weeks (pre-pairing)	–	–	–	–	96.2	87.4*	91.8	91.3
0–20 weeks (gestation)	–	–	–	–	107.3	112.1	106.5	103.2
1–21 weeks (lactation)	–	–	–	–	31.1	30.6	32.2	36.3
F_1 generation								
0–17 weeks	364.3	367.2	354.5	328.6**	–	–	–	–
0–10 weeks (pre-pairing)	–	–	–	–	157.7	159.8	161.8	157.4
0–20 weeks (gestation)	–	–	–	–	101.4	99.7	105.5	99.0
1–21 weeks (lactation)	–	–	–	–	33.3	30.7	33.9	36.6
Body weight (g)[a]								
F_1 generation before weaning								
Lactation day 1	–[a]	–[a]	–[a]	–[a]	6.69	6.64	6.72	6.45
Lactation day 4	–[a]	–[a]	–[a]	–[a]	10.17	9.98	10.30	9.27
Lactation day 7	–[a]	–[a]	–[a]	–[a]	16.83	15.99	16.50	14.89**
Lactation day 14	–[a]	–[a]	–[a]	–[a]	33.97	32.75	33.11	29.91**
Lactation day 21	–[a]	–[a]	–[a]	–[a]	51.63	50.63	50.28	46.29**

Parameters / generation	Males				Females			
	0 ppm	150 ppm	750 ppm	4 500 ppm	0 ppm	150 ppm	450 ppm	1 500 ppm
F_2 generation before weaning								
Lactation day 1	– [a]	– [a]	– [a]	– [a]	6.23	6.45	6.60	6.35
Lactation day 4	– [a]	– [a]	– [a]	– [a]	8.16	7.81	8.76	8.05
Lactation day 7	– [a]	– [a]	– [a]	– [a]	12.60	12.32	13.47	12.95
Lactation day 14	– [a]	– [a]	– [a]	– [a]	30.32	29.58	30.93	29.33
Lactation day 21	– [a]	– [a]	– [a]	– [a]	48.94	47.27	49.60	47.32
Hepatocyte hypertrophy (minimal to slight) [b]								
F_0 generation	0/24	0/24	0/24	19/24	0/24	0/24	0/24	8/24
F_1 generation	0/24	0/24	0/24	18/24	0/24	0/24	0/24	0/24
Follicular hypertrophy in thyroid (minimal) [b]								
F_0 generation	1/24	1/24	2/24	7/24	0/24	0/24	0/24	0/24
F_1 generation	2/24	0/24	1/24	7/24	0/24	0/24	0/24	0/24

F_0: parental generation; F_1: first filial generation; F_2; second filial generation; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Body weights given under the "Females" heading are mean values for males and females combined.

[b] The number of animals with the finding/the number of rats examined.

Source: Hackford (2015)

The NOAEL for parental toxicity was 750 ppm (equal to 46.1 mg/kg bw per day), based on reduced body weight and effects on the thyroid in males at 4500 ppm (equal to 277 mg/kg bw per day). The NOAEL for reproductive toxicity was 1500 ppm (equal to 115 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 450 ppm (equal to 33.7 mg/kg bw per day), based on reduced pup body weight before weaning in the F_1 generation at 1500 ppm (equal to 115 mg/kg bw per day) (Hackford, 2015).

(b) Developmental toxicity

Rats

In a dose range–finding study of developmental toxicity in rats, mated female Crl:CD (SD) rats (six per dose) were administered pydiflumetofen (lot/batch no. 2491-DC/110; purity 98.6%) in 1% aqueous carboxymethylcellulose by oral gavage at a dose of 0, 100, 200, 500 or 1000 mg/kg bw per day from GD 6 to GD 19, then terminated on day 20 for caesarean section and examination of the fetuses. Mortality, body weight, clinical signs and feed consumption were monitored regularly during the study. At termination, gravid uterus weights were measured, and the fetuses were examined for viability, visceral and skeletal malformations and variations, weighed and sexed. Preimplantation and postimplantation losses were calculated.

There were no effects on mortality, clinical signs, maternal gross pathology, implantation loss, number of live fetuses, sex ratio, fetal weights, gravid uterus weights or the incidence of malformations or variations. Body weight gains were slightly reduced at 500 mg/kg bw per day (control: 6 g; 500 mg/kg bw per day: 0.2 g). Body weight loss was observed at 1000 mg/kg bw per day (3 g) between days 6 and 7, but body weights were comparable to control values by the end of the study.

There were no treatment-related fetal abnormalities in any group. Variations were similar in incidence across all dose groups (Manton, 2011).

A developmental toxicity study was conducted in which mated female Sprague Dawley rats (24 per dose) were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in 1% aqueous carboxymethylcellulose by oral gavage at a dose of 0, 10, 30 or 100 mg/kg bw per day from GD 6 to GD 19. On day 20, following a caesarean section, all fetuses were examined.

There were two unscheduled deaths, with both rats being terminated prematurely because of clinical signs of morbidity: one dam at 10 mg/kg bw per day as a result of dosing injury on GD 15, and one dam at 100 mg/kg bw per day from unknown causes on GD 6 after the first dose. The dam at 100 mg/kg bw per day had clinical signs of decreased activity, cold body surface, unsteady and abnormal gait, hunched posture, partially closed eyes and piloerection, but necropsy was unable to identify any macroscopic changes indicating the causes of these signs. Given the absence of similar mortality in other rat studies at similar or higher doses, neither death is considered to be toxicologically relevant.

There were no effects of treatment on mortality, clinical signs, terminal body weights and body weight gains, gravid uterus weights, feed consumption, maternal gross pathology, implantations and implantation loss, and fetal, litter or placental weights. Body weight gains were reduced at 100 mg/kg bw per day between days 6 and 10, associated with a slightly lower feed intake, but returned to control values from day 11. Major fetal abnormalities were noted in one fetus each at 0 and 10 mg/kg bw per day, two fetuses from two litters at 30 mg/kg bw per day and two fetuses from the same dam at 100 mg/kg bw per day.

The summary of findings from the developmental toxicity study in rats is shown in Table 20.

Table 20. Summary of findings from the developmental toxicity study of pydiflumetofen in rats

Parameters	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
Body weight gain (g), F_0 generation				
GDs 0–6				
Mean	27.6	30.0	27.0	28.1
SD	7.8	6.8	6.7	6.2
GDs 6–7				
Mean	6.1	3.5	3.4	0.5**
SD	3.5	4.0	4.3	6.5
GDs 6–10				
Mean	24.1	22.1	22.8	19.7*
SD	4.8	5.3	5.2	6.9
GDs 6–15				
Mean	59.5	59.5	59.9	57.0
SD	6.4	7.8	7.9	10.7
GDs 6–20				
Mean	132.8	135.7	132.3	128.4
SD	14.3	14.4	14.7	19.3
Feed consumption (g/rat per day), F_0 generation				
GDs 6–9				
Mean	26.5	26.5	26.1	24.1*

Parameters	0 mg/kg bw per day	10 mg/kg bw per day	30 mg/kg bw per day	100 mg/kg bw per day
SD	2.3	3.8	3.0	3.0
GDs 9–12				
Mean	27.6	27.7	28.0	26.8
SD	2.2	2.7	3.1	3.5
GDs 6–20				
Mean	28.7	29.5	29.2	28.2
SD	2.11	2.21	2.68	2.91
Identification number of dams and fetuses with fetal abnormalities and their findings				
Diaphragmatic hernia	16/L5[a]	–	–	–
Multiply malformed fetus	–	48/L8	–	–
Anophthalmia; orbital cavity reduced in size; malformed cervical neural arch; absent cervical neural arches; bent scapula	–	–	66/L4	–
Scapulae severely bent; humeri malformed; femurs bowed	–	–	69/L12	–
Exencephaly; open eye; cleft palate; malformed interparietals, parietals, frontals and nasals; cleft palatine	–	–	–	82/L1
Exencephaly; open eye; malformed palate; malformed parietals and frontals; absent interparietals; cleft palatine	–	–	–	82/R3

GD: gestation day; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$

[a] Dam identification number/fetus identification number.

Source: Davies (2015)

Historical control data from Charles River Laboratories, which supplied the animals for the current study, demonstrated the sporadic occurrence of various abnormalities, such as those observed in this study (Barnes, 2015; Davies, 2016). The single severely malformed fetus at 10 mg/kg bw per day was concluded to be incidental, given the lack of similarly affected fetuses at 30 and 100 mg/kg bw per day. The two affected fetuses at 30 mg/kg bw per day had different malformations that were not observed at 100 mg/kg bw per day and again are concluded to be incidental.

The sponsor argued that the exencephaly in two fetuses from the same litter at 100 mg/kg bw per day was incidental based on historical control incidences at the testing laboratory (and other Charles River facilities) and the lack of statistical significance. The historical control data provided for exencephaly indicated a very low frequency of occurrence and only one fetus per litter affected in each case. Exencephaly is known to occur spontaneously at low litter incidences, and it has previously been reported to occur not uncommonly, with more than one fetus per litter affected (Lang, 1993); the effect is confined to a single litter in the current study. On this basis, although the finding is notable, the incidence at 100 mg/kg bw per day was concluded to be likely to be incidental. The absence of similar findings at 1000 mg/kg bw per day in the dose range–finding study is supportive of this conclusion.

The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on the reduction in body weight gain and lower feed consumption in dams at the beginning of treatment at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested (Davies, 2015).

Rabbits

In a non-GLP-compliant dose range–finding developmental toxicity study, mated female New Zealand white rabbits (10 per dose) were administered pydiflumetofen (lot/batch no. 2637-AA/110, SMU2EP; purity 98.5–99.3%) by oral gavage at a dose of 0, 250, 500 or 1000 mg/kg bw per day from GD 6 to GD 27 at a dosing volume of 5 mL/kg bw. Additional groups of mated rabbits at 0 and 1000 mg/kg bw per day (10 per dose) were added to the study when it became apparent that only half the does were pregnant in the initial 1000 mg/kg bw per day group. Animals were checked daily for mortality and clinical signs of toxicity and weighed. Feed consumption was measured. At necropsy of the dams on GD 28, fetuses were examined.

There were no effects of treatment on feed consumption, gross pathology, clinical signs, placental or gravid uterus weights, fetal weights, sex ratio, and preimplantation or postimplantation losses. A single animal at 1000 mg/kg bw per day was found dead on GD 12, with dark lungs, pale kidneys, an accentuated lobular pattern of the liver and cream material in the abdominal cavity found at necropsy. Given the absence of clinical signs or gross pathology in other dams at this dose, this death was concluded to be a treatment-related technical error. Pregnancy was confirmed in 10, nine, nine and five does at 0, 250, 500 and 1000 mg/kg bw per day in the initial dose groups, respectively. In the additional dose groups, pregnancy was confirmed in nine and eight does at 0 and 1000 mg/kg bw per day, respectively. Although body weight gains in the first 1000 mg/kg bw per day group were unaffected, body weight gain during GDs 6–28 in the second 1000 mg/kg bw per day group was approximately 30% lower than that in the concurrent control group. There were no treatment-related adverse effects on the fetuses (Penn, 2015b).

In a developmental toxicity study, mated female New Zealand white rabbits (24 per dose) were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in 1% aqueous carboxymethylcellulose by oral gavage at a dose of 0, 10, 100 or 500 mg/kg bw per day from GD 6 to GD 27 at a dosing volume of 5 mL/kg bw. Blood samples (0.1 mL) were taken from six animals per group on GD 27 at 2, 6 and 12 hours post-dosing for analysis of toxicokinetics. Terminal blood samples (GD 28) were taken immediately after each animal was killed.

There were no effects of treatment on mortality, clinical signs, body weight, body weight gain, feed consumption, maternal gross necropsy, preimplantation or postimplantation losses, live fetuses, sex ratio or fetal, litter or placental weights. There were two early deaths during the study, one at 100 mg/kg bw per day on day 12 as a result of dosing trauma and another at 500 mg/kg bw per day, with termination on day 19 after observed signs of abortion. Both deaths were considered incidental to treatment.

Major fetal abnormalities were observed in all groups, including controls: in five fetuses from three litters in the control group, four fetuses from three litters in the group given 10 mg/kg bw per day, three fetuses from three litters in the group given 100 mg/kg bw per day and two fetuses from two litters in the group given 500 mg/kg bw per day.

Historical control data were supplied for this laboratory and for this strain and source of rabbit. With the exception of diaphragmatic hernia, these major abnormalities have been noted at low incidence in this strain of rabbit in the conducting laboratory. Although diaphragmatic hernia has not been seen within the background data for this strain of rabbit from this supplier, it has been shown to occur spontaneously in rabbits of this strain from other suppliers, and the study report includes information indicating a low level of spontaneous occurrence. The single instance of diaphragmatic hernia at 500 mg/kg bw per day is concluded to be incidental, and this conclusion is supported by the results of the dose range–finding study, in which similar or related abnormalities were not observed at 1000 mg/kg bw per day. Because the nature and intergroup distribution of the fetal abnormalities are consistent with the historical control ranges, they were concluded to be incidental.

The toxicokinetics of pydiflumetofen was characterized by a highly sub-dose-proportional increase in peak concentration and total systemic exposure across the 50-fold dose range. Mean AUC

increased only 4.2-fold across this dose range, and toxicokinetic parameters varied markedly across individuals in each dose group, with a within-group variation of 6- or 7-fold between individuals. As a result, there was considerable overlap in values across dose groups, with individuals in the 10 mg/kg bw per day group achieving AUCs comparable to or higher than those of individuals in the 100 mg/kg bw per day group.

The NOAEL for maternal and embryo/fetal toxicity was 500 mg/kg bw per day, the highest dose tested (Penn, 2015c).

2.6 Special studies

(a) Neurotoxicity

Acute neurotoxicity

In an acute neurotoxicity study, Han Wistar rats (10 of each sex per dose) were administered pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in aqueous 1% carboxymethylcellulose by oral gavage as a single dose of 0, 300, 1000 or 2000 mg/kg bw for males and 0, 100, 1000 or 2000 mg/kg bw for females. Parameters to investigate neurotoxicity included a FOB, quantitative assessment of landing foot splay, body temperature, grip strength and locomotor activity. Fifteen days after treatment (i.e. day 16), the animals were necropsied. Nervous system tissues were fixed by perfusion in situ and were examined for histopathological changes in the control and high-dose groups only.

For males, treatment-related findings were limited to slight decreases in body weight gain over the first day at 1000 and 2000 mg/kg bw. Various clinical signs of toxicity were confined to the females, mainly at 1000 and 2000 mg/kg bw **(**Table 21). The slightly increased severity grade for piloerection at 1000 mg/kg bw and above might be associated with a worsened general condition after dosing. The statistically significant decrease in body temperature at 1000 mg/kg bw was considered a treatment-related adverse effect.

Table 21. Clinical observations and body temperature in female rats treated with pydiflumetofen in an acute neurotoxicity study

Parameter	0 mg/kg bw	100 mg/kg bw	1 000 mg/kg bw	2 000 mg/kg bw
Clinical observations (maximum severity grade)				
Recumbency (1)	0	0	1/9 (1.0)	0
Hunched posture (1)	0	1/10 (1.0)	0	2/10 (1.0)
Piloerection (2)	2/10 (1.0)	3/10 (1.0)	4/9 (1.3)	4/10 (1.3)
Vocalization (2)	3/10 (1.0)	0	1/9 (1.0)	2/10 (1.0)
Reduced muscle tone (1)	0	0	1/9 (1.0)	0
Reduced activity (2)	0	1/10 (1.0)	2/9 (1.5)	1/10 (1.0)
Decreased rearing (1)	2/10 (1.0)	1/10 (1.0)	2/9 (1.0)	1/10 (1.0)
Abnormal gait (2)	0	0	1/9 (1.0)	1/10 (1.0)
Skin cold on touch (1)	0	0	1/9 (1.0)	0
Pupillary reflex impaired (1)	0	0	1/9 (1.0)	0
Mydriasis (1)	0	0	1/9 (1.0)	0
Body temperature at 6 hours post-dosing on day 1				
Mean body temperature (°C) ± SD (% change from control)	38.2 ± 0.7 (100)	37.9 ± 0.5 (−0.8)	37.2 ± 1.3 (−2.6)	37.0 ± 1.3* (−3.1)

Parameter	0 mg/kg bw	100 mg/kg bw	1 000 mg/kg bw	2 000 mg/kg bw
Locomotor activity over a 30-minute session at 6 hours post-dosing on day 1				
Total distance (cm)	2 581	1 767	1 346**	1 060
Total centre time (s)	129	82	101	34
Total number of rearings	47	25	16**	9**

bw: body weight; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$
Source: Broich (2015a)

Locomotor activity was statistically significantly reduced in females at 1000 and 2000 mg/kg bw 6 hours after dosing on day 1. Male values were within the historical control range and not statistically significantly different from control values.

There were no treatment-related effects on feed consumption, organ weights, gross pathology or histopathology in either sex. The findings (reduced locomotor activity) in females at 1000 mg/kg bw were considered treatment related and of a general toxicity nature; however, there was no clear evidence indicating neurotoxicity in these findings.

The NOAEL for systemic toxicity was 100 mg/kg bw, based on altered locomotor activity, clinical signs and reduced body temperature in females at 1000 mg/kg bw. The NOAEL for neurotoxicity was 2000 mg/kg bw per day, the highest dose tested (Broich, 2015a).

A follow-up acute neurotoxicity study was conducted using female rats only. Female Han Wistar rats (10 per dose) were administered a single dose of pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) in aqueous 1% carboxymethylcellulose by oral gavage at a dose of 0, 100, 300 or 1000 mg/kg bw and observed for 8 days. Animals were observed for clinical signs and mortality at least daily. Body weights and feed consumption were measured daily. The FOB was conducted after 6 hours on day 1 and on day 8, including detailed clinical observation and tests for reflexes, hearing, grip strength, landing foot splay, body temperature and locomotor activity. The animals were necropsied for gross pathology. As the previous full study had not identified effects on organ weights or histopathology, these parameters were not assessed in this study.

There were no treatment-related effects on mortality, grip strength, foot splay, body weight, feed consumption or gross pathology. Detailed clinical examination on the day of dosing (as part of the FOB) identified a number of effects across all groups (e.g. vocalization, reduced activity, decreased rearing and tremor). There were no definitive dose–response relationships for any of the parameters assessed, but some of the signs in treated animals did correlate with findings in other stages of the FOB. Two animals at 100 mg/kg bw (animal numbers 15 and 17) and one animal at 1000 mg/kg bw (animal number 34) showed markedly lower body temperatures (34.3, 33.9 and 33.9 °C), but this was not observed in the 300 mg/kg bw group. Therefore, the lower temperature without a dose–response relationship at 100 mg/kg bw was considered to be incidental. The mean body temperature was recalculated excluding these three animals. The mean body temperature 6 hours post-dosing was slightly, but statistically significantly, lower at 300 mg/kg bw and above. This finding was considered treatment related because the results are consistent with those of the previous study (Table 22).

Grip strength in general was unaffected by treatment. Although hindlimb grip strength was slightly decreased on day 8 at 1000 mg/kg bw compared with controls, the results were similar to the pretest data. The deviation from the control mean was only slight, and there were no findings indicative of neurotoxicity at the detailed clinical examinations or on assessment of locomotor activity. Consequently, the observation was concluded to be incidental. Locomotor activity was slightly decreased at 300 and 1000 mg/kg bw at the 6-hour examination. Although the difference was not statistically significant, the decrease was consistent with the results from the previous study and considered to be treatment related (Table 22). The marked decrease in locomotor activity observed in

two animals at 100 mg/kg bw was considered incidental because there was no enhancement of the severity at higher doses. There was no evidence of overt neurotoxicity, with all treatment-related effects consistent with discomfort or other acute effects of dosing related to general toxicity.

Table 22. Clinical observations, body temperature and locomotor activity in abbreviated acute neurotoxicity study of pydiflumetofen in female rats

Parameter	0 mg/kg bw	100 mg/kg bw	300 mg/kg bw	1 000 mg/kg bw
Clinical observations (maximum severity grade)				
Piloerection (2)	0	1/10 (2.0)	0	1/10 (2.0)
Vocalization (2)	1/10 (1.0)	1/10 (1.0)	1/10 (1.0)	2/10 (1.0)
Reduced activity (2)	1/10 (1.0)	4/10 (1.0)	1/10 (1.0)	3/10 (1.0)
Decreased rearing (1)	4/10 (1.0)	7/10 (1.0)	4/10 (1.0)	5/10 (1.0)
Tremor (2)	0	0	0	1/10 (1.0)
Skin cold on touch (1)	0	2/10 (1.0)	0	1/10 (1.0)
Pupillary reflex impaired (1)	0	0	1/10 (1.0)	0
Miosis (1)	0	0	1/10 (1.0)	0
Extensor thrust reflex impaired (1)	0	2/10 (1.0)	0	1/10 (1.0)
Body temperature at 6 hours post-dosing on day 1				
Mean body temperature (°C) ± SD[a]	38.0 ± 0.3	37.7 ± 0.3	37.6 ± 0.2*	37.6 ± 0.3*
Locomotor activity over a 30-minute session at 6 hours post-dosing on day 1				
Total distance (cm)	2 533	2 444	1 874	1 821
Total centre time (s)	103	169	70	117
Total number of rears	51	51	32	30

bw: body weight; SD: standard deviation; *: $P < 0.05$
[a] Exclusion of animal nos 15, 17 and 34 as outliers.
Source: Broich (2015b)

The NOAEL for systemic toxicity was 100 mg/kg bw, based on altered locomotor activity, clinical signs and reduced body temperature at 300 mg/kg bw. The NOAEL for neurotoxicity was 1000 mg/kg bw, the highest dose tested (Broich, 2015b).

Subacute neurotoxicity

Subacute neurotoxicity studies were not conducted, but detailed FOB examinations were conducted in the short-term toxicity studies in mice, rats and dogs and the long-term toxicity study in rats. No changes associated with neurotoxicity were observed in those studies, suggesting that pydiflumetofen is not neurotoxic at the doses tested in those studies.

(b) Immunotoxicity

No study for the assessment of immunotoxicity was provided. No findings indicating immunotoxicity were observed in any of the submitted studies, suggesting that pydiflumetofen is not immunotoxic.

(c) Toxicity of metabolites

A number of toxicity studies have been conducted on metabolites of pydiflumetofen that were detected in livestock and rats after exposure to pydiflumetofen. Summaries of the data are presented below. The metabolites CSAA798670 and SYN508272 are common to a number of other succinate dehydrogenase inhibitors.

2,4,6-Trichlorophenol (2,4,6-TCP)

2,4,6-TCP was the major circulating metabolite of pydiflumetofen in rats (representing up to 5.3% in plasma) and is identified in animal commodities only. 2,4,6-TCP sulfate was identified in plasma at 32–44% (MacDonald & Jewkes, 2015; Tomlinson et al., 2015).

No studies on the toxicity of 2,4,6-TCP were submitted. Oral exposure to 2,4,6-TCP has been shown to cause tumours in two rodent species. When administered in the diet, 2,4,6-TCP caused hepatocellular adenomas or carcinomas in both male and female mice and lymphoma or mononuclear cell leukaemia in male rats (NCI, 1979; IARC, 1982). In the NCI (1979) study, the number of F344 rats and B6C3F1 mice in the control groups was only 20 (the number in treated groups was 50). In addition, the given doses were high (in rats: 5000 and 10 000 ppm; in mice: 5000, 10 000 and 20 000 ppm for the first 38 weeks, reduced to 2500, 5000 and 10 000 ppm thereafter). These doses were considered to exceed the maximum tolerable dose. The data quality, including histopathological criteria, was considered inadequate when compared with current toxicological evaluations. However, increased incidences of leukaemia/lymphoma in male F344 rats at 5000 and 10 000 ppm and liver tumours in both sexes of B6C3F1 mice at 5000 and 10 000 ppm suggested the carcinogenic potential of this compound in rodents at very high doses (NCI, 1979).

