



Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

Prepared according to **Regulation (EC) 1107/2009**
as it applies in Great Britain

Pydiflumetofen

Volume 3 – B.6 (AS) – part I

Toxicology & Metabolism Data

Great Britain

June 2023

Version History

When	What
October 2022	Initial DAR
June 2023	Post Expert Committee on Pesticides (ECP) Independent Scientific Advice (ISA)

Table of contents

B.6. TOXICOLOGY AND METABOLISM DATA.....	4
B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS	4
B.6.1.1. Absorption, distribution, metabolism and excretion by oral route	6
B.6.1.2. Absorption, distribution, metabolism and excretion by other routes.....	128
B.6.1.3. ADME summary	137
B.6.2. ACUTE TOXICITY	141
B.6.2.1. Oral.....	141
B.6.2.2. Dermal.....	144
B.6.2.3. Inhalation	147
B.6.2.4. Skin irritation	152
B.6.2.5. Eye irritation.....	155
B.6.2.6. Skin sensitization	159
B.6.2.7. Phototoxicity	163
B.6.2.8. Summary of acute toxicity	168
B.6.3. SHORT-TERM TOXICITY.....	170
B.6.4. GENOTOXICITY.....	260
B.6.4.1. In vitro studies	260
B.6.4.2. In vivo studies in somatic cells.....	286
B.6.4.3. In vivo studies in germ cells.....	306
B.6.4.4. Summary of genotoxicity	306
B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS.....	308
B.6.6. REPRODUCTIVE TOXICITY.....	379
B.6.6.1. Generational studies	379
B.6.6.2. Developmental toxicity studies	379
B.6.7. NEUROTOXICITY	379
B.6.7.1. Neurotoxicity studies in rodents.....	379
B.6.7.2. Delayed polyneuropathy studies	379
B.6.8. OTHER TOXICOLOGICAL STUDIES.....	379
B.6.8.1. Toxicity studies on metabolites and relevant impurities	379
B.6.8.2. Supplementary studies on the active substance	379
B.6.8.3. Studies on endocrine disruption	379
B.6.9. MEDICAL DATA AND INFORMATION.....	379
B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies.....	379
B.6.9.2. Data collected on humans	379
B.6.9.3. Direct observation	380
B.6.9.4. Epidemiological studies	380
B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test.....	380
B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment	380
B.6.10. REFERENCES RELIED ON	380

B.6. TOXICOLOGY AND METABOLISM DATA

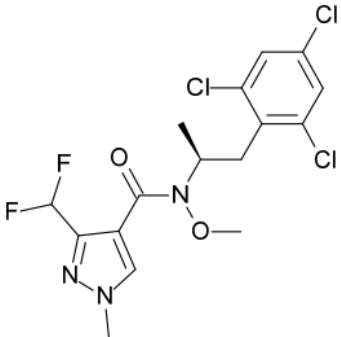