WHO has established a guideline value of 0.2 mg/L 2,4,6-TCP in drinking-water, although the compound is generally found in drinking-water at concentrations below 1 µg/L. Like other chlorophenols (e.g. 2-chlorophenol and 2,4-dichlorophenol), 2,4,6-TCP is most likely to occur in drinking-water as a by-product of chlorination (WHO, 2017).

It was concluded that the present mammalian toxicity data on pydiflumetofen have sufficiently assessed the toxicity of 2,4,6-TCP and its conjugates and that the toxicity of 2,4,6-TCP and its conjugates would be covered by the parent compound.

SYN508272

SYN508272 is an identified metabolite in goats, hens and rats. A synonym for this metabolite is Reg. No. 5621781.

Acute oral toxicity. Groups of three female fasted Crl:WI (Han) rats were dosed sequentially with SYN508272 (lot/batch no. L81-108; purity 99.4%) in 0.5% aqueous carboxymethylcellulose by gavage at a dose of 2000 mg/kg bw (one group) or 500 mg/kg bw (two groups) and observed for 14 days.

All animals in the 2000 mg/kg bw test group were found dead within 24 hours after administration. Animals showed impaired and poor general state, dyspnoea, ataxia, tremor, staggering, twitching, abdominal position and piloerection.

No mortality occurred in the six females administered 500 mg/kg bw. In the first group, no adverse clinical signs were noted. However, in the second group, one animal showed impaired general state, dyspnoea, piloerection, chromodacryorrhoea and reduced faeces from the fourth hour after treatment until the next day.

There were no treatment-related effects on body weight in surviving animals up to 14 days. There were no significant pathological findings in either the surviving or dead animals.

The oral LD_{50} was therefore concluded to be greater than 500 mg/kg bw and less than 2000 mg/kg bw (Cords & Lammer, 2009).

Short-term studies of toxicity. In a 28-day toxicity study, Crl:WI (Han) rats (five of each sex per dose) were administered SYN508272 (lot/batch no. MES114/2; purity 94%) in the diet at a concentration of 0, 100, 500 or 2000 (males) / 4000 (females) ppm (equal to 0, 7.3, 37.4 and 143 mg/kg bw per day for males and 0, 7.8, 42.5 and 244 mg/kg bw per day for females, respectively). Study parameters included mortality and clinical signs at least once daily, body weight and feed consumption weekly, ophthalmoscopy pretrial in all animals and in control and high-dose animals in week 4, FOB pretrial and in week 4, clinical pathology prior to termination (haematology, clinical chemistry, urine analysis, coagulation), and organ weights (all), gross pathology (all) and histopathology (control and high-dose rats only) at termination.

There were no effects of treatment on mortality, clinical signs, FOB, urine analysis, organ weights, gross pathology or histopathology. In females at 4000 ppm, body weight and body weight gain were reduced throughout the treatment period. Body weight gain was 78% lower than control values by the end of the study. In males at 2000 ppm, body weight gain after 12 days was 40.6% lower than control values, but body weight recovered during the remainder of the study, and overall body weight gain at the end of the study was only 5.5% lower than control values (not statistically significant). Feed consumption was lower throughout treatment in females at 4000 ppm and slightly lower for the first 8 days in males at 2000 ppm, but then similar to control values, indicating lower palatability.

White blood cell counts were raised in males at 2000 ppm and in females at 4000 ppm. The value for males at 2000 ppm was outside the historical control range, but that for females at 4000 ppm was within the historical control range. The differential white blood cell counts were correspondingly higher for both neutrophil and lymphocytes (nonsignificant) in males at 2000 ppm and for neutrophil and lymphocytes in females at 4000 ppm. Although the pattern of effects is consistent with a treatment relationship at the high dose, there were no histological or clinical correlates indicative of either infection or inflammation that would support the findings. No supporting findings related to an increase in white blood cell count in either sex at the high doses were observed, indicating that the increase was not toxicologically relevant.

Reticulocyte counts were elevated at the high doses in both sexes. However, they were within the historical control range, although close to the upper bound, and were without any correlating changes in other parameters related to tissues in monocyte–macrophage systems or anaemia. Therefore, this change was not considered to be toxicologically relevant.

Globulin levels were lower and the albumin/globulin ratios were higher in both sexes at the high doses, and both were outside the historical control ranges. However, the lack of corresponding findings related to immunosuppression, including histopathology in the immune system, suggests that the changes were not treatment related. Creatinine levels were higher in females at 4000 ppm, again above the historical control range. In males at 2000 ppm, urea values were reduced and below the historical control range. AST activity was higher in both sexes at the high doses, but the increase was of small magnitude, and values were within historical control ranges and not correlated with effects on either the other liver enzymes or histopathology. The increase in AST activity was considered not to be toxicologically relevant. Phosphate levels in plasma were elevated in both sexes at the high doses but were within the historical control range, and the differences were of small magnitude. Given the consistency across sexes and the statistical significance, the increase in phosphate levels is concluded to be potentially treatment related, but not of toxicological relevance.

The absolute thymus weights were higher in both sexes at 500 and 2000/4000 ppm and were above the historical control range. However, the male control absolute thymus weight was lower than the historical control range, and the female control absolute thymus weight was close to the bottom of the historical control range. Thymus weights are known to vary greatly in young rats, depending on age

and associated rapid increases in body weight. The study author concluded that the higher thymus weights at 500 ppm and 2000/4000 ppm in males and females were within the historical control ranges as a percentage of body weight and, furthermore, were not treatment related due to normal variation in thymus weights in young rats and the lack of any histopathological changes in the thymus. Given the lack of a dose–response relationship at the two highest doses, the lack of histological correlates and the fact that the control values were at or below the lower bound of the historical control range, the study author's conclusion is considered reasonable.

The NOAEL was 2000 ppm (equal to 143 mg/kg bw per day), the highest dose tested. Several findings observed in both sexes at the high doses were not considered adverse due to palatability, small magnitude or lack of toxicological relevance (Dymarkowska, 2015).

This short-term study of SYN508272 in rats (Dymarkowska, 2015) indicated that the key toxicological end-point, reduced body weight gain, was the same as for the parent compound, pydiflumetofen, although the NOAEL of the metabolite was lower than that of the parent compound, for which a NOAEL of 8000 ppm (equal to 619 mg/kg bw per day) was identified (Strepka, 2012b).

Genotoxicity. Three genotoxicity studies of SYN508272, in vitro (two tests) and in vivo (one test), were conducted (summarized in Table 23).

Table 23. Summary of genotoxicity assays with SYN508272

				Results		
Test system	**Test object**	**Concentration/ dose**	**Purity (%)**	**Without S9**	**With S9**	**Reference**
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvr*A/pKM101, WP2pKM101	5 000 µg/plate	94	Negative	Negative	Sokolowski (2014b)
Chromosomal aberration, mammalian (in vitro)	Human lymphocytes	1 860 µg/mL	94	Positive[a]	Negative[a]	Bohnenberger (2013b)
Point mutation (*tk* locus) assay	Mouse lymphoma L5178Y cells	1 860 µg/mL	94	Negative	Negative	Wollny (2013b)
Micronucleus assay in rat	Rat bone marrow cells	1 250 mg/kg bw	94	Negative	NA	Dony (2014b)

bw: body weight; NA: not applicable; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *tk*: thymidine kinase
[a] Precipitation observed at and above 1062.9 µg/plate.

An in vitro chromosomal aberration test in human lymphocytes was conducted to assess the potential of SYN508272 (lot/batch no. MES114/2; purity 94%) to induce structural chromosomal aberrations in cultured human lymphocytes in the absence and presence of an exogenous metabolic activation system (liver S9 mix from phenobarbital/β-naphthoflavone-treated male rats). In each experimental group, two parallel cultures were analysed. Per culture, at least 100 metaphases were evaluated for structural chromosomal aberrations, except for the positive controls in experiment II

without S9 mix, where only 50 metaphases were evaluated. The highest applied concentration of the test substance in this study was 1860.0 µg/mL, approximately 10 mmol/L.

In experiment I, in the absence and presence of S9 mix, and in experiment II, in the presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration. In experiment II, in the absence of S9 mix, and under long exposure conditions, concentrations showing clear cytotoxic effects were not evaluable for cytogenetic damage. However, the mitotic index was markedly reduced to 53.9% and 57.3% of control values at the two highest evaluated concentrations.

In experiment I, either with or without metabolic activation, no clastogenicity was observed at the concentrations evaluated. In experiment II, in the absence of S9 mix, one single statistically significant increase in chromosomal aberrations (4.5% aberrant cells, excluding gaps) was observed after treatment with 1062.9 µg/mL. Results at this concentration clearly exceeded the historical control range (0.0–2.5% aberrant cells, excluding gaps). In experiment II, in the presence of S9 mix, one single statistically significant increase in chromosomal aberrations (4.3% aberrant cells, excluding gaps) was observed after treatment with 347.1 µg/mL. Results at this concentration slightly exceeded the historical control range (0.0–3.5% aberrant cells, excluding gaps). However, in the presence of S9 mix, the effect was not reproducible in the two independent experiments performed. The response seen at 347.1 µg/mL, in the presence of S9 mix, was not part of a concentration-related response.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test substance compared with the control cultures. Appropriate mutagens were used as positive controls. They induced statistically significant increases ($P < 0.05$) in cells with structural chromosomal aberrations.

Under the experimental conditions reported, the test substance induced structural chromosomal aberrations in human lymphocytes in vitro. Therefore, SYN508272 was considered to be clastogenic in this chromosomal aberration test when tested up to precipitating or the highest evaluable concentrations (Bohnenberger, 2013b).

In conclusion, SYN508272, a metabolite in rats, was clastogenic in a chromosomal aberration test at the highest concentrations, but not mutagenic in an Ames test. Negative results in an in vivo genotoxicity test were obtained, indicating that this metabolite is not genotoxic in vivo.

It was concluded that the toxicity of SYN508272 would be covered by the parent compound.

CSAA798670

Metabolite CSAA798670 is also known as DF-pyrazole acid, CA4312, NOA449410 and M700F001 (Reg. No. 5069089, a metabolite of BAS 700 F). This metabolite has been previously identified in the rat biotransformation pathway for fluxapyroxad (BAS 700 F) (Annex 1, reference *127*). This chemical was also evaluated as part of the 2013 JMPR assessment of benzovindiflupyr as a general metabolite (Annex 1, reference *130*), although it is not identified in the rat biotransformation pathway. Similarly, this metabolite is associated with pydiflumetofen, but is not explicitly identified in either the mouse or rat biotransformation pathways provided for pydiflumetofen (see Figs 3 and 4, respectively).

Acute oral toxicity. An acute oral toxicity study of DF-pyrazole acid was conducted. Four fasted Sprague Dawley CD strain rats (two males and two females) were treated with DF-pyrazole acid (CA4312) (lot/batch no. and purity not reported) at a dose of 2000 mg/kg bw. Clinical signs and body weight were monitored during the study. All animals were subjected to gross necropsy.

There were no deaths and no clinical signs of systemic toxicity. All animals gained weight, and no effect on body weight was observed in surviving animals. There were no abnormalities seen at termination. The acute oral LD_{50} of DF-pyrazole acid (CA4312) in the Sprague Dawley CD strain rat was greater than 2000 mg/kg bw (Pooles, 2008).

Short-term studies of toxicity. In a short-term toxicity study, groups of five male and five female RccHan:Wistar (SPF) rats were administered CSAA798670 in the diet at a concentration of 0, 2000, 6000 or 12 000 ppm (equal to 0, 167, 511 and 1007 mg/kg bw per day for males and 0, 175, 572 and 1043 mg/kg bw per day for females, respectively) for at least 28 days. In addition to test guideline parameters, detailed clinical observations comprising open-field evaluation of clinical signs were performed once prior to treatment start and once weekly (during weeks 1–3) thereafter. FOBs, including the quantitative assessment of grip strength in the forelimbs and hindlimbs, were performed on all animals in randomized order in week 4. Locomotor activities were assessed after the FOB evaluation during week 4. Feed consumption values were recorded once prior to treatment start and daily throughout the study. Body weights were recorded twice prior to treatment start, weekly thereafter and before necropsy. Ophthalmoscopic examinations were performed in all animals once prior to treatment start and in all animals in week 4. At the end of the treatment period, all surviving animals were necropsied. Selected organs and tissues were processed and examined microscopically.

All animals survived. There were no treatment-related effects on clinical observations, ophthalmoscopic findings, FOB findings, including grip strength and locomotor activity, feed consumption, feed utilization, body weight, body weight gain, clinical pathology, macroscopic findings or organ weights. Hepatocellular hypertrophy was noted in livers of males and females at 6000 and 12 000 ppm, and this was accompanied by a lower incidence of glycogen deposition at 12 000 ppm. Considering the absence of any effect on liver weight or clinical biochemistry parameters related to liver function, it was considered that minimal hepatocellular hypertrophy is an adaptive response that does not represent an adverse effect. No effects on liver histopathology or any other parameters measured in this study were observed at 2000 ppm.

The NOAEL was 12 000 ppm (equal to 1007 mg/kg bw per day), the highest dose tested (Sommer, 2010).

In a 13-week oral toxicity study in rats, M700F001, Reg. No. 5069089 (metabolite of BAS 700 F) (lot/batch no. L80-68; purity 99.2%) was administered to Crl:WI (Han) rats at a nominal dose of 0, 100, 300 or 1000 mg/kg bw per day for at least 91 days. Dietary concentrations of the test substance were adjusted weekly based on actual body weight and feed consumption to meet the intended nominal doses.

No treatment-related changes or any indications of adverse effects were observed in any parameters examined.

The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Kaspers et al., 2009).

Developmental toxicity. In a developmental toxicity study, presumably pregnant Crl:KBL New Zealand white rabbits (31 does for treated groups and 32 does for control group) were administered the BAS 700 F metabolite M700F001 = Reg. No. 5069089 (lot/batch no. L80-68; purity 99.2%) by gavage at a daily dose of 0, 40, 100 or 250 mg/kg bw from GD 6 to GD 28.

No effects on maternal feed consumption or body weight development were observed. Likewise, no treatment-related necropsy findings were noted. Even though the conception rate of the time-mated rabbits was relatively low (72%, 87%, 90% and 84% at 0, 40, 100 and 250 mg/kg bw per day, respectively), a sufficient number of litters for evaluation was available (20, 25, 26 and 25 at 0, 40, 100 and 250 mg/kg bw per day, respectively). No treatment-related effects on gestational parameters (number of corpora lutea, implantation sites, preimplantation and postimplantation losses, early and late resorptions, number of dead and viable fetuses, placental or fetal weights, or sex ratio) were observed.

Fetal examination did not reveal any treatment-related external, visceral or skeletal malformations, variations or unclassified observations.

The NOAEL for maternal and embryo/fetal toxicity was 250 mg/kg bw per day, the highest dose tested (Schneider, Fabian & Mellert, 2009).

Genotoxicity. Genotoxicity assays conducted with CSAA798670 are summarized in Table 24. Overall, CSAA798670 was negative in all genotoxicity studies in vitro.

Table 24. Summary of genotoxicity assays with CSAA798670

				Results		
Test system	**Test object**	**Concentration/ dose**	**Purity (%)**	**Without S9**	**With S9**	**Reference**
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2*uvr*A/pKM101, WP2pKM101	Exp. I/IA: 3, 10, 33, 100, 333, 1 000, 2 500 and 5 000 µg/plate Exp. II: 33, 100, 333, 1 000, 2 500 and 5 000 µg/plate	99.6	Negative	Negative	Sokolowski (2007)
Chromosomal aberration, mammalian (in vitro)	Human lymphocytes	11.8, 20.6, 36.0, 63.0, 110.3, 193.0, 337.7, 591.0, 1 034.3, 1 810.0 µg/mL	97.4	Negative	Negative	Bohnenberger (2009)
Point mutation (*tk* locus) assay	Mouse lymphoma L5178Y cells	113.1, 226.3, 425.5, 905.0 and 1 810.0 µg/mL	97.4	Negative	Negative	Wollny (2009)

Exp.: Experiment; S9: 9000 × *g* supernatant fraction from rat liver homogenate; *tk*: thymidine kinase

The toxicological profile of CSAA798670 indicated that it was less potent than pydiflumetofen.

SYN547897

SYN547897 is present at greater than 10% in rat faeces (from bile). It was concluded that this metabolite and its conjugates would be covered by the parent compound.

Other metabolites

No toxicity data were provided for the metabolites SYN547948, SYN547890, SYN545547, SYN547891, SYN548265, SYN548263, SYN548264, SYN547893 or SYN547894. These metabolites were identified in the rat metabolism study.

(d) Mechanistic studies

A number of studies were conducted to investigate the MOA of liver tumour induction in male mice in the carcinogenicity study (Robertson, 2015) and follicular cell hypertrophy in the thyroid of male rats (Hackford, 2015; Shearer & Robertson, 2015).

Liver effects in mice

In a non-GLP-compliant study to examine liver effects, including induction of drug metabolism enzymes, histopathological changes and cell proliferation activity, male CD-1 mice (30 males per dose) were treated with pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at a dietary concentration of 0, 75 or 2250 ppm (equal to 0, 10.0 and 324.0 mg/kg bw per day, respectively) for up to 28 days. The animals were killed (10 per dose) on days 2, 7 and 28 after commencement of the treatment. The histopathological analysis was subjected to internal and external peer review.

There was no effect of pydiflumetofen on terminal body weights in either treatment group. Absolute and relative liver weights were increased at 2250 ppm after 7 and 28 days. AST levels in blood were statistically significantly lower than the control values, reaching 70% and 60% of control values at 75 ppm and 2250 ppm after 28 days of administration. This decrease was considered not to be toxicologically relevant. No other blood biochemistry parameter was changed. Total cytochrome P450 levels were unaffected by treatment at 75 ppm at all time points. Pentoxyresorufin *O*-depentylation (PROD) activities were minimally (1.6- to 2.4-fold), but not statistically significantly, increased at 75 ppm. At 2250 ppm, pydiflumetofen treatment statistically significantly elevated total cytochrome P450 levels (up to 2.1-fold) and PROD activity (up to 37-fold) after all three treatment durations. 7-Benzyloxyresorufin *O*-debenzylation (BROD) activity was not affected by the treatment. No morphological changes were found in the liver stained by haematoxylin and eosin at any time point at 75 ppm. At 2250 ppm, centrilobular hypertrophy was induced and its severity was enhanced with time up to the terminal time point. After 2 days of treatment at 2250 ppm, an increase in mitotic figures in the liver was also observed, but the increase was not observed following 7 or 28 days of dietary administration. The hepatocellular labelling index (S-phase) using uptake of 5-bromo-2′-deoxyuridine (BrdU) in the liver was slightly increased at 75 ppm by 3.1-, 2.3- and 5.6-fold relative to time-matched controls after 2, 7 and 28 days, respectively. These differences were statistically significant after 7 and 28 days. At 2250 ppm, substantial and statistically significant increases in the labelling index were observed. A maximal increase (13.6-fold) was observed after 2 days. After 7 and 28 days, the labelling index was increased to a lesser extent (5.4- and 5.6-fold relative to control values).

In conclusion, pydiflumetofen induced liver hypertrophy, increased cytochrome P450 levels and increased PROD activity after 2, 7 and 28 days of treatment. In addition, DNA synthesis (S-phase) was maximal after 2 days of treatment and accompanied by increased mitosis of hepatocytes. The treatment induced minimal impact on the liver at 75 ppm (Elcombe, 2015).

Cell proliferation and enzyme induction in mouse hepatocytes in vitro

Effects on cytotoxicity, PROD and BROD enzyme activities and hepatocyte proliferation were investigated in isolated male CD-1 mouse hepatocyte cultures in a non-GLP-compliant study. Phenobarbital sodium salt and epidermal growth factor were used as positive controls. Primary monolayer cultures of hepatocytes were prepared in 25 cm^2 flasks and 96-well and six-well plastic tissue culture plates in Leibowitz CL15 medium. Hepatocytes were exposed to pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at four concentrations (5, 10, 25 and 35 µmol/L), phenobarbital sodium salt at two concentrations (100 and 1000 µmol/L) or vehicle (0.5% v/v DMSO) alone for 96 hours. There were three replicates for each concentration in 25 cm^2 flasks for the measurement of PROD and BROD activities, five replicates for each concentration in six-well plates for replicative DNA synthesis analysis, and six replicates for each concentration in 96-well plates for cytotoxicity measurements as the change in cellular adenosine-5′-triphosphate (ATP).

Treatment with 35 µmol/L pydiflumetofen showed no cytotoxicity and no statistical reductions in intracellular ATP levels. PROD and BROD enzyme activities were increased at 5 and 10 µmol/L pydiflumetofen (up to 1.98-fold). In contrast, both PROD and BROD activities were lower than the vehicle control values following treatment with 25 and 35 µmol/L pydiflumetofen. The decreased PROD and BROD activities observed at higher concentrations were considered to be substrate competition between residual pydiflumetofen in the cell lysate preparation and pentoxyresorufin and benzyloxyresorufin, resulting in decreased enzymatic conversion of these substrates to form resorufin. Treatment of hepatocytes with 25 and 35 µmol/L pydiflumetofen resulted in a significant increase in hepatocyte proliferation as measured by replicative DNA synthesis (maximal at 25 µmol/L, resulting in a 1.90-fold increase relative to controls). The positive controls showed expected responses.

This study indicated that the treatment of cultured male CD-1 mouse hepatocytes with pydiflumetofen increased cell proliferation, measured as the change in replicative DNA synthesis (S-phase of the cell cycle), and increased PROD/BROD activity as an indicator of increased CYP2B/CYP3A activity in mouse hepatocytes, although dose dependency was not clear (Lowes, 2015a).

Cell proliferation and enzyme induction in human hepatocytes in vitro

Effects on cytotoxicity, PROD and BROD activities and replicative DNA synthesis (S-phase of the cell cycle) were investigated in cryopreserved male human hepatocyte cultures in a non-GLP-compliant study. Phenobarbital sodium salt and epidermal growth factor were used as positive controls. Primary monolayer cultures of hepatocytes were prepared in 25 cm^2 flasks and 96-well and six-well plastic tissue culture plates in Leibowitz HCL15 medium. Hepatocytes were exposed to pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at four concentrations (5, 10, 25 and 35 µmol/L), phenobarbital sodium salt at two concentrations (100 and 1000 µmol/L) or vehicle (0.5% v/v DMSO) alone for 96 hours. There were three replicates for each concentration in 25 cm^2 flasks for measurement of PROD and BROD activities, five replicates for each concentration in six-well plates for replicative DNA synthesis analysis and six replicates for each concentration in 96-well plates for cytotoxicity measurements, measured as the change in cellular ATP.

The treatment resulted in cytotoxicity at 25 µmol/L and above due to a statistically significant reduction of intracellular ATP levels (58.5% of the vehicle control value). The treatment with 5, 10, 25 and 35 µmol/L pydiflumetofen statistically significantly increased PROD activities in hepatocytes by 2.9-, 3.3-, 2.5- and 2.7-fold, respectively. Phenobarbital sodium salt treatment resulted in statistically significant increases in PROD activity. The treatment with 5, 10 and 25 µmol/L pydiflumetofen statistically significantly increased BROD activities by 3.4-, 5.9-, and 4.3-fold, respectively. Phenobarbital sodium salt treatment resulted in statistically significant increases in BROD activity. There were no statistically significant increases in cell proliferation, measured as the change in replicative DNA synthesis, following the treatment at any concentration. Positive control compounds showed expected results.

This study indicated that the treatment of cultured male human hepatocytes with pydiflumetofen had no effect on cell proliferation, measured as the change in replicative DNA synthesis (S-phase of the cell cycle). PROD and BROD activities were increased by the treatment, and these were an indicator of increased CYP2B6/CYP3A4 activity in human hepatocytes, although dose dependencies were not clear (Lowes, 2015b).

In vitro cell proliferation and enzyme induction activity in male human hepatocytes and male CD-1 mouse hepatocytes are compared in Table 25.

Table 25. Comparison of in vitro cell proliferation and enzyme induction activity in male human hepatocytes and male CD-1 mouse hepatocytes treated with pydiflumetofen[a]

	Male CD-1 mouse hepatocytes		Male human hepatocytes	
Treatment	**ATP (luminescence units released)[b]**	**S-phase labelling index (%)[c]**	**ATP (luminescence units released)[b]**	**S-phase labelling index (%)[c]**
Vehicle control (0.5% v/v DMSO)	674 253 ± 68 995 (100 ± 10.2)	2.65 ± 0.1 (100 ± 3.77)	268 057 ± 19 089 (100 ± 7.1)	0.27 ± 0.06 (100 ± 23.08)
PB				
100 µmol/L	595 367 ± 16 637* (88.0 ± 2.5)	3.39 ± 0.27** (127.93 ± 10.34)	352 620 ± 47 400* (132 ± 17.7)	0.30 ± 0.09 (109.38 ± 33.37)
1 000 µmol/L	547 991 ± 27 707** (81.0 ± 4.1)	4.41 ± 0.43** (166.48 ± 16.39)	291 060 ± 70 476 (109 ± 26.3)	0.31 ± 0.06 (116.01 ± 24.01)
Pydiflumetofen				
5 µmol/L	588 475 ± 21 538** (87 ± 3.2)	3.54 ± 0.81 (133.92 ± 30.55)	329 623 ± 44 433** (123 ± 16.6)	0.26 ± 0.06 (97.90 ± 21.65)
10 µmol/L	611 652 ± 24 815* (91.0 ± 3.7)	3.54 ± 0.57 (133.89 ± 21.64)	284 562 ± 29 279 (106 ± 10.9)	0.32 ± 0.14 (120.27 ± 50.20)
25 µmol/L	567 202 ± 19 849** (84.0 ± 2.9)	5.03 ± 0.51** (189.86 ± 19.38)	204 833 ± 25 385** (76 ± 9.5)	0.10 ± 0.06** (36.14 ± 23.31)
35 µmol/L	555 771 ± 23 090** (82.0 ± 3.4)	3.99 ± 0.50** (150.90 ± 18.84)	178 281 ± 18 062** (67 ± 6.7)	0.07 ± 0.05** (25.58 ± 17.88)
EGF				
25 ng/mL	–	19.69 ± 3.14** (743.73 ± 118.49)	–	1.76 ± 0.24** (650.01 ± 89.84)

	Male CD-1 mouse hepatocytes		Male human hepatocytes	
Treatment	**PROD (pmol resorufin/mg per minute)[d]**	**BROD (pmol resorufin/mg per minute)[d]**	**PROD (pmol resorufin/mg per minute)[d]**	**BROD (pmol resorufin/mg per minute)[d]**
Vehicle control (0.5% v/v DMSO)	21.26 ± 3.35 (100 ± 15.8)	86.40 ± 36.82 (100 ± 42.6)	0.11 ± 0.017 (100.0 ± 15.3)	1.15 ± 0.062 (100 ± 5.4)
PB				
100 µmol/L	40.36 ± 4.65** (189.9 ± 21.9)	160.93 ± 19.54* (186.3 ± 22.6)	0.21 ± 0.025** (189.9 ± 22.8)	3.05 ± 0.16** (265.5 ± 14.2)
1 000 µmol/L	77.13 ± 8.76** (362.9 ± 41.2)	274.3 ± 25.49** (317.5 ± 29.5)	0.37 ± 0.045** (332.4 ± 41.4)	8.53 ± 0.65** (743.7 ± 56.4)
Pydiflumetofen				
5 µmol/L	39.25 ± 10.23** (184.7 ± 48.1)	171.48 ± 46.89* (198.5 ± 54.3)	0.32 ± 0.086* (293.6 ± 78.0)	3.87 ± 1.05* (336.9 ± 91.7)
10 µmol/L	36.41 ± 5.22* (171.3 ± 24.5)	170.23 ± 32.23* (197.3 ± 37.3)	0.37 ± 0.054** (332.3 ± 48.9)	6.80 ± 0.79** (593.1 ± 69.0)

Treatment	Male CD-1 mouse hepatocytes		Male human hepatocytes	
	ATP (luminescence units released)[b]	S-phase labelling index (%)[c]	ATP (luminescence units released)[b]	S-phase labelling index (%)[c]
25 µmol/L	11.54 ± 3.23	63.12 ± 7.74	0.28 ± 0.055**	4.90 ± 0.67**
	(54.3 ± 15.2)	(73.1 ± 9.0)	(254.8 ± 49.9)	(427.4 ± 57.9)
35 µmol/L	3.34 ± 2.01	16.78 ± 11.20	0.29 ± 0.018**	3.66 ± 1.70
	(15.7 ± 9.5)	(19.4 ± 13.0)	(267.2 ± 16.1)	(318.9 ± 148.0)

ATP: adenosine 5′-triphosphate; BROD: benzyloxyresorufin *O*-debenzylation; DMSO: dimethyl sulfoxide; EGF: epidermal growth factor; PB: phenobarbital sodium salt; PROD: pentoxyresorufin *O*-dependentylation; S-phase: replicative DNA synthesis; SD: standard deviation; v/v: volume per volume; *: $P < 0.05$; **: $P < 0.01$ (analysis of variance with Dunnett's post-test)

[a] Values are mean ± SD. Values in parentheses are mean % control ± SD.

[b] $n = 6$ per group.

[c] $n = 5$ per group.

[d] $n = 3$ per group.

Source: Lowes (2015a,b)

Transactivation assay with mouse, rat and human constitutive androstane receptors

The ability to directly activate the constitutive androstane receptor (CAR, also known as nuclear receptor subfamily 1, group I, member 3, or NR1I3) by pydiflumetofen treatment was investigated in a non-GLP-compliant study using a reporter assay. Briefly, complementary DNA expression vectors for constitutive androstane receptor-3 (CAR3) variants of mouse, rat and human CAR were transfected into COS-1 cells, along with necessary cofactors and a *Cyp2b6* response element–luciferase reporter construct. After a suitable expression time, the cells were incubated with pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at a concentration of 1, 3, 10 or 30 µmol/L. The direct CAR activator artemisinin was also incubated at these same concentrations, and model direct-acting substrates of CAR3 for mouse (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, or TCPOBOP), rat (clotrimazole) or human ((6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl) oxime), or CITCO) were each incubated at a single concentration (0.5, 10 and 5 µmol/L for TCPOBOP, clotrimazole and CITCO, respectively). Light emission from the luciferase reporter was quantified to indicate the extent of CAR activation upon incubation with suspected ligands, including pydiflumetofen. Results were reported as normalized luciferase activity and fold change compared with a DMSO solvent control. The CAR3 reporter assay is accepted as a sensitive method for detecting direct activators of CAR, and it does not respond to indirect activators of CAR such as phenobarbital salt, because the second messenger systems that are believed to be responsible for indirect activation are not present in COS-1 cells.

In the human, mouse and rat assays using the respective species' CAR3 reporter constructs, a strong concentration-dependent activation of rodent and human CAR3 was observed. In rodents, pydiflumetofen produced up to 42-fold activation of rat CAR3 and up to 34-fold activation of mouse CAR3. The response in mouse CAR3 approached a maximum at the lower test concentrations of 1 µmol/L (24-fold) to 3 µmol/L (34-fold) and was slightly lower at the highest mouse test concentration of 30 µmol/L (20-fold), which correlated with decreased viability in the COS-1 cells at 10–30 µmol/L. The treatment activated human CAR3 in a concentration-responsive manner, and the maximal response (15-fold) at 30 µmol/L was higher than that of the human CAR positive control compound, CITCO (10-fold). The model activators CITCO, TCPOBOP and clotrimazole produced robust responses in human, mouse and rat CAR3 constructs, respectively. Artemisinin produced a concentration-dependent response that was much more marked with rat CAR3 than with human or mouse CAR3.

This study showed that pydiflumetofen was a direct activator of CAR from mice, rats and humans with high efficacy (i.e. maximal fold change compared with model activators) in all three species.

The summary of this study is shown in Table 26 (Omiecinski, 2014).

Table 26. Comparison of CAR3 transactivation assays among mice, rats and humans treated with pydiflumetofen

Construct	Treatment	Dose	Normalized luciferase activity	Fold change
Empty vector	DMSO	–	0.011 60	1.000
	Phenobarbital	1 mmol/L	0.010 39	0.895 7
	CITCO	5 µmol/L	0.010 29	0.886 6
	TCPOBOP	0.5 µmol/L	0.008 662	0.746 6
	Clotrimazole	10 µmol/L	0.016 25	1.400
	Pydiflumetofen	30 µmol/L	0.008 446	0.727 9
	Artemisinin	30 µmol/L	0.010 04	0.865 6
Human CAR3	DMSO	–	0.006 284	1.000
	Phenobarbital	1 mmol/L	0.005 459	0.868 7
	CITCO	5 µmol/L	0.064 98**	10.34
	Pydiflumetofen	1.0 µmol/L	0.009 235	1.470
		3.0 µmol/L	0.030 06	4.784
		10 µmol/L	0.079 40**	12.64
		30 µmol/L	0.093 05**	14.81
	Artemisinin	1.0 µmol/L	0.007 721	1.229
		3.0 µmol/L	0.010 19	1.621
		10 µmol/L	0.017 77	2.829
		30 µmol/L	0.051 29**	8.162
Mouse CAR3	DMSO	–	0.011 44	1.000
	Phenobarbital	1 mmol/L	0.023 63**	2.065
	TCPOBOP	0.5 µmol/L	0.518 7**	45.33
	Pydiflumetofen	1.0 µmol/L	0.275 0**	24.03
		3.0 µmol/L	0.385 7**	33.70
		10 µmol/L	0.363 9**	31.80
		30 µmol/L	0.229 4**	20.05
	Artemisinin	1.0 µmol/L	0.021 70	1.897
		3.0 µmol/L	0.045 02**	3.934
		10 µmol/L	0.125 8**	11.00
		30 µmol/L	0.147 7**	12.91
Rat CAR3	DMSO	–	0.005 455	1.000
	Phenobarbital	1 mmol/L	0.032 47**	5.953
	Clotrimazole	10 µmol/L	0.520 5**	95.43
	Pydiflumetofen	1.0 µmol/L	0.015 53	2.847
		3.0 µmol/L	0.077 95**	14.29
		10 µmol/L	0.020 08**	36.82

Construct	Treatment	Dose	Normalized luciferase activity	Fold change
		30 µmol/L	0.228 8**	41.95
	Artemisinin	1.0 µmol/L	0.015 97	2.927
		3.0 µmol/L	0.061 15	11.21
		10 µmol/L	0.206 8**	37.91
		30 µmol/L	0.286 0**	52.44

CAR: constitutive androstane receptor; CITCO: 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl) oxime; DMSO: dimethyl sulfoxide; TCPOBOP: 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene; **: $P < 0.01$
Source: Omiecinski (2014)

Ex vivo enzyme analysis of mouse liver from the 28-day dietary study

Effects of pydiflumetofen on liver peroxisomal and microsomal enzyme expression in male and female mice were investigated in a non-GLP-compliant study. Male and female mice were administered diet containing pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at 500–7000 ppm for 28 days. Animals in the satellite study group were administered pydiflumetofen at 7000 ppm for 3 or 7 days. At termination, liver tissue was dissected, snap frozen and dispatched to CXR Biosciences (Dundee, United Kingdom) for analysis. The frozen liver chunks were processed to heavy pellets and microsomes. The heavy pellet samples prepared from these livers were assayed for total protein content and the activity of CN^--insensitive palmitoyl coenzyme A (CoA) oxidase. The microsomal samples prepared from these livers were assayed for total protein content, total cytochrome P450 content, the activities of ethoxyresorufin *O*-deethylation (EROD), PROD, benzyloxyquinoline *O*-debenzylation (BQ) and lauric acid 12-hydroxylation (LAH).

Administration of pydiflumetofen at 500–7000 ppm did not stimulate hepatic peroxisomal palmitoyl CoA oxidation and minimally increased (less than 4-fold) microsomal LAH in both sexes. This indicates that pydiflumetofen is not a peroxisome proliferator. Pydiflumetofen induced total cytochrome P450, PROD and BQ in a dose-dependent manner. A marked induction (up to 20-fold) of PROD, a marker for CYP2B enzymes, was observed. Increases in EROD, a marker for CYP1A enzymes, were minimal (up to 1.4-fold). Similar dose–response relationships were seen in both sexes except in the case of BQ, where much greater fold increases were found in females. This was considered to be due to lower constitutive CYP3A activities, which were more commonly seen in female mice than in male mice. Cytochrome P450, EROD, PROD and BQ activities increased time dependently in both sexes.

A summary of the enzyme analysis is shown in Table 27.

Table 27. Summary of ex vivo hepatic enzyme analysis in mice treated with pydiflumetofen

	Males					Females				
Enzymes	**0 ppm**	**500 ppm**	**1 500 ppm**	**4 000 ppm**	**7 000 ppm**	**0 ppm**	**500 ppm**	**1 500 ppm**	**4 000 ppm**	**7 000 ppm**
PCO (nmol NAD^+ reduced/min per mg protein)	15.47	9.97**	12.95	11.22*	10.69**	14.10	8.31	7.16**	7.97*	6.78*
Total P450 (nmol/mg protein)	0.51	0.78**	0.84**	0.84**	0.90**	0.55	0.65	0.74*	0.95**	0.87**
EROD (pmol resorufin	25.5	18.02*	18.36	21.22	34.77	23.79	26.22	19.14	32.84*	33.06**

Enzymes	Males					Females				
	0 ppm	500 ppm	1 500 ppm	4 000 ppm	7 000 ppm	0 ppm	500 ppm	1 500 ppm	4 000 ppm	7 000 ppm
formed/min per mg protein)										
PROD (pmol resorufin formed/min per mg protein)	2.77	32.68**	25.55**	24.97**	42.50**	7.50	38.77**	40.30**	31.70**	23.57**
BQ (nmol 7-hydroxy-quinoline formed/min per mg protein)	1.73	2.58**	2.05	4.63**	6.13**	4.31	4.28	6.38	10.79**	10.82**
LAH (nmol 12-OH lauric acid formed/10 min per mg protein)	4.12	5.39	10.35**	10.16**	14.46**	4.19	2.91	4.13	2.14**	4.22

BQ: benzyloxyquinoline *O*-debenzylation; EROD: ethoxyresorufin *O*-deethylation; LAH: lauric acid 12-hydroxylation; NAD^+: nicotinamide adenine dinucleotide (oxidized); P450: cytochrome P450; PCO: CN^--insensitive palmitoyl coenzyme A oxidation; ppm: parts per million; PROD: pentoxyresorufin *O*-depentylation; *: $P < 0.05$; **: $P < 0.01$
Source: Haines (2012)

This study indicated that pydiflumetofen did not demonstrate the prototypical properties of peroxisome proliferators in male or female mice. Pydiflumetofen was considered to have similar properties to those of phenobarbital (Haines, 2012).

Proposed MOA for the formation of mouse liver tumours

MOA and relevance to humans for liver tumour induction in mice by pydiflumetofen were postulated based on the international framework of the International Programme on Chemical Safety (IPCS) of WHO and the International Life Sciences Institute (ILSI). The data, including mechanistic studies and all toxicity studies of pydiflumetofen provided in the present evaluation, as well as current knowledge regarding liver tumour induction, support a proposed MOA involving the following key events:

- activation of CAR;
- an early, transient increase in hepatocellular proliferation;
- increased hepatocellular foci as a result of clonal expansion of spontaneously mutated (initiated) cells; and
- eventual progression to liver tumours.

Based on the qualitative species differences in the hepatocellular proliferation response to pydiflumetofen, it has been established that this MOA is not relevant to humans. In conclusion, these data support the conclusion that pydiflumetofen does not pose a hepatocarcinogenic hazard to humans.

Details of this MOA are described in Appendix 2 (Cowie, 2015).

Rat thyroid peroxidase activity

Effects on rat thyroid peroxidase activity were investigated in vitro. The aim of this study was to evaluate the effect of pydiflumetofen (lot/batch no. SMU2EP12007; purity 98.5%) at 0, 0.007, 0.1,

1.5 and 10 µmol/L on rat thyroid peroxidase activity in vitro. A pooled thyroid gland microsomal preparation from five rats was assayed for thyroid peroxidase activity by determining the monoiodination of L-tyrosine using a method based on studies by Doerge et al. (1998) and Freyberger & Ahr (2006). As a positive control, the effect of 6-propyl-2-thiouracil (PTU) at 10 µmol/L on rat thyroid peroxidase activity was also determined.

Preparations of thyroid gland microsomes from male Wistar Han rats (age range 67–74 days) were obtained from Charles River UK Ltd. A whole homogenate of the pooled thyroid glands from five rats was prepared in 0.154 mol/L potassium chloride containing 50 mmol/L Tris-hydrochloride (pH 7.4).

Pydiflumetofen at 0.007, 0.1, 1.5 and 10 µmol/L had no significant effect on thyroid peroxidase activity. In contrast, the addition of 10 µmol/L PTU, the positive control compound, resulted in a 99.9% inhibition of thyroid peroxidase activity (Table 28).

Table 28. Effects of pydiflumetofen and PTU on rat thyroid peroxidase activity

Addition[a]	Thyroid peroxidase activity (nmol/min per mg protein)[b,c]
Control (DMSO only)	7.07 ± 0.546 (100)
Pydiflumetofen 0.007 µmol/L	6.81 ± 0.329 (96)
Pydiflumetofen 0.1 µmol/L	6.72 ± 0.699 (95)
Pydiflumetofen 1.5 µmol/L	6.76 ± 0.217 (96)
Pydiflumetofen 10 µmol/L	7.05 ± 0.197 (100)
PTU 10 µmol/L	0.007 ± 0.001 5** (0.1)

DMSO: dimethyl sulfoxide; PTU: 6-propyl-2-thiouracil; SD: standard deviation; **: $P < 0.01$

[a] Pydiflumetofen and PTU were added in DMSO (2.5 µL/incubation).

[b] Results are presented as mean ± SD of either quadruplicate (control) or triplicate (all concentrations of pydiflumetofen and PTU) incubations.

[c] Values in parentheses are percentage of control levels.

Source: Lake (2014)

Pydiflumetofen was not an inhibitor of rat thyroid peroxidase activity in vitro (Lake, 2014).

Rat hepatic microsomal protein content and UGT activity

Using the liver samples of male rats obtained from the 91-day toxicity study (Shearer & Robertson, 2015), hepatic microsomal protein content and hepatic microsomal UGT activity were measured.

The treatment of male rats with 1500 or 8000 ppm pydiflumetofen for 91 days statistically significantly increased hepatic microsomal protein content. The treatment of male rats with pydiflumetofen induced hepatic microsomal UGT activity at all treated doses in a dose-dependent manner.

This study demonstrated that pydiflumetofen was an inducer of hepatic microsomal UGT activity towards thyroxine as a substrate in male rats. Treatment with 250 ppm pydiflumetofen for 91 days resulted in statistically significant increases in hepatic microsomal UGT activity towards thyroxine as substrate expressed as specific activity and per gram of liver, whereas treatment with 1500 and 8000 ppm pydiflumetofen resulted in statistically significant increases in enzyme activity expressed as specific activity, per gram of liver, per total liver weight and per relative liver weight (Table 29).

Table 29. Effects of pydiflumetofen on hepatic microsomal protein content and UGT activity in rats

	Males					Females				
Parameter	**0 ppm**	**250 ppm**	**1 500 ppm**	**8 000 ppm**	**16 000 ppm**	**0 ppm**	**250 ppm**	**1 500 ppm**	**8 000 ppm**	**16 000 ppm**
Hepatic microsomal protein content (mg/g liver)										
Mean	33.4	35.8	38.0	40.8**	NE	NE	NE	NE	NE	NE
(%)	(100)	(107)	(114)	(122)						
SD	3.82	3.43	2.89**	2.96						
UGT in the liver[a]										
A, Mean	5.6	8.5*	9.6**	16.1**	NE	NE	NE	NE	NE	NE
SD	1.4	2.8	2.71	2.37						
B, Mean	189	306*	366**	656**	NE	NE	NE	NE	NE	NE
SD	58.9	115	117	111						
C, Mean	2.77	4.39	6.20	11.66**	NE	NE	NE	NE	NE	NE
SD	1.03	1.66	1.57	2.21						
D, Mean	6.34	10.46	15.2	30.8**	NE	NE	NE	NE	NE	NE
SD	2.16	3.46	4.45	5.40						

bw: body weight; NE: not examined; ppm: parts per million; SD: standard deviation; UGT: uridine diphosphate glucuronosyltransferase activity (substrate thyroxine); *: $P < 0.05$; **: $P < 0.01$

[a] Units are as follows: A, pmol/min per mg protein; B, pmol/min per g liver; C, nmol/min per total liver weight; D, nmol/min per total liver weight per kg bw.

Source: Lake (2015)

These increases in microsome protein and UGT activity in the rat liver obtained from the present study indicated that the thyroid finding was secondary to phase II enzyme induction of metabolism in the liver (Lake, 2015). Whereas thyroxine and thyroid stimulating hormone levels in blood were not measured, the evidence suggested that follicular cell hypertrophy found in males at 1500 ppm and above and in females at 8000 ppm and above was a secondary effect on the thyroid resulting from overexcretion of thyroxine from blood due to liver phase II enzyme induction.

(e) Microbiological effects

The fungicide pydiflumetofen was evaluated at the 2018 JMPR to determine its impact on microbiota in the gastrointestinal tract. As no data were submitted by the sponsors, a literature search was performed using a number of search engines. These included BioOne (http://www.bioone.org/), Google (https://www.google.com/), Google Scholar (http://scholar.google.com/), PubMed (http://www.ncbi.nlm.nih.gov/pubmed), ScienceDirect (http://www.sciencedirect.com/) and Web of Science (https://apps.webofknowledge.com).

The search strategy used the input keywords of the fungicide chemical name (pydiflumetofen), chemical structure, antimicrobial mode of action, antimicrobial spectrum of activity, antimicrobial resistance, resistance mechanisms and genetics, microbiome, microbiota, gut microbiota, gut microbiome, gastrointestinal microbiota, gastrointestinal microbiome, etc., as well as the Boolean operators AND, OR and NOT.

The extensive search and review of the scientific literature did not find any reports on the effects of pydiflumetofen on the intestinal microbiome to include in the toxicological risk assessment.

3. Observations in humans

In a database of incidents involving chemical exposure of workers (2002–2017), no records of adverse health effects were reported during synthesis or formulation activities (Syngenta, 2018). Furthermore, no epidemiological studies have been conducted with pydiflumetofen.

Comments

Biochemical aspects

In rats administered radiolabelled pydiflumetofen by gavage, the oral absorption of total radioactivity was 85–90% at a dose of 5 mg/kg bw. Absorption became limited at 100 mg/kg bw (females: 50–55%) and 300 mg/kg bw (males: 19–24%). Pydiflumetofen was widely distributed, with highest concentrations of radioactivity observed in the liver and kidney. There was no evidence for accumulation. Following a single oral gavage dose of 5 mg/kg bw, excretion was rapid (at least 91% within 48 hours). Excretion was predominantly in faeces via the bile. Systemic exposure (AUC_{0-t}) of pydiflumetofen was nonlinear from 300 mg/kg bw in males and from 100 mg/kg bw in females. Following a single oral administration of radiolabelled pydiflumetofen, C_{max} was observed at 0.5–2 hours at 5 mg/kg bw and at 8 hours at 100 mg/kg bw and above (Punler & Harris, 2014b; Hutton, 2015a,b; Hutton & O'Hagan, 2015; MacDonald & Jewkes, 2015; Penn, 2015a; Webbley & Williams, 2015).

In rats, unchanged parent accounted for less than 4% of the 5 mg/kg bw dose by oral administration. The primary metabolic routes included demethoxylation, *N*-dealkylation, monohydroxylation and dihydroxylation, *O*-demethylation, and oxidative and reductive dechlorination. Numerous metabolites were detected, with only two metabolites each accounting for between 10% and 15% of the dose: 1) 2,4,6-TCP sulfate (phenyl label) and 2) SYN548263 (pyrazole label). The majority of these metabolites were also monohydroxylated and dihydroxylated and in many cases were conjugated with glucuronic acid or sulfate (MacDonald & Jewkes, 2015).

Studies in mice demonstrated similar toxicokinetics and biotransformation as were found in rats (Punler & Harris, 2014a; Tomlinson et al., 2015). The toxicokinetics of pydiflumetofen in pregnant rabbits indicated a subproportional increase in systemic exposure with dose, with no increase in systemic exposure with doses of 750 mg/kg bw or higher (Penn, 2015a).

Toxicological data

In rats, the acute oral LD_{50} of pydiflumetofen was greater than 5000 mg/kg bw (Petus-Árpásy, 2012), the acute dermal LD_{50} was greater than 5000 mg/kg bw (Petus-Árpásy, 2013) and the 4-hour acute inhalation LC_{50} was greater than 5.11 mg/L (Nagy, 2013). Pydiflumetofen showed no skin irritation in rabbits (Török-Bathó, 2012a), showed mild irritation to rabbit eyes (Török-Bathó, 2012b) and was not sensitizing in mice (Hargitai, 2013).

The main toxic effects of pydiflumetofen in short- and long-term toxicity studies were reduced body weight and effects in the liver in mice, rats and dogs.

In a 91-day toxicity study in mice fed pydiflumetofen at a dietary concentration of 0, 100, 500, 4000 or 7000 ppm (equal to 0, 17.5, 81.6, 630 and 1158 mg/kg bw per day for males and 0, 20.4, 106, 846 and 1483 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 81.6 mg/kg bw per day), based on increased cholesterol concentrations associated with liver hypertrophy at 4000 ppm (equal to 630 mg/kg bw per day) (Shearer, 2015).

In a 28-day toxicity study in rats administered pydiflumetofen at a dietary concentration of 0, 500, 4000, 8000 or 16 000 ppm (equal to 0, 43, 343, 677 and 1322 mg/kg bw per day for males and 0, 40, 322, 619 and 1174 mg/kg bw per day for females, respectively), the NOAEL was 8000 ppm (equal to 619 mg/kg bw per day), based on reduction of body weight gain at 16 000 ppm (equal to 1174 mg/kg bw per day) (Strepka, 2012b).

In a 91-day toxicity study in rats administered pydiflumetofen in the diet at a concentration of 0, 250, 1500, 8000 or 16 000 ppm (equal to 0, 18.6, 111, 587 and 1187 mg/kg bw per day for males and 0, 21.6, 127, 727 and 1325 mg/kg bw per day for females, respectively), the NOAEL was 250 ppm (equal to 18.6 mg/kg bw per day), based on follicular cell hypertrophy in the thyroid at 1500 ppm (equal to 111 mg/kg bw per day) (Shearer & Robertson, 2015). The Meeting noted that MOA studies for the effect of pydiflumetofen on the thyroid indicated that the induction of follicular cell hypertrophy by pydiflumetofen at high doses was secondary to increased hepatic microsomal UGT activity, not a direct effect on the thyroid. Therefore, the Meeting concluded that the follicular cell hypertrophy in the thyroid of rats would not be relevant to humans (Lake, 2014, 2015).

In a 13-week oral toxicity study in dogs treated with pydiflumetofen in capsules at 0, 30, 300 or 1000 mg/kg bw per day, the NOAEL was 300 mg/kg bw per day, based on reduced body weight gain and increased triglycerides at 1000 mg/kg bw per day (Blunt, 2015a).

In a 1-year oral toxicity study in dogs administered pydiflumetofen in capsules at a dose of 0, 30, 100 or 300 mg/kg bw per day, the NOAEL was 300 mg/kg bw per day, the highest dose tested (Blunt, 2015b).

The overall NOAEL for pydiflumetofen in dogs was 300 mg/kg bw per day, and the overall lowest-observed-adverse-effect level (LOAEL) was 1000 mg/kg bw per day.

In an 18-month carcinogenicity study in mice administered pydiflumetofen at a dietary concentration of 0, 75, 375 or 2250 ppm (equal to 0, 9.2, 45.4 and 287.9 mg/kg bw per day for males and 0, 9.7, 48.4 and 306.2 mg/kg bw per day for females, respectively), the NOAEL for toxicity was 375 ppm (equal to 45.4 mg/kg bw per day), based on reduced body weight gain in males at 2250 ppm (equal to 287.9 mg/kg bw per day). The NOAEL for carcinogenicity was 75 ppm (equal to 9.2 mg/kg bw per day), based on an increased incidence of liver tumours in male mice at 375 ppm (equal to 45.4 mg/kg bw per day) (Robertson, 2015).

The results of several mechanistic studies of the liver effects of pydiflumetofen in mice (Cowie, 2015; Elcombe, 2015; Lowes, 2015a) indicate that pydiflumetofen has a CAR-mediated MOA in the induction of liver tumours. The MOA for these tumours was assessed using the IPCS/ILSI human relevance framework. It was concluded that these carcinogenic responses are not relevant to humans.

In a 2-year toxicity and carcinogenicity study in rats administered pydiflumetofen at a dietary concentration of 0, 200, 1000 or 6000 ppm for males (equal to 0, 9.9, 51.0 and 319 mg/kg bw per day, respectively) or 0, 150, 450 or 1500 ppm for females (equal to 0, 10.2, 31.0 and 102 mg/kg bw per day, respectively), the NOAEL for toxicity was 200 ppm (equal to 9.9 mg/kg bw per day), based on lower body weight and reduced body weight gain in males at 1000 ppm (equal to 51.0 mg/kg bw per day). No treatment-related increases in tumour incidence were observed (Robertson, 2016).

The Meeting concluded that pydiflumetofen is carcinogenic in male mice, but not in female mice or rats.

Pydiflumetofen was tested for genotoxicity in an adequate range of in vitro and in vivo assays (Roth, 2012; Sokolowski, 2012, 2014a; Bohnenberger, 2013a; Wollny, 2013a; Dony, 2014a). It gave a positive response in a chromosomal aberration mammalian test using human lymphocytes without S9 (Bohnenberger, 2013a), but a negative response in the micronucleus test in vivo (Roth, 2012; Dony, 2014a).

The Meeting concluded that pydiflumetofen is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo, the absence of carcinogenicity in rats and female mice and evidence that the tumours in male mice are not relevant to humans, the Meeting concluded that pydiflumetofen is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study, rats were given pydiflumetofen in the diet at a concentration of 0, 150, 750 or 4500 ppm (equal to 0, 9.1, 46.1 and 277 mg/kg bw per day, respectively) for males and 0, 150, 450 or 1500 ppm (equal to 0, 12.6, 33.7 and 115 mg/kg bw per day, respectively)

for females. The NOAEL for parental toxicity was 750 ppm (equal to 46.1 mg/kg bw per day), based on reduced body weight and effects on the thyroid (increased thyroid weight and minimal thyroid follicular cell hypertrophy) in males at 4500 ppm (equal to 277 mg/kg bw per day). The NOAEL for offspring toxicity was 450 ppm (equal to 33.7 mg/kg bw per day), based on reduced pup body weight before weaning in the F_1 generation at 1500 ppm (equal to 115 mg/kg bw per day). The NOAEL for reproductive toxicity was 1500 ppm (equal to 115 mg/kg bw per day), the highest dose tested (Hackford, 2015).

In a developmental toxicity study, pregnant rats were administered pydiflumetofen by gavage at a dose of 0, 10, 30 or 100 mg/kg bw per day during GDs 6–19. The NOAEL for maternal toxicity was 30 mg/kg bw per day, based on reductions in body weight gain and feed consumption at the beginning of dosing at 100 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 100 mg/kg bw per day, the highest dose tested (Davies, 2015).

In a developmental toxicity study in which rabbits were administered pydiflumetofen by gavage at a dose of 0, 10, 100 or 500 mg/kg bw per day during GDs 6–27, the NOAEL for maternal and embryo/fetal toxicity was 500 mg/kg bw per day, the highest dose tested (Penn, 2015c).

The Meeting concluded that pydiflumetofen is not teratogenic.

In an acute neurotoxicity study in rats administered pydiflumetofen by gavage at a dose of 0, 100 (females) or 300 (males), 1000 or 2000 mg/kg bw, the NOAEL for systemic toxicity was 100 mg/kg bw, based on clinical signs, lower body temperature and decreased locomotor activity in females at 1000 mg/kg bw. There was no evidence of neuropathological changes. The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested (Broich, 2015a).

In an additional acute neurotoxicity study in female rats administered pydiflumetofen by gavage at a dose of 0, 100, 300 or 1000 mg/kg bw, the NOAEL for systemic toxicity was 100 mg/kg bw, based on clinical signs, lower body weight and decreased locomotor activity at 300 mg/kg bw. The NOAEL for neurotoxicity was 1000 mg/kg bw, the highest dose tested (Broich, 2015b).

The overall NOAEL for systemic toxicity in the two acute neurotoxicity studies in rats was 100 mg/kg bw.

Although a subchronic neurotoxicity study was not submitted, the Meeting noted that FOBs were performed in repeated-dose studies in mice, rats and dogs. No effects were observed.

The Meeting concluded that pydiflumetofen is not neurotoxic.

Although no immunotoxicity study was submitted, there was no indication of immunotoxicity in the submitted toxicity studies. The Meeting concluded that pydiflumetofen is not immunotoxic.

There was no information about the effect of pydiflumetofen on the microbiome of the human gastrointestinal tract based on a search of the literature.

Toxicological data on metabolites and/or degradates

2,4,6-Trichlorophenol (2,4,6-TCP)

2,4,6-TCP was the major circulating metabolite of pydiflumetofen in rats (representing up to 5.3% in plasma) and is identified in animal commodities only. 2,4,6-TCP sulfate was identified in plasma at 32–44% (MacDonald & Jewkes, 2015; Tomlinson et al., 2015).

WHO has established a drinking-water guideline value of 0.2 mg/L for 2,4,6-TCP, although it is generally found in drinking-water at concentrations below 0.001 mg/L. Like other chlorophenols (e.g. 2-chlorophenol and 2,4-dichlorophenol), 2,4,6-TCP is most likely to occur in drinking-water as a by-product of chlorination (WHO, 2017).

The Meeting noted that this metabolite was identified in animal commodities and concluded that the toxicity of 2,4,6-TCP and its conjugates would be covered by the parent compound.

SYN547897 (livestock and rat metabolite)

SYN547897 is present at greater than 10% in rat faeces (from bile). Therefore, the Meeting concluded that this metabolite and its conjugates would be covered by the parent compound.

SYN508272 (Reg. No. 5621781)

SYN508272 was found in rats. The precursor of this metabolite was SYN548263, a major metabolite in rats. The acute oral LD_{50} of SYN508272 was greater than 500 mg/kg bw and less than 2000 mg/kg bw (Cords & Lammer, 2009).

In a 28-day toxicity study in which rats were administered diets containing SYN508272 at a concentration of 0, 100, 500 or 2000 ppm (males) / 4000 ppm (females) (equal to 0, 7.3, 37.4 and 143 mg/kg bw per day for males and 0, 7.8, 42.5 and 244 mg/kg bw per day for females, respectively), marginally lower body weights and lower feed consumption were observed in females only during the first part of the study at 4000 ppm (equal to 244 mg/kg bw per day). The Meeting noted that these effects on body weight and feed consumption may have been due to palatability issues. The NOAEL was 2000 ppm (equal to 143 mg/kg bw per day), the highest dose tested (Dymarkowska, 2015).

The toxicological profile of this metabolite was the same as that of pydiflumetofen. The NOAEL of the metabolite in the 28-day rat study (2000 ppm, equal to 143 mg/kg bw per day, the highest dose tested) (Dymarkowska, 2015) was slightly lower than that of the parent compound in a similar study (NOAEL of 8000 ppm, equal to 619 mg/kg bw per day) (Strepka, 2012b).

SYN508272 showed one positive result in an in vitro genotoxicity study and negative results in two other in vitro studies and an in vivo study (Bohnenberger, 2013b; Wollny, 2013b; Dony, 2014b; Sokolowski, 2014b).

Human data

In reports on manufacturing plant personnel, no adverse health effects during the manufacture of pydiflumetofen or its product formulation were noted by the sponsor (Syngenta, 2018).

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

Toxicological evaluation

The Meeting established an ADI of 0–0.1 mg/kg bw on the basis of the NOAEL of 9.9 mg/kg bw per day in a 2-year study in rats for decreased body weight in males observed at 51.0 mg/kg bw per day. A safety factor of 100 was applied.

The Meeting established an ARfD of 0.3 mg/kg bw on the basis of the NOAEL of 30 mg/kg bw per day in a rat developmental toxicity study for reduction in maternal body weight gain and feed consumption early during treatment, observed at 100 mg/kg bw per day. A safety factor of 100 was applied.

The ADI and the ARfD apply to the metabolites 2,4,6-TCP and SYN547897 and their conjugates, expressed as pydiflumetofen.

Levels relevant to risk assessment of pydiflumetofen

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of carcinogenicity[a]	Toxicity	375 ppm, equal to 45.4 mg/kg bw per day	2 250 ppm, equal to 287.9 mg/kg bw per day
		Carcinogenicity	75 ppm, equal to 9.2 mg/kg bw per day	375 ppm, equal to 45.4 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	200 ppm, equal to 9.9 mg/kg bw per day	1 000 ppm, equal to 51.0 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 102 mg/kg bw per day[b]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	1 500 ppm, equal to 115 mg/kg bw per day[b]	–
		Parental toxicity	750 ppm, equal to 46.1 mg/kg bw per day	4 500 ppm, equal to 277 mg/kg bw per day
		Offspring toxicity	450 ppm, equal to 33.7 mg/kg bw per day	1 500 ppm, equal to 115 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	30 mg/kg bw per day	100 mg/kg bw per day
		Embryo and fetal toxicity	100 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	500 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	500 mg/kg bw per day[b]	–
Dog	Three-month and 1-year studies of toxicity[d,e]	Toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Two or more studies combined.
[e] Capsule administration.

Acceptable daily intake (ADI) (applies to pydiflumetofen and the metabolites 2,4,6-TCP and SYN547897 and their conjugates, expressed as pydiflumetofen)

0–0.1 mg/kg bw

Acute reference dose (ARfD) (applies to pydiflumetofen and the metabolites 2,4,6-TCP and SYN547897 and their conjugates, expressed as pydiflumetofen)

0.3 mg/kg bw

Information that would be useful for the continued evaluation of pydiflumetofen

Further characterization of the toxicity of metabolites found in plant and animal commodities; results from epidemiological, occupational health and other such observational studies of human exposure

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	85–90% absorbed; T_{max} within 2 hours at low dose
Distribution	Widely distributed; highest levels in liver and kidney
Potential for accumulation	No indication for accumulation in tissues
Rate and extent of excretion	Rapidly excreted (>90% within 48 hours)
Metabolism in animals	Demethoxylation, *N*-dealkylation, monohydroxylation and dihydroxylation, *O*-demethylation, oxidative and reductive dechlorination
Toxicologically significant compounds in animals and plants	Pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP)
Acute toxicity	
Rat, LD_{50}, oral	>5 000 mg/kg bw
Rat, LD_{50}, dermal	>5 000 mg/kg bw
Rat, LC_{50}, inhalation	>5.11 mg/L
Rabbit, dermal irritation	Not irritating to skin
Rabbit, ocular irritation	Slightly irritating to eye
Mouse, dermal sensitization	Not sensitizing (LLNA)
Short-term studies of toxicity	
Target/critical effect	Thyroid follicular cell hyperplasia (rat)
Lowest relevant oral NOAEL	18.6 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weight and body weight gain (rat)
Lowest relevant NOAEL	9.9 mg/kg bw per day (rat)
Carcinogenicity	Carcinogenic in male mice, but not in female mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity in vivo[a]
Reproductive toxicity	
Target/critical effect	Thyroid follicular cell hypertrophy (rat)
Lowest relevant parental NOAEL	46.1 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	33.7 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	115 mg/kg bw per day, highest dose tested (rat)

Developmental toxicity	
Target/critical effect	Reduced body weight gain and feed consumption (rat)
Lowest relevant maternal NOAEL	30 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	100 mg/kg bw per day, highest dose tested (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	1 000 mg/kg bw, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	No evidence for neurotoxicity in repeated-dose studies in mice, rats and dogs
Other toxicological studies	
Immunotoxicity NOAEL	No evidence for immunotoxicity in repeated-dose toxicity studies
Studies on toxicologically relevant metabolites	
2,4,6-Trichlorophenol (2,4,6-TCP)	Major metabolite (rat) WHO drinking-water guideline value: 0.2 mg/L
SYN508272	500 mg/kg bw < LD_{50} < 2 000 mg/kg bw 28-day study NOAEL 143 mg/kg bw per day, highest dose tested (rat) No evidence of genotoxicity in vivo
Human data	
	No adverse health effects on manufacturing plant personnel have been reported

[a] Unlikely to pose a carcinogenic risk to humans.

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw[a]	Two-year study of toxicity and carcinogenicity in rats	100
ARfD	0.3 mg/kg bw[a]	Developmental toxicity study in rats	100

[a] Applies to pydiflumetofen and the metabolites 2,4,6-TCP and SYN547897 and their conjugates, expressed as pydiflumetofen.

References

Barnes E (2015). SYN545974: additional historical control data to support developmental toxicity studies in the rat. Unpublished report no. TK0103655. Syngenta Ltd, Jealott's Hill International Research Centre, Bracknell, Berkshire, United Kingdom. Syngenta File No. SYN545974_10244. Submitted to WHO by Syngenta Ltd.

Blunt H (2015a). SYN545974 – 90 day oral (capsule) study in the dog. Unpublished report no. BFI0113. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10207. Submitted to WHO by Syngenta Ltd.

Blunt H (2015b). SYN545974 – 52 week oral (capsule) toxicity study in the dog. Unpublished report no. BFI0114. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10205. Submitted to WHO by Syngenta Ltd.

Bohnenberger S (2009). CSAA798670: chromosome aberration test in human lymphocytes in vitro. Unpublished report no. 1266902. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. NOA449410_10001. Submitted to WHO by Syngenta Ltd.

Bohnenberger S (2013a). SYN545974 – chromosome aberration test in human lymphocytes in vitro. Unpublished report no. 1498902. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN545974_10048. Submitted to WHO by Syngenta Ltd.

Bohnenberger S (2013b). SYN508272: chromosome aberration test in human lymphocytes in vitro. Unpublished report no. 1555902. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN508272_10904. Submitted to WHO by Syngenta Ltd.

Broich K (2015a). SYN545974 – acute oral (gavage) neurotoxicity study in the Wistar rat (report amendment 1). Unpublished report no. D84504. Harlan Laboratories Ltd, Itingen, Switzerland. Syngenta File No. SYN545974_10198. Submitted to WHO by Syngenta Ltd.

Broich K (2015b). SYN545974 – an abbreviated acute oral (gavage) neurotoxicity study in the female Wistar rat (report amendment 1). Unpublished report no. D97813. Harlan Laboratories Ltd, Itingen, Switzerland. Syngenta File No. SYN545974_10197. Submitted to WHO by Syngenta Ltd.

Cords S, Lammer E (2009). Reg. No. 5621781 (metabolite of BAS 700 F): acute oral toxicity study in rats. Unpublished report no. 2009/1084176. Bioassay – Labor für Biologische Analytik GmbH, Heidelberg, Germany. Syngenta File No. SYN508272_10903. Submitted to WHO by Syngenta Ltd.

Cowie D (2015). SYN545974 – human relevance framework assessment of liver tumour induction in CD-1 mice. Unpublished report no. TK0258437. Syngenta Crop Protection Ltd, Bracknell, Berkshire, United Kingdom. Syngenta File No. SYN545974_10290. Submitted to WHO by Syngenta Ltd.

Davies S (2015). SYN545974: oral (gavage) prenatal developmental toxicity study in the rat. Unpublished report no. BFI0118. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10190. Submitted to WHO by Syngenta Ltd.

Davies S (2016). SYN545974 – historical control data for SYN545974_10190. Syngenta, Bracknell, Berkshire, United Kingdom. Syngenta File No. SYN545974_10453. Submitted to WHO by Syngenta Ltd.

Doerge D, Chang H, Divi R, Churchwell M (1998). Mechanism for inhibition of thyroid peroxidase by leucomalachite green. Chem Res Toxicol. 11:1098–104.

Dony E (2014a). SYN545974 – micronucleus assay in bone marrow cells of the mouse. Unpublished report no. 1648702. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN545974_10141. Submitted to WHO by Syngenta Ltd.

Dony E (2014b). SYN508272: micronucleus assay in bone marrow cells of the rat. Unpublished report no. 1602600. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN508272_10910. Submitted to WHO by Syngenta Ltd.

Dymarkowska K (2015). SYN508272: a 28 day dietary toxicity study in rats. Unpublished report no. 35015. Charles River Laboratories, Preclinical Services, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN508272_10919. Submitted to WHO by Syngenta Ltd.

Elcombe B (2015). SYN545974: a 28-day dietary liver mode of action study in male CD-1 mice. Unpublished report no. CXR1508. CXR Biosciences, Dundee, United Kingdom. Syngenta File No. SYN545974_10267. Submitted to WHO by Syngenta Ltd.

European Commission (2018). Pydiflumetofen. Volume 1. Combined draft assessment report prepared according to Regulation (EC) No. 1107/2009 and proposal for harmonized classification and labelling (CLH report) according to Regulation (EC) No. 1272/2008 (https://echa.europa.eu/documents/10162/aeb9f88b-912e-4228-af6c-d75a2ce217ce, accessed 2 February 2019).

Freyberger A, Ahr HJ (2006). Studies on the goitrogenic mechanism of action of *N*,*N*,*N′*,*N′*-tetramethylthiourea. Toxicology. 217(2–3):169–75.

Hackford S (2015). SYN545974: oral (dietary) two-generation reproduction toxicity study in the rat. Unpublished report no. BFI0171. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10246. Submitted to WHO by Syngenta Ltd.

Haines C (2012). SYN545974: ex-vivo enzyme analysis of liver samples taken at termination of a 28 day dietary study of SYN545974 and SYN546022 in the mouse. Unpublished report no. CXR1117. CXR Biosciences, Dundee, United Kingdom. Syngenta File No. SYN545974_10209. Submitted to WHO by Syngenta Ltd.

Hargitai J (2013). SYN545974 – local lymph node assay in the mouse. Unpublished report no. 12/344-037E. CiToxLAB Hungary Ltd, Szabadságpuszta, Hungary. Syngenta File No. SYN545974_10052. Submitted to WHO by Syngenta Ltd.

Hutton E (2015a). SYN545974 – the absorption and excretion of [phenyl U ^{14}C] and [pyrazole 5 ^{14}C] SYN545974 following single oral administration in the rat. Unpublished report no. 34214. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10248. Submitted to WHO by Syngenta Ltd.

Hutton E (2015b). SYN545974 – tissue depletion of [phenyl U ^{14}C] and [pyrazole 5 ^{14}C] SYN545974 following single oral administration in the rat. Unpublished report no. 34340. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10252. Submitted to WHO by Syngenta Ltd.

Hutton E, O'Hagan P (2015). SYN545974 – the pharmacokinetics of [phenyl U ^{14}C] and [pyrazole 5 ^{14}C] SYN545974 following single oral and intravenous administration in the rat. Unpublished report no. 34107. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10250. Submitted to WHO by Syngenta Ltd.

IARC (1982). 2,4,5-Trichlorophenol and 2,4,6-trichlophenol. In: Chemicals, industrial processes and industries associated with cancer in humans: an updating of IARC Monographs Volumes 1 to 29. IARC Monogr Eval Carcinogen Risk Chem Hum. 4(Suppl):249–50.

Kaspers U, Strauss V, Graters S, Fabian E, van Ravenzwaay B (2009). Reg. No. 5069089 (metabolite of BAS 700 F): repeated dose 90-day oral toxicity study in Wistar rats – administration in the diet. Unpublished report no. 50S0451/07119. BASF SE, Ludwigshafen/Rhein, Germany. Syngenta File No. R958945_11273. Submitted to WHO by Syngenta Ltd.

Lake B (2014). SYN545974: effect on rat thyroid peroxidase activity in vitro. Unpublished report no. 5523/1/2/2014 (amendment 1). Molecular Sciences Department, Leatherhead Food Research (LFR), Leatherhead, Surrey, United Kingdom. Syngenta File No. SYN545974_10120. Submitted to WHO by Syngenta Ltd.

Lake B (2015). SYN545974: effect on hepatic UDPglucuronosyltransferase activity towards thyroxine as substrate after dietary administration for 90 days to male rats. Unpublished report no. 5522/1/2/2014 (amendment 1). Molecular Sciences Department, Leatherhead Food Research (LFR), Leatherhead, Surrey, United Kingdom. Syngenta File No. SYN545974_10264. Submitted to WHO by Syngenta Ltd.

Lowes D (2015a). SYN545974: in vitro hepatocyte proliferation indexing and enzyme activity measurements in male CD-1 mouse hepatocyte cultures. Unpublished report no. CXR1503. CXR Biosciences, Dundee, United Kingdom. Syngenta File No. SYN545974_10272. Submitted to WHO by Syngenta Ltd.

Lowes D (2015b). SYN545974: in vitro hepatocyte proliferation indexing and enzyme activity measurements in male human hepatocyte cultures. Unpublished report no. CXR1504. CXR Biosciences, Dundee, United Kingdom. Syngenta File No. SYN545974_10270. Submitted to WHO by Syngenta Ltd.

MacDonald M, Jewkes Y (2015). SYN545974 – biotransformation of [^{14}C]-SYN545974 in rat. Unpublished report no. 34216. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10259. Submitted to WHO by Syngenta Ltd.

Manton J (2011). SYN545974, SYN546022: preliminary oral (gavage) prenatal developmental toxicity dose range finding study in the rat. Unpublished report no. BFI0031. Sequani Ltd, Bromyard Road, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10003. Submitted to WHO by Syngenta Ltd.

Nagy K (2013). SYN545974 – acute inhalation toxicity study (nose-only) in the rat. Unpublished report no. 12/334-004P. CiToxLAB Hungary Ltd, Szabadságpuszta, Hungary. Syngenta File No. SYN545974_10051. Submitted to WHO by Syngenta Ltd.

NCI (1979). Bioassay of 2,4,6-trichlorophenol for possible carcinogenicity. CAS No. 88-06-2. NCI-CG-TR 155. Technical Report Series No. 155; DHEW Publications No. (NIH) 79-1711. Bethesda (MD): United States Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute (https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr155.pdf, accessed September 2018).

Omiecinski C (2014). SYN545974: CAR3 transactivation assay with mouse, rat and human CAR. Unpublished report no. TK0219831. Department of Veterinary & Biomedical Sciences, Penn State University, University Park, PA, USA. Syngenta File No. SYN545974_50044. Submitted to WHO by Syngenta Ltd.

Penn L (2015a). SYN545974 – oral (gavage) toxicokinetic study in the pregnant rabbit (final report amendment 3). Unpublished report no. BFI0126. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10125. Submitted to WHO by Syngenta Ltd.

Penn L (2015b). SYN545974: preliminary oral (gavage) prenatal developmental toxicity study in the rabbit (final report amendment 2). Unpublished report no. BFI0046. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10192. Submitted to WHO by Syngenta Ltd.

Penn L (2015c). SYN545974: oral (gavage) prenatal developmental toxicity study in the rabbit. Unpublished report no. BFI0116. Sequani Ltd, Ledbury, Herefordshire, United Kingdom. Syngenta File No. SYN545974_10177. Submitted to WHO by Syngenta Ltd.

Petus-Árpásy M (2012). SYN545974 – acute oral toxicity study in the rat (up and down procedure). Unpublished report no. 12/344-001P. CiToxLAB Hungary Ltd, Szabadságpuszta, Hungary. Syngenta File No. SYN545974_10043. Submitted to WHO by Syngenta Ltd.

Petus-Árpásy M (2013). SYN545974 – acute dermal toxicity study in rats. Unpublished report no. 12/344-002P. CiToxLAB Hungary Ltd, Szabadságpuszta, Hungary. Syngenta File No. SYN545974_10046. Submitted to WHO by Syngenta Ltd.

Pooles A (2008). DF-pyrazole acid (CA4312): screening acute oral toxicity study in the rat. Unpublished report no. 2364/0169. SafePharm Laboratories Ltd, Shardlow, Derbyshire, United Kingdom. Syngenta File No. CA4312/0003. Submitted to WHO by Syngenta Ltd.

Punler M, Harris J (2014a). SYN545974 – pharmacokinetics of SYN545974 in the mouse following multiple oral and single intravenous administration. Unpublished report no. 33408. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10124. Submitted to WHO by Syngenta Ltd.

Punler M, Harris J (2014b). SYN545974 – pharmacokinetics of SYN545974 in the rat following multiple oral and single intravenous administration. Unpublished report no. 33409. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10104. Submitted to WHO by Syngenta Ltd.

Robertson B (2015). SYN545974: 80 week mouse dietary carcinogenicity study (report amendment 1). Unpublished report no. 35914. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10237. Submitted to WHO by Syngenta Ltd.

Robertson B (2016). SYN545974: 104 week rat dietary carcinogenicity study with a combined 52 week toxicity study (report amendment 1). Unpublished report no. 36248. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10245. Submitted to WHO by Syngenta Ltd.

Roth M (2012). SYN545974 – micronucleus assay in bone marrow cells of the mouse. Unpublished report no. 1498904. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN545974_10045. Submitted to WHO by Syngenta Ltd.

Sakamoto Y, Inoue K, Takahashi M, Taketa Y, Kodama Y, Nemoto K et al. (2013). Different pathways of constitutive androstane receptor–mediated liver hypertrophy and hepatocarcinogenesis in mice treated with piperonyl butoxide or decabromodiphenyl ether. Toxicol Pathol. 41(8):1078–92.

Schneider S, Fabian E, Mellert W (2009). Reg. No. 5069089 (metabolite of BAS 700 F) – prenatal developmental toxicity study in New Zealand white rabbits – oral administration (gavage). Unpublished report no. 2009/1072507. BASF SE, Ludwigshafen/Rhein, Germany. Syngenta File No. NOA449410_10005. Submitted to WHO by Syngenta Ltd.

Shearer J (2015). SYN545974: a 13 week toxicity study of SYN545974 by oral (dietary) administration in mice (final report amendment 2). Unpublished report no. 33011. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10211. Submitted to WHO by Syngenta Ltd.

Shearer J, Robertson B (2015). SYN545974: a 13 week toxicity study of SYN545974 by oral (dietary) administration in rats (final report amendment 2). Unpublished report no. 33012. Charles River, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10210. Submitted to WHO by Syngenta Ltd.

Shearer J, Robertson B (2016). SYN545974 – historical control data for SYN545974_10210 and SYN545974_10211. Unpublished report. Syngenta, Jealott's Hill, Bracknell, United Kingdom. Syngenta File No. SYN545974_10448. Submitted to WHO by Syngenta Ltd.

Sieber M (2013). SYN545974: 28-day dermal toxicity study in the Wistar rat (final report amendment 1). Unpublished report no. D62072. Harlan Laboratories Ltd, Itingen, Switzerland. Syngenta File No. SYN545974_10047. Submitted to WHO by Syngenta Ltd.

Sokolowski A (2007). *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay with DF-pyrazole acid (CA4312). Unpublished report no. 1077403. RCC, Cytotest Cell Research GmbH (RCC-CCR), Rossdorf, Germany. Syngenta File No. SYN520453/0096. Submitted to WHO by Syngenta Ltd.

Sokolowski A (2012). SYN545974: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. Unpublished report no. 1498901. Harlan, Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN545974_10018. Submitted to WHO by Syngenta Ltd.

Sokolowski A (2014a). SYN545974 – *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. Unpublished report no. 1648701. Harlan Cytotest Cell Research GmbH, Rossdorf, Germany. Syngenta File No. SYN545974_10127. Submitted to WHO by Syngenta Ltd.

Sokolowski A (2014b). SYN508272: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay. Unpublished report no. 1555901. Harlan Cytotest Cell Research GmbH, Rossdorf, Germany. Syngenta File No. SYN508272_10908. Submitted to WHO by Syngenta Ltd.

Sommer E (2010). CSAA798670: 28-day oral (dietary) toxicity study in the Wistar rat. Unpublished report no. C57852. Harlan Laboratories Ltd, Itingen, Switzerland. Syngenta File No. NOA449410_10003. Submitted to WHO by Syngenta Ltd.

Strepka C (2012a). SYN545974, SYN546022: 28 day dietary toxicity study in mice. Unpublished report no. 32153. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10042. Submitted to WHO by Syngenta Ltd.

Strepka C (2012b). SYN545974, SYN546022: 28 day dietary toxicity study in rats. Unpublished report no. 32168. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10044. Submitted to WHO by Syngenta Ltd. Submitted to WHO by Syngenta Ltd.

Strepka C, Shearer J (2015). SYN545974 – a 28 day toxicity study of SYN545974 by the oral (capsule) route in dogs. Unpublished report no. 33167. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Submitted to WHO by Syngenta Ltd.

Sun G, Thai SF, Lambert GR, Wolf DC, Tully DB, Goetz AK et al. (2006). Fluconazole-induced hepatic cytochrome P450 gene expression and enzymatic activities in rats and mice. Toxicol Lett. 164(1):44–53.

Syngenta (2018). Pydiflumetofen. SYN545974. JMPR 2018. Information on animal metabolism and toxicology for evaluation. Working paper. Submission to the WHO Panel of the JMPR for review as new compound submission to the WHO for the review of pydiflumetofen. Submitted to WHO by Syngenta Ltd.

Tomlinson J, Hutton E, Green M, Allan G (2015). SYN545974 – the excretion and biotransformation of [phenyl-U-^{14}C] and [pyrazole-5-^{14}C]-SYN545974 following single oral administration in the mouse. Unpublished report no. 35415. Charles River Laboratories Edinburgh Ltd, Tranent, Edinburgh, United Kingdom. Syngenta File No. SYN545974_10257. Submitted to WHO by Syngenta Ltd.

Török-Bathó M (2012a). SYN545974 – primary skin irritation study in rabbits. Unpublished report no. 12/344-006N. CiToxLAB Hungary Ltd, Szabadságpuszta Hungary. Syngenta File No. SYN545974_10035. Submitted to WHO by Syngenta Ltd.

Török-Bathó M (2012b). SYN545974 – acute eye irritation study in rabbits. Unpublished report no. 12/344-005N. CiToxLAB Hungary Ltd, Szabadságpuszta, Hungary. Syngenta File No. SYN545974_10033. Submitted to WHO by Syngenta Ltd.

Webbley K, Williams D (2015). SYN545974 – a preliminary study of pharmacokinetics, absorption, metabolism and excretion in rats following single oral and intravenous administration of ^{14}C-SYN545974. Unpublished report no. SGA-64. Quotient Bioresearch (Rushden) Ltd, Rushden, Northamptonshire, United Kingdom. Syngenta File No. SYN545974_10188. Submitted to WHO by Syngenta Ltd.

WHO (2017). Guidelines for drinking-water quality, 4th edition, incorporating the first addendum. Geneva: World Health Organization (http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/, accessed September 2018).

Wollny H (2009). CSAA798670: cell mutation assay at the thymidine kinase locus (*TK*$^{+/-}$) in mouse lymphoma L5178Y cells. Unpublished report no. 1266901. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. NOA449410_10000. Submitted to WHO by Syngenta Ltd.

Wollny H-E (2013a). SYN545974 – cell mutation assay at the thymidine kinase locus (*TK*$^{+/-}$) in mouse lymphoma L5178Y cells. Unpublished report no. 1498903. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN545974_10049. Submitted to WHO by Syngenta Ltd.

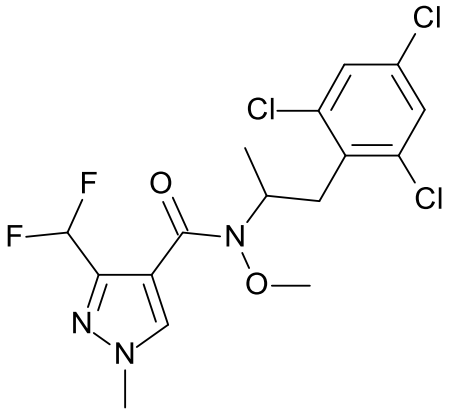
Wollny H (2013b). SYN508272: cell mutation assay at the thymidine kinase locus (*TK*$^{+/-}$) in mouse lymphoma L5178Y cells. Unpublished report no. 1555903. Harlan Cytotest Cell Research GmbH (Harlan CCR), Rossdorf, Germany. Syngenta File No. SYN508272_10906. Submitted to WHO by Syngenta Ltd.

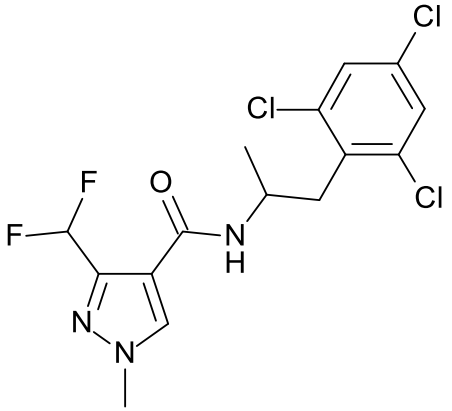
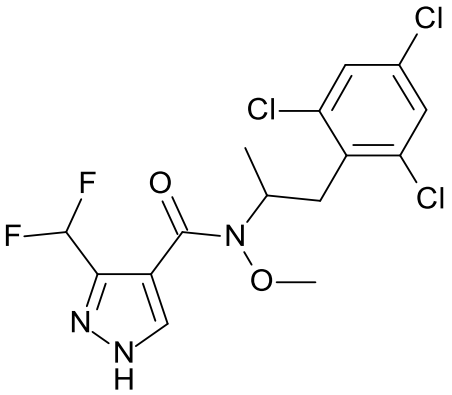
Yamamoto Y, Moore R, Goldsworthy TL, Negishi M, Maronpot RR (2004). The orphan nuclear receptor constitutive active/androstane receptor is essential for liver tumor promotion by phenobarbital in mice. Cancer Res. 64(20):7197–200.

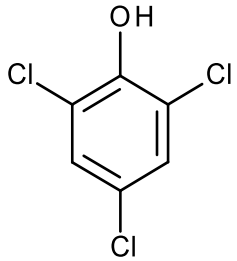
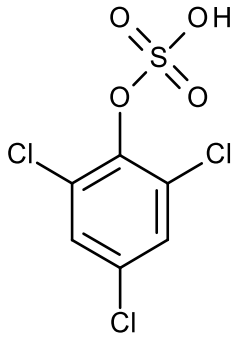
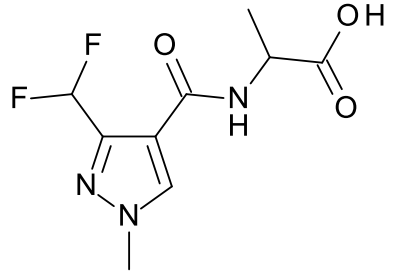
Appendix 1: List of animal metabolites

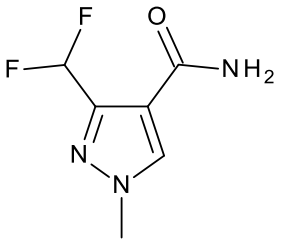
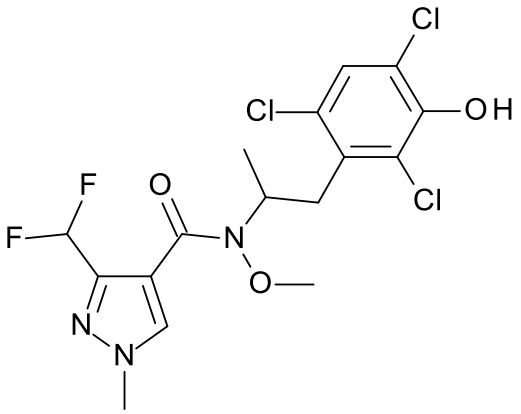
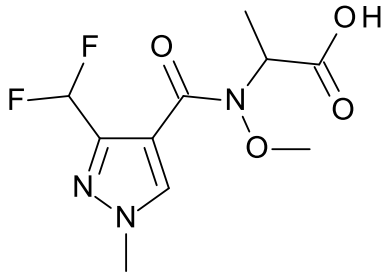
All metabolites of pydiflumetofen that have been identified in the rat are listed in Table A1-1.

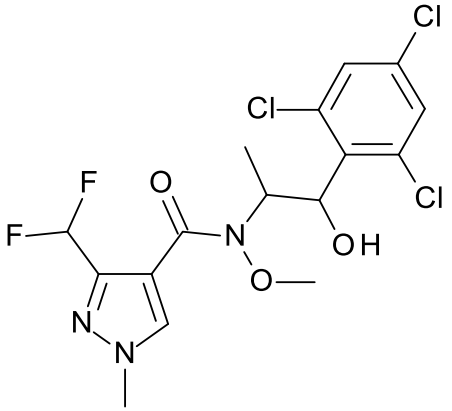
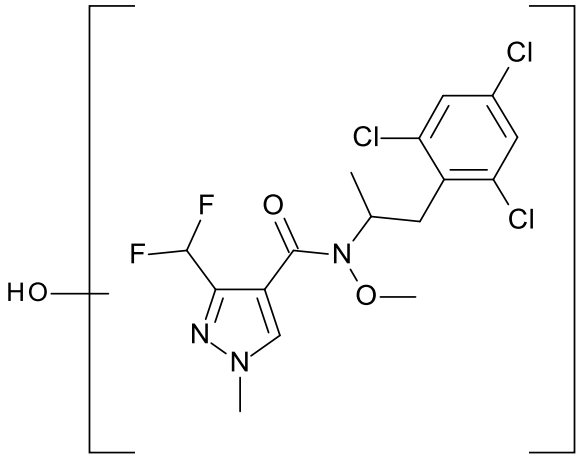
Table A1-1. Summary of metabolites found in various matrices (structures, codes and synonyms)

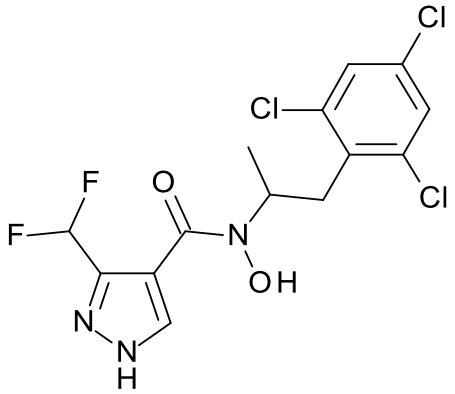
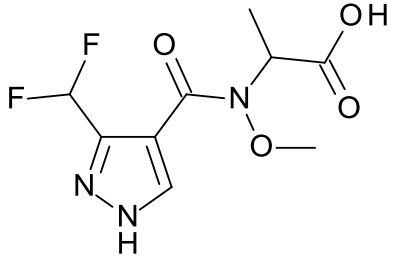
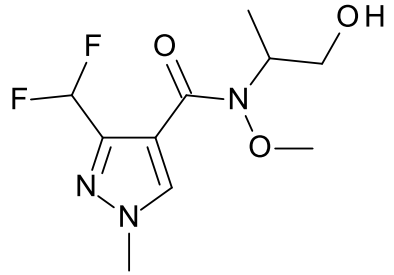
Code number (synonyms)	Description	Compound found in:	Structure
Pydiflumetofen SYN545974 CSCD678790	(*S*)-3-Difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-amide (*R*)-3-Difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-amide	Soil (sterile, aerobic, anaerobic, photolysis) Water (photolysis) Water–sediment systems Crops (wheat, oilseed rape, tomato, rotated crops) Livestock (hen, goat) Rat	

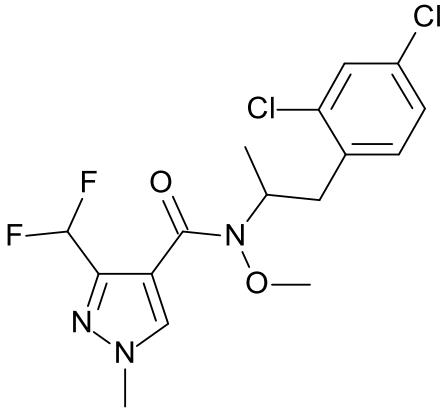
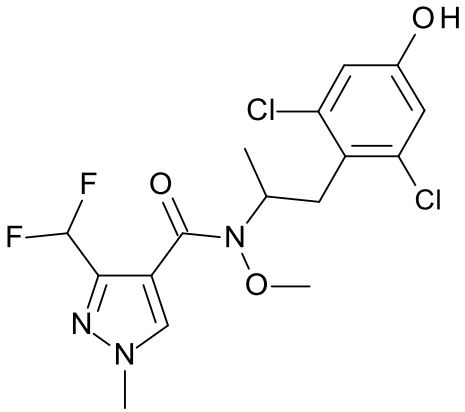
Code number (synonyms)	Description	Compound found in:	Structure
SYN545547 CSCD550897	3-(Difluoromethyl)-1-methyl-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]pyrazole-4-carboxamide	Soil (sterile, aerobic, anaerobic, photolysis) Water (photolysis) Water–sediment systems Crops (wheat, oilseed rape, tomato, rotated crops) Livestock (hen, goat) Rat	
SYN547891 CSCV764139	3-(Difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide	Crops (wheat, oilseed rape, tomato, rotated crops) Livestock (hen, goat) Rat	

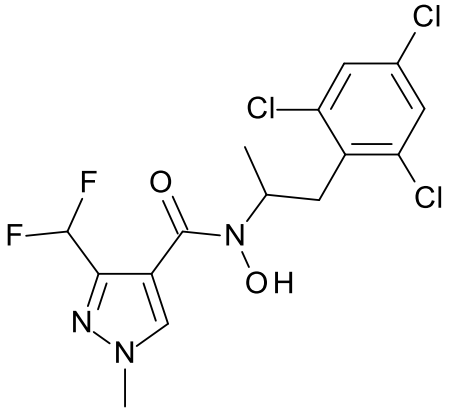
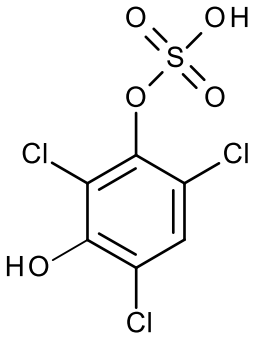
Code number (synonyms)	Description	Compound found in:	Structure
2,4,6-Trichlorophenol 2,4,6-TCP	2,4,6-Trichlorophenol	Livestock (hen, goat) Rat	
2,4,6-TCP sulfate	Sulfate conjugate of 2,4,6-trichlorophenol	Livestock (hen, goat) Rat	
SYN548264 CSCD548196 *N*-Desmethoxy SYN548263	2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]propanoic acid	Livestock (goat) Rat	

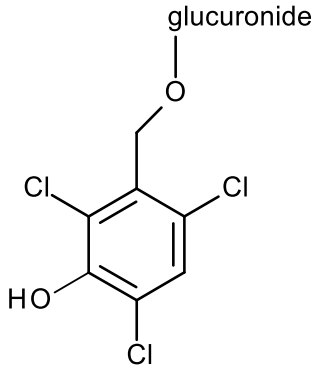
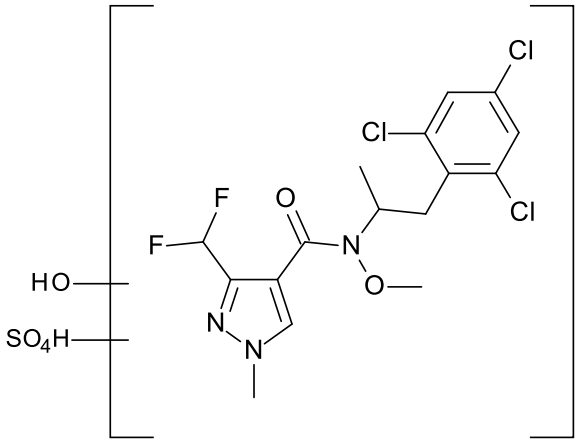
Code number (synonyms)	Description	Compound found in:	Structure
SYN508272 CSCC210616 R423363	3-(Difluoromethyl)-1-methyl-pyrazole-4-carboxamide	Livestock (hen, goat) Rat	
SYN547897 CSCV764146	3-(Difluoromethyl)-*N*-methoxy-1-methyl-*N*-[1-methyl-2-(2,4,6-trichloro-3-hydroxy-phenyl)ethyl]pyrazole-4-carboxamide	Livestock (hen, goat) Rat	
SYN548263 CSCZ159698	2-[[3-(Difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid	Livestock (goat) Rat	

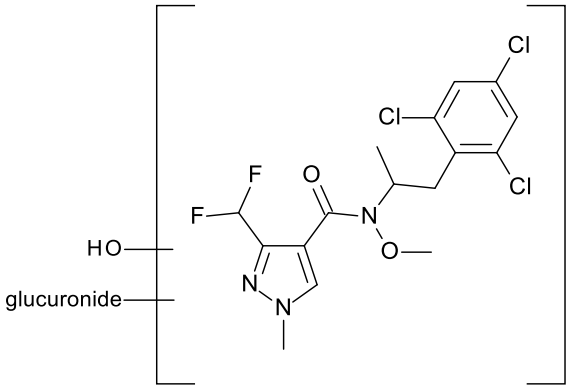
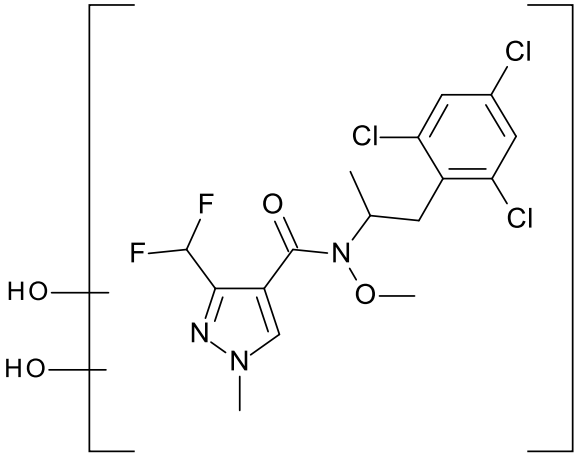
Code number (synonyms)	Description	Compound found in:	Structure
SYN547948 CSCY608054	3-(Difluoromethyl)-*N*-[2-hydroxy-1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-*N*-methoxy-1-methyl-pyrazole-4-carboxamide	Livestock (hen, goat) Rat	
Hydroxylated SYN545974	Hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	Livestock (goat) Rat	

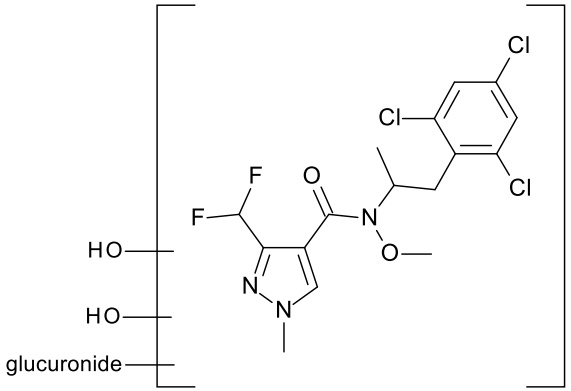
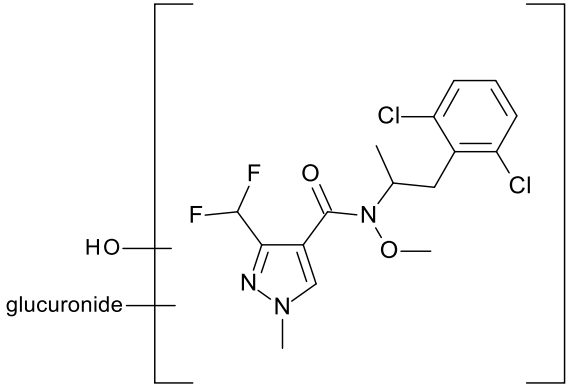
Code number (synonyms)	Description	Compound found in:	Structure
N-Desmethyl SYN547890	3-(Difluoromethyl)-*N*-hydroxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide	Rat	
Desmethyl SYN548263 CSCZ159698	Desmethyl 2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid	Rat	
SYN548265	3-(Difluoromethyl)-*N*-(2-hydroxy-1-methyl-ethyl)-*N*-methoxy-1-methyl-pyrazole-4-carboxamide	Rat	

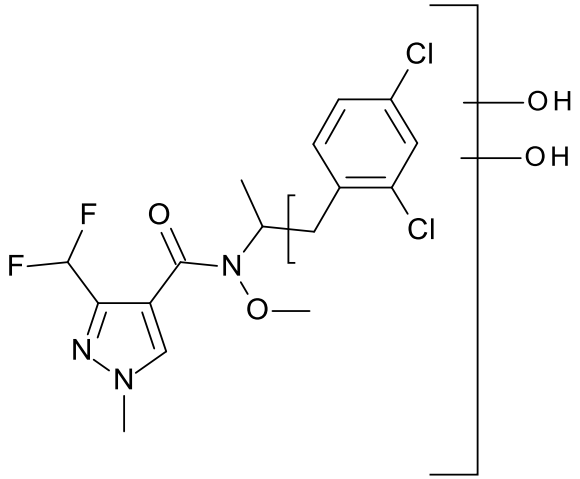
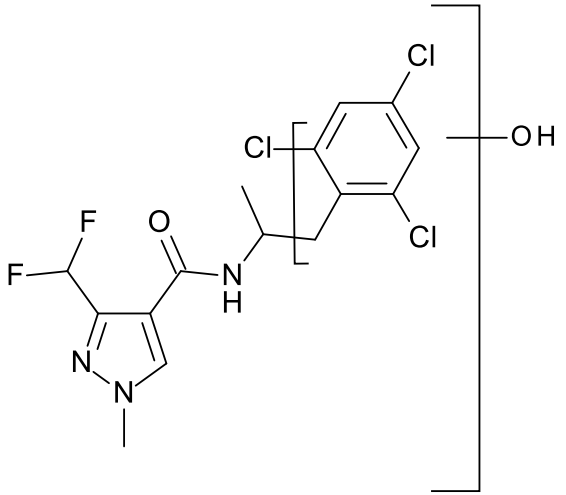
Code number (synonyms)	Description	Compound found in:	Structure
SYN547893 CSCD677133	*N*-[2-(2,4-Dichlorophenyl)-1-methyl-ethyl]-3-(difluoromethyl)-*N*-methoxy-1-methyl-pyrazole-4-carboxamide	Rat	
SYN547894 CSCV764141	*N*-[2-(2,6-Dichloro-4-hydroxy-phenyl)-1-methyl-ethyl]-3-(difluoromethyl)-*N*-methoxy-1-methyl-pyrazole-4-carboxamide	Rat	

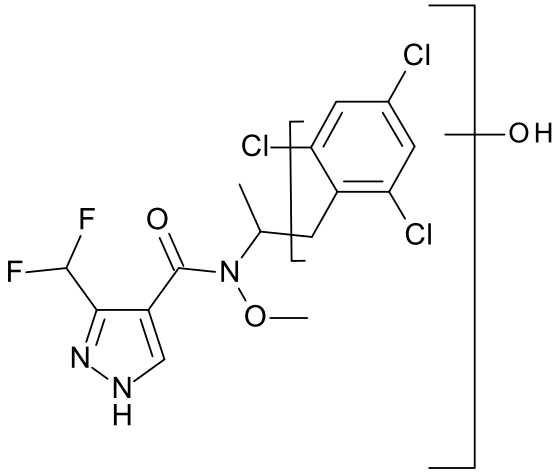
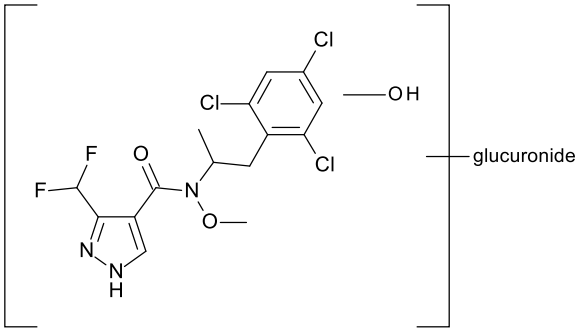
Code number (synonyms)	Description	Compound found in:	Structure
SYN547890	3-(Difluoromethyl)-*N*-hydroxy-1-methyl-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]pyrazole-4-carboxamide	Rat	
Hydroxylated TCP-sulfate HTCP sulfate	(2,4,6-Trichloro-3-hydroxyphenyl) hydrogen sulfate	Rat	

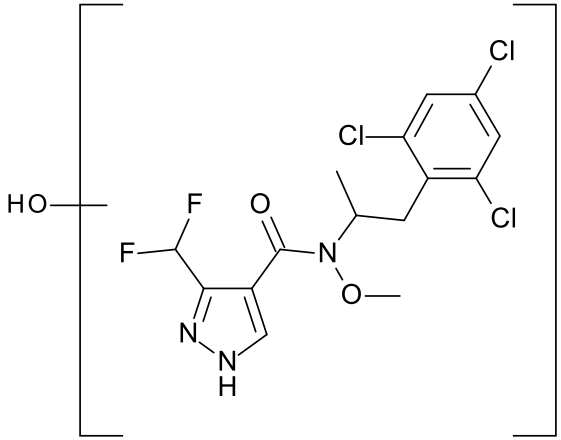
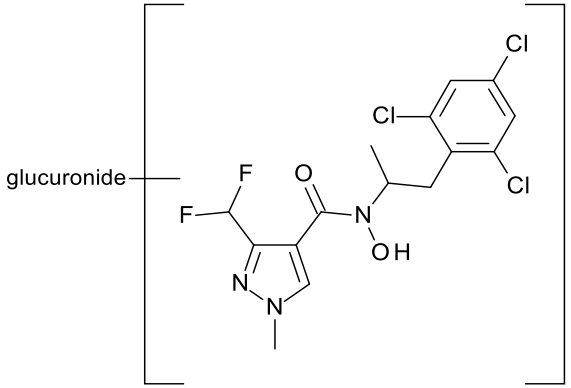
Code number (synonyms)	Description	Compound found in:	Structure
TCPM glucuronide	2,4,6-Trichloro-3-(methoxymethyl)phenol glucuronide	Rat	
Hydroxylated SYN545974 Sulfate conjugate	Hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Sulfate conjugate	Rat	

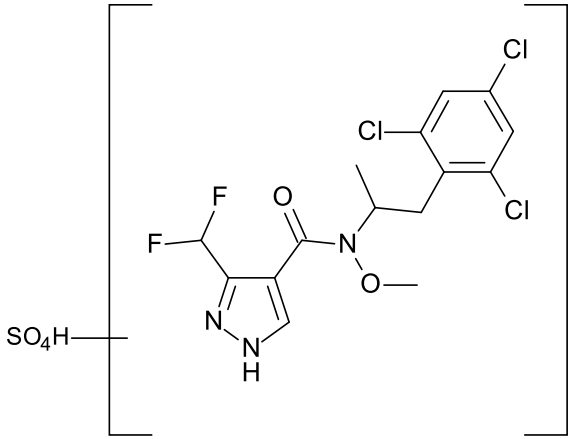
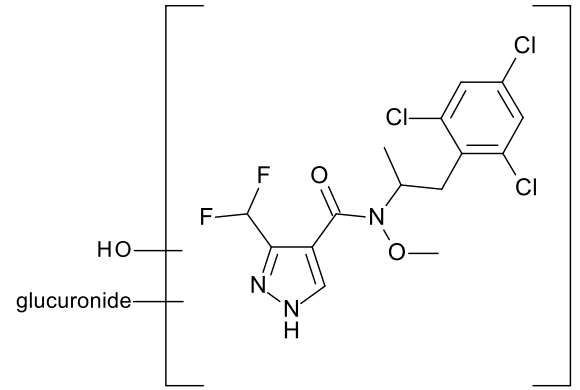
Code number (synonyms)	Description	Compound found in:	Structure
Hydroxylated SYN545974 Glucuronide conjugate	Hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	
Dihydroxy SYN545974	Dihydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	Rat	

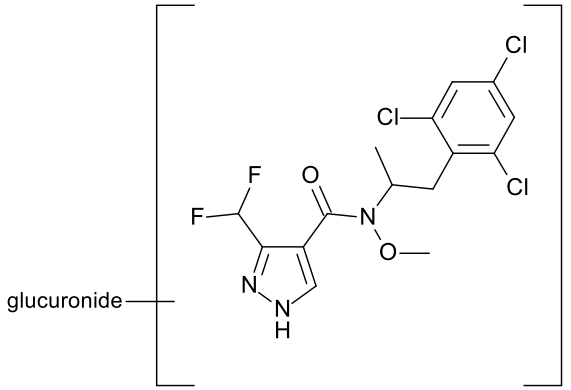
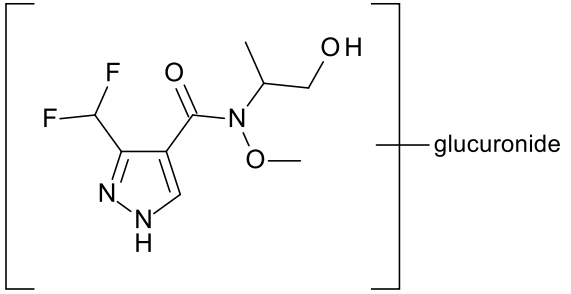
Code number (synonyms)	Description	Compound found in:	Structure
Dihydroxy SYN545974 Glucuronide conjugate	Dihydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	
Dechlorinated hydroxy SYN545974 Glucuronide conjugate	Dechlorinated hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	

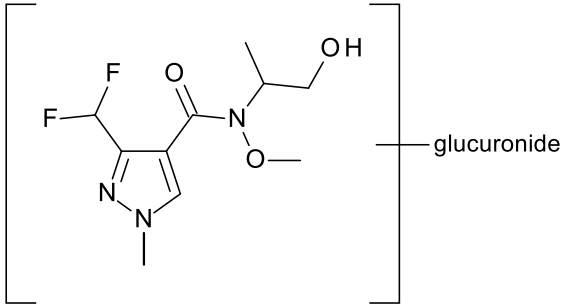
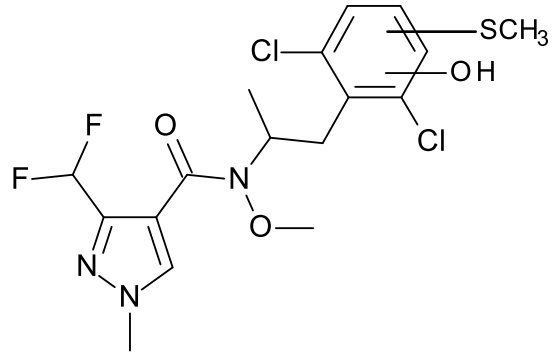
Code number (synonyms)	Description	Compound found in:	Structure
[Code not provided]	[Description not provided]	Rat	
Hydroxy SYN545547	Hydroxylated 3-(difluoromethyl)-1-methyl-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]pyrazole-4-carboxamide	Rat	

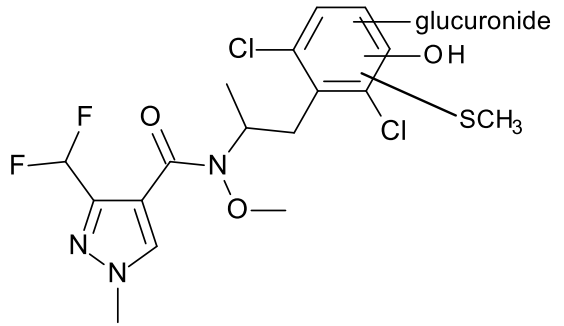
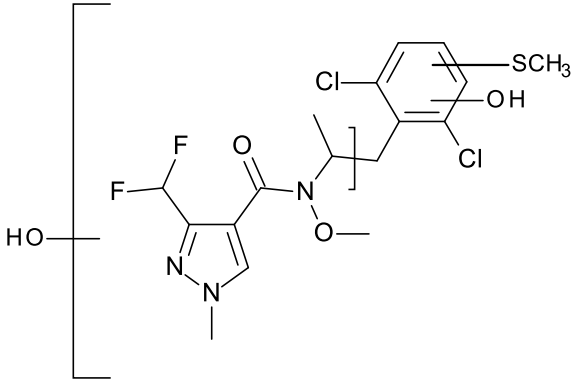
Code number (synonyms)	Description	Compound found in:	Structure
Hydroxy SYN547891	Hydroxylated 3-(difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide	Rat	
SYN547891 Glucuronide conjugate	3-(Difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide Glucuronide conjugate	Rat	

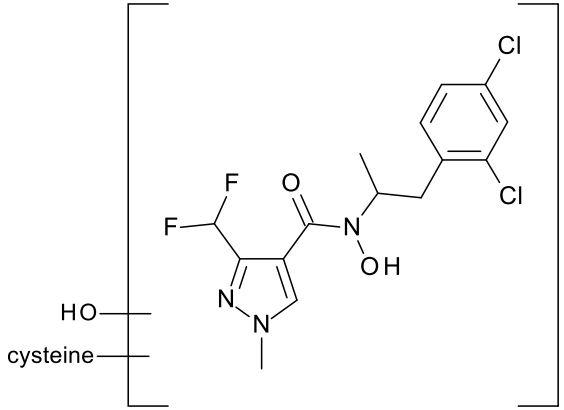
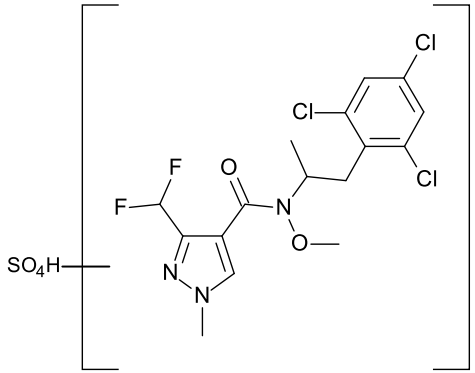
Code number (synonyms)	Description	Compound found in:	Structure
Desmethyl hydroxy SYN545974	Hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	Rat	
Desmethyl SYN545974 Glucuronide conjugate	Desmethyl *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	

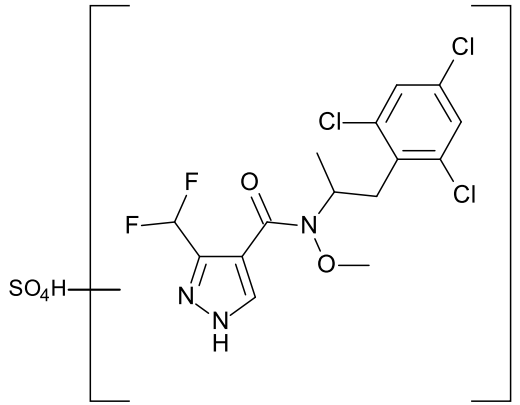
Code number (synonyms)	Description	Compound found in:	Structure
Desmethyl hydroxy SYN545974 Sulfate conjugate	3-(Difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide Sulfate conjugate	Rat	
Desmethyl hydroxy SYN545974 Glucuronide conjugate	Hydroxylated *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	

Code number (synonyms)	Description	Compound found in:	Structure
Desmethyl SYN545974 Glucuronide conjugate	3-(Difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide Glucuronide conjugate	Rat	
Desmethyl SYN548265 Glucuronide conjugate	Desmethyl 3-(difluoromethyl)-*N*-(2-hydroxy-1-methyl-ethyl)-*N*-methoxy-1-methyl-pyrazole-4-carboxamide Glucuronide conjugate	Rat	

Code number (synonyms)	Description	Compound found in:	Structure
SYN548265 Glucuronide conjugate	3-(Difluoromethyl)-*N*-(2-hydroxy-1-methyl-ethyl)-*N*-methoxy-1-methyl-pyrazole-4-carboxamide Glucuronide conjugate	Rat	
Dechlorinated hydroxy thiomethyl SYN545974	Dechlorinated hydroxy thiomethyl *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	Rat	

Code number (synonyms)	Description	Compound found in:	Structure
Dechlorinated hydroxy thiomethyl SYN545974 Glucuronide conjugate	Dechlorinated hydroxy thiomethyl *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Glucuronide conjugate	Rat	
Dechlorinated dihydroxy thiomethyl SYN545974	Dechlorinated dihydroxy thiomethyl *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide	Rat	

Code number (synonyms)	Description	Compound found in:	Structure
Dechlorinated hydroxy SYN545974 Cysteine conjugate	Dechlorinated hydroxy *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Cysteine conjugate	Rat	
Hydroxy SYN545974 Sulfate conjugate	Hydroxy *N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide Sulfate conjugate	Rat	

Code number (synonyms)	Description	Compound found in:	Structure
Hydroxy SYN547891 Sulfate conjugate	Hydroxy 3-(difluoromethyl)-*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1*H*-pyrazole-4-carboxamide Sulfate conjugate	Rat	

Note: The majority of the nomenclature used in the table above was provided by the sponsor.

Appendix 2: Mode of action of pydiflumetofen-induced mouse liver tumours: analysis of their human relevance using the IPCS/ILSI framework[1]

The hypothesized MOA for pydiflumetofen-induced liver tumours and their relevance to humans have been assessed using an established international framework: that of WHO's IPCS and ILSI.

Carcinogenicity of pydiflumetofen

The carcinogenicity of pydiflumetofen has been evaluated in 104- and 80-week bioassays in the rat and mouse, respectively (Robertson, 2015, 2016). The treatment-related neoplastic finding was liver tumours in male mice at 375 ppm and higher (Table A2-1). No treatment-related neoplastic findings were observed in rats of either sex or in female mice. The NOAEL for liver tumours in male mice was 75 ppm.

Table A2-1. Liver carcinogenicity[a] of pydiflumetofen in the male CD-1 mouse

Tumour type	0 ppm	75 ppm	375 ppm	2 250 ppm	Charles River Laboratories (Edinburgh) historical control data
No. of animals examined	*50*	*50*	*49*	*50*	*247*
Hepatocellular adenoma	4 (8%) [0]	6 (12%) [0]	9 (18.4%) [7]**	22**, $$$ (44%) [14]**	45 (18.2%) (range = 10.0–28.0%)
Hepatocellular carcinoma	2 (4%) [0]	3 (6%) [0]	4 (8.2%) [0]	10* (20%) [2]	19 (7.7%) (range = 6.0–10.0%)

ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$ (Fisher's exact test); $$$: $P \leq 0.001$ (Peto trend test)

[a] Tumour incidences are expressed as total per group (percentage incidence) [number with multiple tumours].

Source: Robertson (2015)

Postulated mode of action and key events

A number of time- and dose-related key events have been identified to characterize the MOA for pydiflumetofen-induced liver tumours in male mice. Integrating all evidence, the proposed non-genotoxic MOA is initiated by CAR activation.

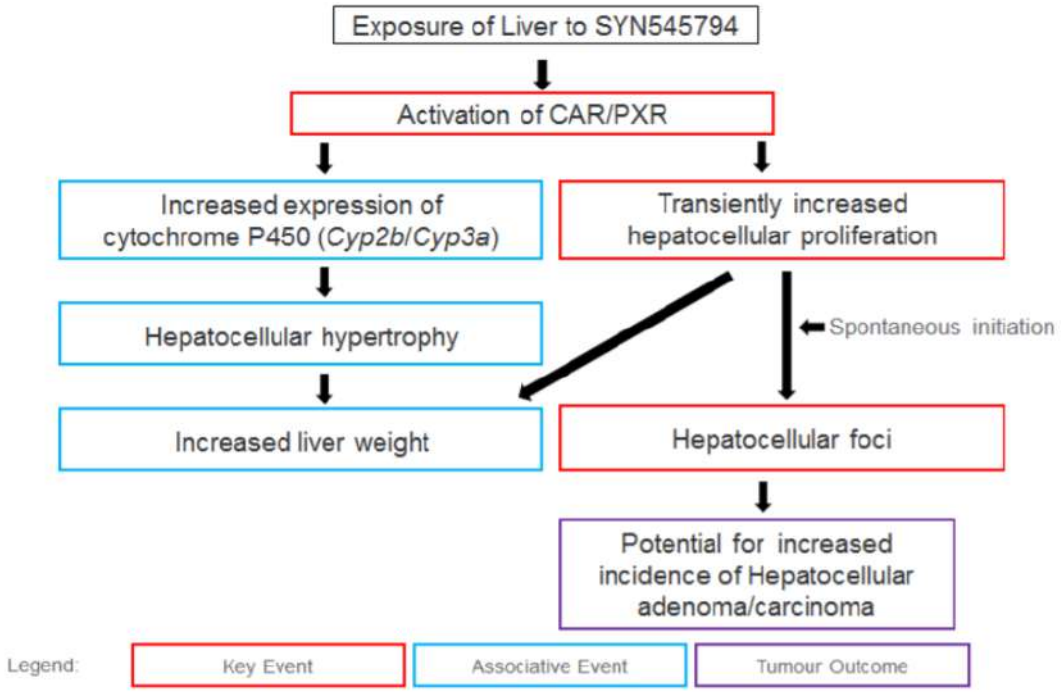
The essential key event, CAR activation, in male mice results in altered expression of CAR-responsive genes, leading to CAR-mediated stimulation of cell proliferation, including replicative DNA synthesis. This promotes an environment permissive for increased cell replication, which can result in a higher rate of spontaneous mutations due to normal replication errors. Suppression of apoptosis promotes an alteration of environment that would allow a spontaneous cell mutation to clonally expand before it could be removed by the normal control process of apoptosis. Over time, transformed cells progress to eosinophilic foci of cellular alteration, a preneoplastic change, with clonal expansion, eventually leading to the development of liver tumours. The eosinophilic foci of hepatocellular alteration or eosinophilic adenomas were reported to be induced in mice treated with phenobarbital or chemicals that activate mouse CAR (Yamamoto et al., 2004; Sakamoto et al., 2013). The activation of CAR and subsequent burst of cellular proliferation are considered to be key events in the tumour MOA, being necessary and directly resulting in the induction of liver tumours in the mouse.

[1] Adapted from Cowie (2015).

In addition to the CAR activation in male mice, a number of other genes, including some coding for members of specific cytochrome P450 families of isozymes, particularly those of *Cyp2b* and, to a lesser extent, *Cyp3a* and *Cyp2a* subfamilies, were induced. The effects on cytochrome P450s are a characteristic hallmark of CAR activation, but associative events of the proposed MOA – that is, they are not central to the induction of liver tumours. A further associative event is liver hepatocellular hypertrophy, which is caused by proliferation of the smooth endoplasmic reticulum as a consequence of cytochrome P450 induction. This hypertrophy, in combination with the increased hepatocyte proliferation, in turn results in an increase in liver weight.

The MOA outlining the key and associative events is shown in Fig. A2-1.

Fig. A2-1. MOA hypothesis for pydiflumetofen-induced liver tumour formation in male mice



CAR: constitutive androstane receptor; PXR: pregnane X receptor
Source: Cowie (2015)

After the key events within a postulated MOA have been described, the IPCS/ILSI framework and Hill criteria require that they be evaluated by a standardized weight of evidence evaluation. In this process, the key events that are causally related to the formation of tumours must be shown to be required steps that lead to the tumour, and the findings must be shown to be reproducible. A weight of evidence analysis for pydiflumetofen-induced liver tumour formation is described in the following sections.

Relevant data for the assessment of the MOA hypothesis for pydiflumetofen

The following studies were considered when evaluating the MOA hypothesis for pydiflumetofen:

- an assessment of the ability of pydiflumetofen to activate CAR (Omiecinski, 2014);
- three studies with MOA end-points that assess the dietary toxicity of pydiflumetofen over a period of 28 days (Haines, 2012; Strepka, 2012a; Elcombe, 2015);

- an assessment of the subchronic (13-week) toxicity in the CD-1 mouse (Shearer, 2015);
- an 80-week carcinogenicity study in the CD-1 mouse (Robertson, 2015); and
- investigative studies in primary cultures of mouse and human hepatocytes to assess mechanistic end-points with respect to human relevance (Lowes, 2015a,b).

Concordance of dose–response relationships

For the key events in a proposed MOA to be causally related to the formation of tumours, they must show concordance with the doses that produce tumours (dose concordance). Table A2-2 summarizes the dose concordance of the key and associative events (as defined in the hypothesized MOA) with the tumour outcome. This table considers concordance with the following doses:

- 75 ppm as the NOAEL for liver tumours in male CD-1 mice; and
- 2250 ppm as a carcinogenic concentration that induces liver tumours in male CD-1 mice.

Table A2-2 demonstrates that it is only at the tumorigenic dose (2250 ppm) that all of the key (and associative) events are observed. Early effects at the non-tumorigenic dose (75 ppm) are confined to a small increase in hepatocellular proliferation, which is probably indicative of a low level of CAR activation of insufficient magnitude to stimulate any of the other key or associative events. This is supported by the lack of any other key or associative events occurring at the non-tumorigenic dose, including no effects on liver weight or liver histopathology findings at any study duration. The absence of any other histopathology changes, including no evidence of increased adenomas or carcinomas, and in light of the CAR-mediated MOA for liver tumours that is fully described in this MOA framework appendix, the marginal increase in cell proliferation at 75 ppm is considered not to be a human-relevant adverse effect. Taken together, it can be concluded that there is strong concordance between key (and associative) events and doses that produce tumours.

Table A2-2. Dose concordance of key and associative events obtained from all data on pydiflumetofen in mice

Event	75 ppm	2 250 ppm
CAR activation (in vivo)[a]	No	Yes
Induction of *Cyp2b/Cyp3a* (PROD activity)	No	Yes
Increase in hepatocellular replicative DNA synthesis (2–28 days)	Yes (slight)	Yes
Increased mitosis[b]	No	Yes
Increased hepatocellular hypertrophy[b]	No	Yes
Increased liver weight	No	Yes
Increased incidence of hepatocellular foci[b]	No	Yes
Increased incidence of liver tumours[b]	No	Yes

CAR: constitutive androstane receptor; Cyp: cytochrome P450; DNA: deoxyribonucleic acid; ppm: parts per million; PROD: pentoxyresorufin *O*-dependentylation

[a] CAR activation as assessed by surrogate markers of CAR activation from in vivo studies (e.g. *Cyp2b*-mediated PROD activity). The intrinsic ability of pydiflumetofen to directly activate mouse CAR also has been demonstrated using an in vitro transactivation assay.

[b] Effects observed in histopathology examinations of the liver.

Source: Cowie (2015)

Temporal association

Liver tumours were clearly induced at 2250 ppm. The observed effects on parameters associated with the key and associative events must occur in a logical, time-dependent manner

consistent with the proposed MOA (see Fig. A2-1). The temporal concordance for pydiflumetofen-induced liver tumours in the male CD-1 mouse database is outlined below:

- Activation of CAR was evident after 2 days of exposure (based on increases in *Cyp2b10* [PROD] activity) and likely occurred as soon as pydiflumetofen was absorbed and transported to the liver. Surrogate markers of specific CAR activity were only measured to day 28 of exposure; however, other, less specific markers of CAR activation, such as liver weight increases, were evident in treatment durations of up to 80 weeks.
- Increases in cell proliferation of hepatocytes were evident from day 2 through day 28 (the last time point tested). Proliferative indices were maximal after day 2, when cell proliferation was associated with increased mitosis, and decreased with time. Consistent with the typical pattern of CAR activation, the histopathological evidence of increased mitosis was not present at day 7 and beyond, indicating a transient stimulation of cell division.
- Elevated cytochrome P450, measured as both total cytochrome content and PROD activity, was evident after 2 days of exposure and likely occurred throughout the exposure period (day 28 was the latest time point tested).
- Elevation of centrilobular hypertrophy was evident after 2 days of exposure to pydiflumetofen, and the corresponding increase in liver weight was statistically significant after 7 days of treatment. These increases persisted throughout the duration of both the subchronic and chronic mouse studies.
- Increased hepatocellular foci and hepatocellular adenomas and carcinomas were evident after 80 weeks of treatment.

Overall, the progression of changes following CAR activation in the liver by pydiflumetofen followed a logical time course, with the earlier key events preceding the ultimate progression to adenomas and carcinomas in the liver (Table A2-3).

Table A2-3. Temporal concordance of key and associative events obtained from all data on pydiflumetofen (2250 ppm) in mice

Key/associative event	Day 2	Day 3	Day 7	Day 28	Day 90	Day 560
CAR activation	Yes	Yes	Yes	Yes	–[a]	–[a]
Hepatocellular replicative DNA synthesis	Yes	–[a]	Yes	Yes	–	–
Increased mitosis	Yes	–	No	No	–	–
Elevated *Cyp2b/Cyp3a* levels (PROD activity)	Yes	Yes	Yes	Yes	–[a]	–[a]
Elevated liver weight	No	Yes	Yes	Yes	Yes	Yes
Centrilobular hypertrophy	No	No	Yes	Yes	Yes	Yes
Hepatocellular foci	No	No	No	No	No	Yes
Tumours	No	No	No	No	No	Yes

–: no data; No: key/associative event not occurring; Yes: key/associative event occurring

[a] Presumed to still be occurring due to effects observed at earlier times and other associative events occurring.

Source: Cowie (2015)

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

All data related to the toxicity or carcinogenicity of pydiflumetofen have been integrated. There is clear consistency across the database. The activation of CAR was measured either directly or via alterations in its surrogate markers of enzyme induction, hepatocyte proliferation, hypertrophy and

hepatomegaly and was seen consistently, across multiple studies, at several time points before the observation of an increased incidence of liver tumours. CAR activation was confirmed both via a direct in vitro transactivation assay and via surrogate markers across multiple studies, which included increased PROD activity. PROD activity is catalysed primarily by CYP2B family enzymes in mice or rats, but with some contribution from other cytochrome P450s, including those in the CYP3A subfamily. In mice, CAR activation has been shown to produce a very large (>100-fold) induction of *Cyp2b10* in particular, with lesser induction of *Cyp3a11* and other cytochrome P450s; therefore, the consistent large increases in PROD activity in multiple mouse studies with pydiflumetofen help confirm the sustained activation of CAR in the liver at higher doses.

The specificity of the hepatocellular adenomas and carcinomas induced by pydiflumetofen in male mice was demonstrated (Robertson, 2015). Studies have shown that although certain key and associative events such as CAR activation and increased liver weight do occur in the rat (Omiecinski, 2014), tumours were not observed in the 104-week carcinogenicity study in the rat with pydiflumetofen (Robertson, 2016). This highlights that CAR activation, while being a necessary causal key event in the proposed MOA for mouse liver tumour formation, is not sufficient by itself. Ample evidence in the literature has shown that mice (particularly male mice) are more prone to liver tumours than rats.

In all data integrated, a very weak proliferative signal after exposure to 75 ppm pydiflumetofen was observed in the 2-day investigative study; however, this key event occurred in isolation, and no statistically significant increases in surrogate markers of CAR activation (*Cyp2b10*-mediated PROD activity), no increases in mitosis and no alterations in liver weight or histopathology in studies up to 90 days in duration were observed. In the 80-week carcinogenicity study, consistent with what was observed in the short-term studies, no effects on liver weight were observed, and, ultimately, no increases in tumour incidence were observed at 75 ppm. This reinforces that a small increase in a necessary causal key event in the proposed MOA for mouse liver tumour formation (hepatocellular proliferation) is not sufficient in isolation to result in a tumorigenic response.

Biological plausibility and coherence

The liver is by far the most common target tissue affected in cancer bioassays. When evaluating liver tumour susceptibility, two metrics are typically used: incidence and multiplicity. The former is most often used to characterize spontaneous liver neoplasia, and the latter to assign relative susceptibility to treatment-induced liver neoplasia. The carcinogenicity database for liver tumour induction in the mouse with pydiflumetofen supports the promotor, non-genotoxic MOA, which is known to increase the incidence of spontaneous age-related tumours without necessarily decreasing the tumour latency. Several MOAs have been identified for the induction of liver tumours in rodents, including activation of nuclear receptors such as CAR, peroxisome proliferator–activated receptor (PPAR) and aryl hydrocarbon receptor (AhR).

Integrated data from multiple studies with pydiflumetofen demonstrating the temporal and dose concordance of the key events are summarized in Tables A2-2 and A2-3, respectively. These data are consistent with the current understanding of the necessary key events in the MOA for CAR activators. Thus, the data for pydiflumetofen support a biologically plausible MOA by a mechanism that is well understood and supported by extensive published research.

Other MOAs

Several MOAs have been identified for liver carcinogenesis, both in humans and in rodent models (Table A2-4**)**. Some of the key events described for pydiflumetofen are common to other known MOAs. However, a full examination of the available database for pydiflumetofen can be used to exclude the alternative MOAs.

Table A2-4. Alternative MOAs for hepatocarcinogenesis

Alternative MOA	Pydiflumetofen: reason for exclusion
DNA reactivity	Pydiflumetofen has been demonstrated to be negative in in vitro bacterial and mammalian mutagenicity assays (Sokolowski, 2012, 2014a; Bohnenberger, 2013a; Wollny, 2013a). Pydiflumetofen has been demonstrated to be negative in in vivo assays for clastogenicity and aneugenicity (Roth, 2012; Dony, 2014a). Late onset of tumours is not indicative of a DNA-reactive mechanism.
Peroxisome proliferator	Pydiflumetofen does not induce peroxisomal palmitoyl CoA oxidase or lauric acid 12-hydroxylase (marker for *Cyp4a*) activities in microsomal mouse liver preparations (Haines, 2012). A slight decrease in palmitoyl CoA oxidase activity with increasing dose was observed, and prior work has established that CAR activators suppress the activation of the PPARα receptor. A 2- to 3-fold increase in lauric acid 12-hydroxylase activity was observed with pydiflumetofen, and similar small increases in this enzyme activity by CAR activators have been shown previously in mice, in contrast to very large increases in this marker of *Cyp4a* activity by PPARα activators (12-fold).
Enzyme induction (AhR)	Pydiflumetofen does not induce substantial EROD activity (a marker for *Cyp1a*) in microsomal male mouse liver preparations (Haines, 2012). Pydiflumetofen caused a 2- to 3-fold increase in EROD activity after 3 or 7 days of treatment, but no effects after 28 days. Small increases in EROD by CAR activators have been demonstrated previously (Sun et al., 2006) and are possibly due to some metabolism via induced CYP2B isoenzymes; these minor changes are in contrast to the large increases by AhR activators.
Statins	Pydiflumetofen was not designed to inhibit HMG-CoA reductase, and pydiflumetofen treatment does not result in decreased cholesterol (Shearer, 2015).
Cytotoxicity	Pydiflumetofen is not a hepatic cytotoxicant based on (a) lack of evidence of elevated hepatic damage in clinical chemistry markers and (b) lack of histopathological evidence of sustained inflammation, necrosis or regenerative proliferation in the liver (Strepka, 2012a; Elcombe, 2015; Robertson, 2015; Shearer, 2015).
Infection	Excluded based on lack of evidence of infection, cellular inflammatory response or regenerative proliferation (Robertson, 2015, 2016; Shearer, 2015).
Increased apoptosis	There is no histological evidence that pydiflumetofen increases hepatocyte apoptosis (Robertson, 2015, 2016; Shearer, 2015).
Estrogenic activity	In the large mammalian toxicological database available for pydiflumetofen, including the studies summarized in this appendix, as well as studies of the effects of pydiflumetofen on reproduction and development (Davies, 2015; Hackford, 2015; Penn, 2015c), there is no evidence for estrogenic stimulation.

AhR: aryl hydrocarbon receptor; CAR: constitutive androstane receptor; CoA: coenzyme A; Cyp: cytochrome P450; DNA: deoxyribonucleic acid; EROD: ethoxyresorufin *O*-deethylation; HMG-CoA: 3-hydroxy-3-methyl-glutaryl-coenzyme A; PPARα: peroxisome proliferator–activated receptor-alpha
Source: Cowie (2015)

Liver carcinogens can be divided into those that are DNA reactive and those that are non–DNA reactive, and both produce their carcinogenic effect by increasing cell proliferation. DNA reactivity can be excluded, as pydiflumetofen tested negative in a battery of in vitro and in vivo genetic toxicity studies. Furthermore, the tumour profile observed in pydiflumetofen carcinogenicity bioassays is typical of a non-genotoxic mechanism (single species, single sex, single organ and lack of decreased latency).

MOAs involving induction of other cytochrome P450 isoforms or peroxisome proliferation can also be excluded based on the nature of the liver findings. Increased liver weight and hepatocellular

hypertrophy are not specific surrogate markers for CAR activation, because the induction of other cytochrome P450 isoforms or peroxisome proliferation can also produce these findings. However, these other MOAs can be ruled out because the experimental evidence shows that pydiflumetofen treatment does not result in any observable biochemical evidence for peroxisome proliferation within the liver hepatocytes; and no substantial increase was observed in EROD activity, which is a marker for *Cyp1a* and AhR activation (Haines, 2012). Hepatocellular cytotoxicity with subsequent regenerative proliferation, such as that caused by chloroform, is another mechanism by which carcinogenesis can occur. This mechanism is characterized by sustained diffuse necrosis and cellular proliferation; however, it can be excluded for pydiflumetofen because of the experimental evidence demonstrating a lack of hepatic damage and regenerative proliferation at all time points investigated. The exclusion of other MOAs for pydiflumetofen induction of liver tumours is detailed in Table A2-4**.**

Uncertainties, inconsistencies and data gaps

The available data support the proposed MOA for the increased incidence of liver tumours in the male CD-1 mouse and exclude the alternative MOAs as described. No critical uncertainties, inconsistencies or data gaps have been identified. The only minor inconsistency in the database for pydiflumetofen is in one 28-day dietary study in CD-1 mice, where there was a lack of observed centrilobular hypertrophy in the presence of increased cytochrome P450 activity and hepatomegaly (Strepka, 2012a). This is in contrast to centrilobular hypertrophy being observed, associated with these events, in numerous other studies of similar or longer duration (Elcombe, 2015; Robertson, 2015; Shearer, 2015).

Assessment of the postulated MOA

The concordance analysis presented above has established that the proposed key events resulting in the liver tumour response are well documented and reproducible across many studies and exhibit a strong dose–response relationship and temporal consistency with the tumour end-point. In addition, this is a well-established MOA based on prior publications, and the parameters essential for describing the MOA have been presented for pydiflumetofen.

Therefore, there is a high level of confidence that the postulated MOA was responsible for the tumour outcome in the male CD-1 mouse.

Human relevance of postulated MOA

Once an MOA in mice has been established, the relevance to humans is assessed by examining each key event. Based on experimental data with pydiflumetofen as well as published information, each key event is assessed to determine whether it is plausible in humans based on 1) qualitative differences or 2) quantitative differences between species in toxicokinetics and toxicodynamics. Table A2-5 describes the similarities and differences between mice and humans in relation to each key event.

Table A2-5. Interspecies comparison of key events for pydiflumetofen

	Mouse		Human		
Key event	**In vivo**	**In vitro**	**In vitro**	**In vivo**	**Comments**
Activation of CAR and altered gene expression	Yes	Yes	Yes	Likely	Activation of both human and mouse CAR occurs
Cytochrome P450 induction (*Cyp2b* and *Cyp3a* isoforms)	Yes	Yes	Yes	Likely	Based on large increase in PROD activities

Key event	Mouse		Human		Comments
	In vivo	In vitro	In vitro	In vivo	
Cell proliferation	Yes	Yes	No	Unlikely[a]	[a] Based on in vitro/in vivo correlation
Tumours	Yes	N/A	N/A	Unlikely[b]	[b] Based on lack of cell proliferation and data on human drugs that are CAR activators

N/A: not applicable
Source: Cowie (2015)

PROD activity has been reported to be catalysed primarily by CYP2B family enzymes in mice and rats, but with some contribution from other cytochrome P450 isoforms, including those in the CYP3A subfamily. With human primary hepatocytes and purified cytochrome P450 isoforms, PROD activity has been shown to correlate with human CYP2B6 activity, although other isoforms may also contribute.

CAR activation

The data generated with in vitro CAR reporter gene assays clearly demonstrate that pydiflumetofen was a direct activator of mouse and human CAR. Further, the concentrations at which maximal CAR activation was attained provide evidence that pydiflumetofen may have greater potency with mouse CAR than with rat or human CAR. The downstream effects that follow human CAR activation were also tested in a human hepatocyte system. Testing human male donor hepatocytes in primary culture with pydiflumetofen resulted in elevations of PROD and BROD activities, which are surrogate markers of *Cyp2b/Cyp3a* induction produced via CAR activation (Lowes, 2015b). Similar results were obtained in the mouse (Lowes, 2015a). As activation of CAR and induction of *Cyp2b/Cyp3a* isoforms occurred in both mouse and human hepatocytes, there is no qualitative difference between human and mouse in terms of the ability of pydiflumetofen to activate CAR.

Therefore, the human is not qualitatively different from the mouse in terms of the initial key event, pydiflumetofen-induced CAR activation.

Hepatocyte proliferation

The ability of pydiflumetofen to increase the proliferation of human hepatocytes was evaluated by treating male donor hepatocytes in primary culture with pydiflumetofen and measuring replicative DNA synthesis by BrdU labelling index as the marker of increased S-phase and proliferation (Lowes, 2015b). Similar experiments were performed using mouse hepatocytes in primary culture (Lowes, 2015a). At doses ranging from 5 to 35 µmol/L, pydiflumetofen did not increase BrdU labelling in male human hepatocytes, whereas a robust increase in labelling index was observed in male mouse hepatocytes (Lowes, 2015a,b). In addition to this, the positive control CAR activator phenobarbital (PB) displayed a similar pattern to pydiflumetofen, causing increased BrdU labelling of mouse hepatocytes, but no effect in male human hepatocytes.

This response is typical of that seen with other CAR activators, such as PB, sulfoxaflor and metofluthrin.

Therefore, the human is qualitatively different from the mouse in terms of the second key event in the MOA for pydiflumetofen, hepatocellular proliferation induced by CAR activation.

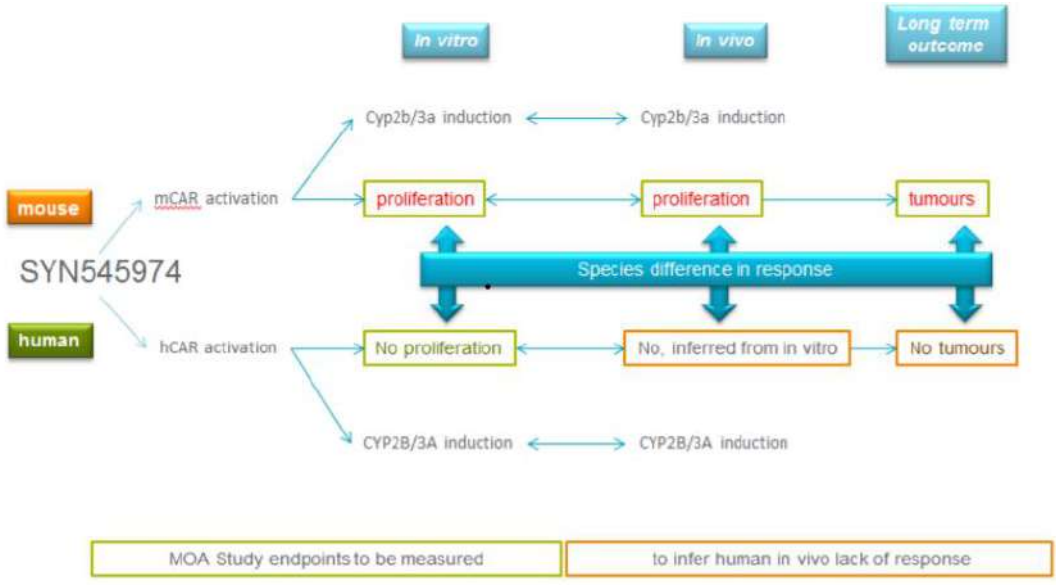
Statement of confidence, analysis and implications

Based on the above, there is strong evidence to support an MOA for pydiflumetofen-induced hepatocellular tumours in male mice that involves *Cyp2b/Cyp3a* induction through CAR activation and an increase in hepatocyte proliferation that results ultimately in an increase in the incidence of liver tumours. Based on the human evidence presented above, using comparative studies in hepatocytes, there is strong evidence that qualitative species differences exist between mouse and human for the pivotal key event of increased hepatocellular proliferation; pydiflumetofen did not produce cell proliferation in human hepatocytes. Based on these species differences in response, pydiflumetofen is unlikely to cause cell proliferation in humans in vivo, and it is therefore unlikely to cause tumours in humans (Table A2-1; Figs A2-1 and A2-2). This conclusion is supported further by epidemiology studies that show a lack of tumour response in patients treated with known CAR activators, which share the same MOA as pydiflumetofen.

Conclusion

Based on the available data, the MOA for liver tumour induction in male CD-1 mice treated with pydiflumetofen was established. This MOA involves key events that include an initial activation of CAR, altered CAR-dependent gene transcription, and a critical key event of increased cell proliferation. Based on the qualitative species differences in the hepatocellular proliferation response to pydiflumetofen, it has been established that this MOA is not relevant to humans (Fig. A2-2).

Fig. A2-2. Summary of species differences in response to pydiflumetofen



Source: Cowie (2015)

Therefore, these data support the conclusion that pydiflumetofen does not pose a hepatocarcinogenic hazard to humans.

Appendix 3: Blood concentrations of pydiflumetofen in toxicity studies

Thirteen-week study in mice (Shearer, 2015)

Blood samples (approximately 0.1 mL) were obtained from the saphenous vein of all animals (five mice of each sex at each time point) in each group for the analysis of pydiflumetofen in whole blood. The samples were collected at 07:00, 11:00, 15:00 and 18:00 on days 2, 16, 30 and 91. Control group (0 ppm) samples were collected prior to those from the treatment groups. Fifty microlitres of whole blood from each sample was mixed with 50 µL of deionized water and stored at −80 ºC prior to analysis. Concentrations of pydiflumetofen were determined using a suitable liquid chromatography–tandem mass spectrometry (LC-MS/MS) analytical method. A research method was established by preparing calibration and quality control samples over the range 5–5000 ng/mL.

The mean concentrations are shown in Table A3-1 and Fig. A3-1.

Table A3-1. Group mean blood/water[a] concentrations of pydiflumetofen in mice over a 13-week period

	Group mean[b] blood/water concentrations (ng/mL)									
	0 ppm		100 ppm		500 ppm		4 000 ppm		7 000 ppm	
Time	M	F	M	F	M	F	M	F	M	F
Day 2										
07:00	<LLOQ	<LLOQ	<LLOQ	12.0	<LLOQ	21.9	<LLOQ	1.9	98.0	103.4
11:00	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	16.3	20.3	73.5	50.9	248.2
15:00	–[c]	<LLOQ	1.1	5.9	3.9	8.5	27.2	116.8	101.5	142.2
18:00	–[c]	–[c]	4.1	5.3	6.2	14.7	31.6	105.1	66.5	390.2
Day 16										
07:00	<LLOQ	<LLOQ	1.3	3.8	8.5	101.7	138.3	335.4	239.2	633.8
11:00	–[c]	–[c]	9.5	4.2	2.4	33.3	256.6	181.5	85.1	329.2
15:00	–[c]	<LLOQ	<LLOQ	13.9	2.8	29.8	39.3	93.2	64.1	182.3
18:00	<LLOQ	–[c]	1.4	3.0	3.6	41.3	100.2	715.2	143.4	705.4
Day 30										
07:00	–[c]	<LLOQ	5.7	<LLOQ	2.2	14.6	21.7	36.2	52.0	108.8
11:00	<LLOQ	–[c]	149.1	3.6	5.4	3.7	30.6	44.5	64.3	310.8
15:00	–[c]	–[c]	<LLOQ	1.5	2.9	15.5	25.6	27.3	43.3	53.1
18:00	<LLOQ	2.8	2.8	1.9	1.9	32.3	17.3	42.2	38.9	217.0
Day 91										
07:00	–[c]	<LLOQ	<LLOQ	1.1	4.3	21.8	65.0	139.8	118.4	770.1
11:00	<LLOQ	<LLOQ	1.7	3.4	<LLOQ	11.1	110.5	138.4	45.2	194.0
15:00	–[c]	<LLOQ	<LLOQ	1.1	3.8	21.5	13.1	46.9	122.4	132.1
18:00	<LLOQ	<LLOQ	<LLOQ	1.3	<LLOQ	8.7	28.4	190.7	60.6	122.2

F: females; LLOQ: lowest limit of quantification (<5.0 ng/mL); M: males; ppm: parts per million

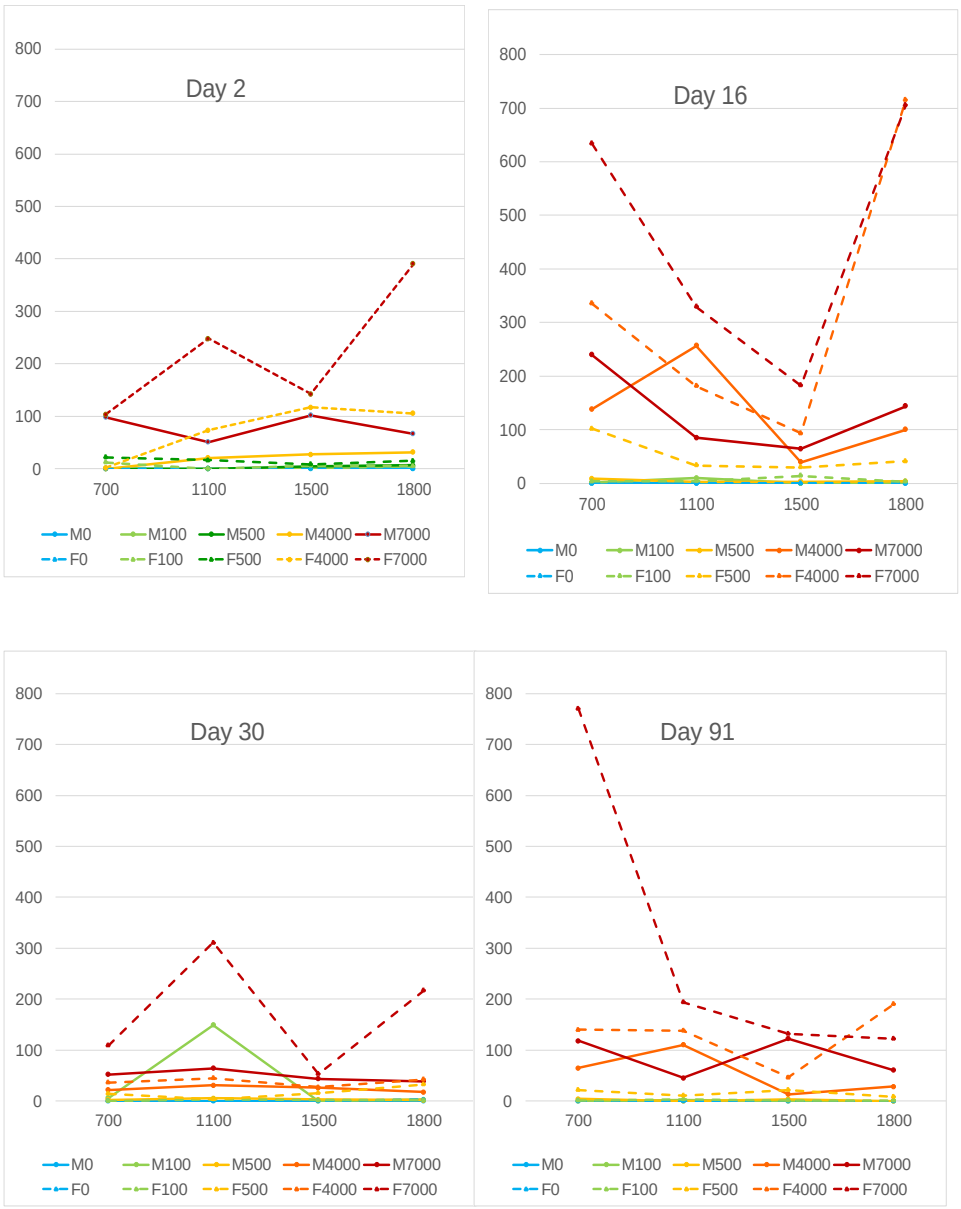
[a] Immediately following collection, 50 µL of whole blood from each sample was measured into a plain plastic tube containing 50 µL of deionized water. Concentrations are based on a whole blood:water (1:1, v/v) solution.

[b] In mean calculations, LLOQ imputed as 0.

[c] Eighteen animals were noted with a blood concentration marginally above the LLOQ.

Source: Shearer (2015)

Fig. A3-1. Group mean blood/water[a] concentrations (ng/mL) of pydiflumetofen in mice over a 13-week period



F: females; M: males
LOQ or LLOQ was expressed as 0.
[a] Immediately following collection, 50 µL of whole blood from each sample was measured into a plain plastic tube containing 50 µL of deionized water. Concentrations are based on a whole blood:water (1:1, v/v) solution.
Source: Shearer (2015)

Thirteen-week study in rats (Shearer & Robertson, 2015)

Blood samples (approximately 0.1 mL) were obtained from the tail vein of all animals (10 rats of each sex at each time point) in each group for the analysis of pydiflumetofen in whole blood. The samples were collected at 07:00, 11:00, 15:00 and 18:00 on days 2, 9, 28 and 91. Samples from the control group (0 ppm) were collected prior to those from the treatment groups. Fifty microlitres of whole blood from each sample was mixed with 50 µL of deionized water and stored at −80 ºC prior to analysis. Concentrations of pydiflumetofen were determined using a suitable LC-MS/MS analytical method. A

research method was established by preparing calibration and quality control samples over the range 5–5000 ng/mL.

The mean concentrations are shown in Table A3-2 and Fig. A3-2.

Thirteen-week study in dogs (Blunt, 2015a)

Blood samples (0.3 mL) were taken from all animals by venipuncture of the cephalic veins before dosing and at 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours after dosing on days 1 and 28 and during week 13 and placed into tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Whole blood (0.05 mL) was mixed with 0.05 mL of deionized water and stored at −70 °C before analysis. All samples from animals given pydiflumetofen were analysed, with only predosing samples analysed for controls. Concentrations of pydiflumetofen were determined from blood samples using a validated LC-MS/MS bioanalytical method.

Pydiflumetofen was detected in the blood of control animals. Concentrations of pydiflumetofen in blood from treated groups at each time point are shown in Table A3-3.

There was no consistent evidence of accumulation over the treatment period. Exposure to pydiflumetofen tended to be higher in males, most notably at 300 mg/kg bw per day.

Table A3-2. Group mean blood/water[a] concentrations of pydiflumetofen in rats over a 13-week period

	Group mean[b] blood/water concentrations (ng/mL)									
	0 ppm		250 ppm		1 500 ppm		8 000 ppm		16 000 ppm	
Time	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Day 2										
07:00	<LLOQ	<LLOQ	14.8	23.9	44.1	86.7	47.5	70.6	58.2	107.1
11:00	<LLOQ	–[c]	3.4	28.9	26.5	69.1	31.6	68.4	35.1	77.3
15:00	<LLOQ	<LLOQ	1.6	17.0	16.8	36.9	21.4	42.6	25.7	53.0
18:00	<LLOQ	–[c]	2.4	21.8	15.6	49.1	29.8	43.2	31.3	50.0
Day 16										
07:00	–[c]	–[c]	13.3	46.3	39.5	84.4	50.0	81.3	59.8	94.9
11:00	–[c]	<LLOQ	8.6	26.8	13.0	74.3	46.9	73.9	53.9	70.1
15:00	–[c]	<LLOQ	0.6	17.9	12.1	39.7	45.1	63.5	49.2	94.1
18:00	<LLOQ	<LLOQ	13.7	19.8	19.1	50.0	28.1	89.7	65.9	65.4
Day 30										
07:00	<LLOQ	–[c]	2.4	20.7	15.0	64.6	32.8	83.1	54.1	103.0
11:00	<LLOQ	–[c]	<LLOQ	23.3	10.5	48.6	23.4	84.5	62.3	102.2
15:00	<LLOQ	–[c]	0.9	14.7	9.8	37.7	30.3	63.9	90.2	67.8
18:00	<LLOQ	–[c]	<LLOQ	18.1	8.3	38.2	27.7	73.8	38.3	67.2

LLOQ: lowest limit of quantification (5.0 ng/mL); ppm: parts per million

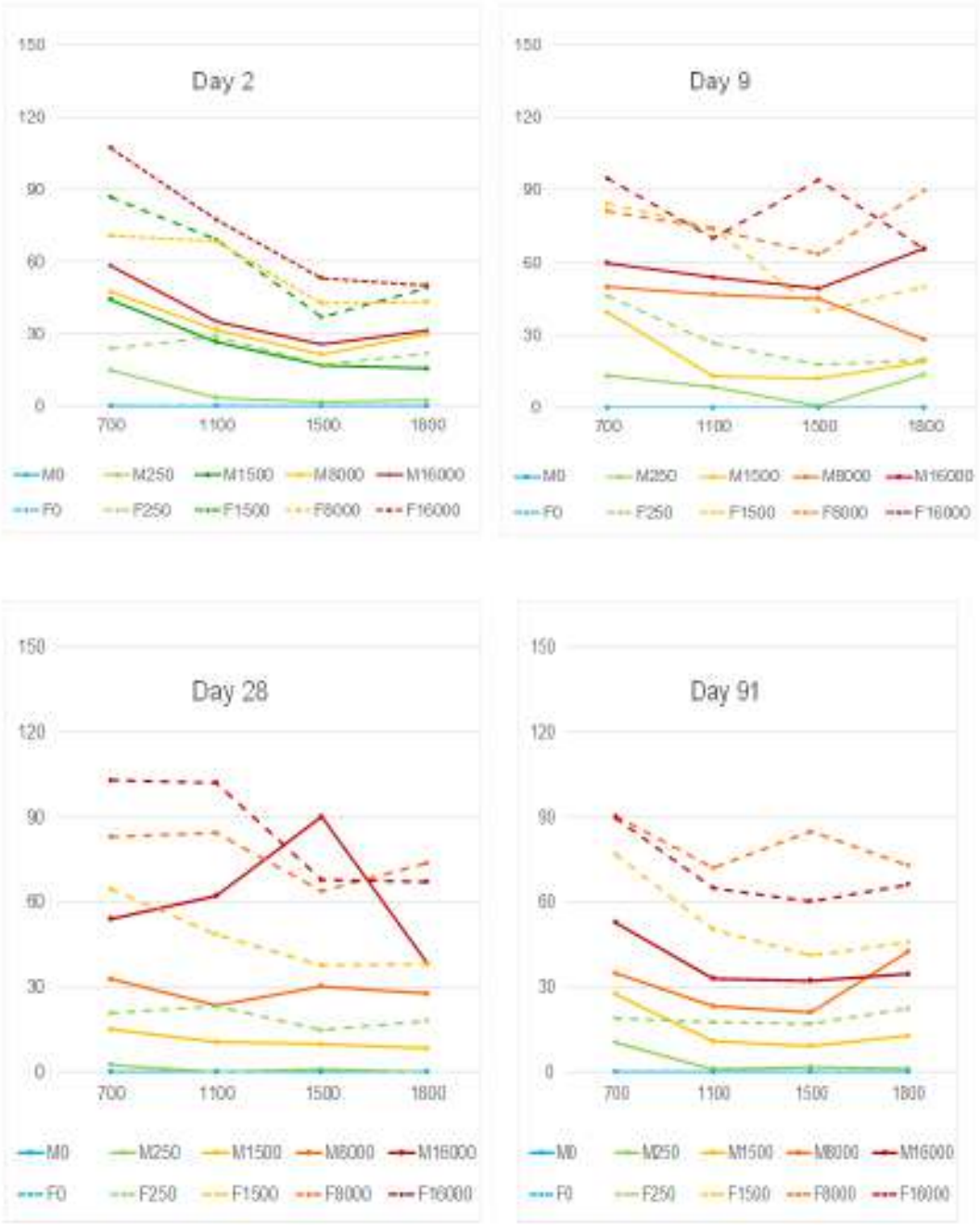
[a] Immediately following collection, 50 µL of whole blood from each sample was measured into a plain plastic tube containing 50 µL of deionized water. Concentrations are based on a whole blood:water (1:1, v/v) solution.

[b] In mean calculations, LLOQ imputed as 0.

[c] Twelve animals were noted with a blood concentration above the LLOQ.

Source: Shearer & Robertson (2015)

Fig. A3-2. Group mean blood/water[a] concentrations (ng/mL) of pydiflumetofen in rats over a 13-week period



F: females; M: males
LOQ or LLOQ was expressed as 0.
[a] Immediately following collection, 50 µL of whole blood from each sample was measured into a plain plastic tube containing 50 µL of deionized water. Concentrations are based on a whole blood:water (1:1, v/v) solution.
Source: Shearer & Robertson (2015)

Table A3-3. Group mean blood/water[a] concentrations (ng/mL) of pydiflumetofen in dogs over a 13-week period

Hours after dosing	Group mean blood/water concentrations (ng/mL)					
	30 mg/kg bw		300 mg/kg bw		1 000 mg/kg bw	
	Males	Females	Males	Females	Males	Females
Day 1						
0	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
0.5	4.50	1.68	7.57	6.11	12.8	5.53
1	10.4	2.95	13.4	15.8	27.4	15,6
1.5	15.1	8.15	13.4	15.8	33.9	39.5
2	24.0	16.1	104	133	140	109
4	11.0	13.6	399	494	2 170	2 200
8	1.78	3.18	266	210	3 040	2 300
12	<5.00	<5.00	488	88.6	1 400	1 230
24	<5.00	<5.00	65.8	33.9	231	169
Day 28						
0	2.68	<5.00	14.9	3.76	49.4	29.5
0.5	5.13	1.34	40.0	13.8	60.9	55.1
1	10.1	7.06	75.8	31.9	128	161
1.5	19.0	18.8	127	89.5	269	513
2	34.5	20.8	144	138	501	834
4	15.1	16.0	207	119	1 700	1 740
8	9.01	3.02	362	18.7	160	1 360
12	3.70	0.38	474	9.01	267	259
24	6.33	<5.00	10.0	8.57	32.4	25.5
Day 91						
0	<5.00	<5.00	11.9	5.78	24.6	24.7
0.5	4.94	5.37	45.1	23.5	103	64.0
1	12.1	13.6	89.0	46.1	239	141
1.5	26.0	11.4	258	56.6	689	255
2	34.5	11.2	390	91.7	1 040	638
4	17.3	10.4	510	129	2 040	825
8	4.88	1.66	370	19.8	1 460	295
12	2.73	<5.00	314	11.3	435	270
24	5.52	4.65	11.1	11.5	19.3	28.3

<5.00: below the limit of quantification; bw: body weight

[a] Values based on 1:1 blood:water mixture.

Source: Blunt (2015a)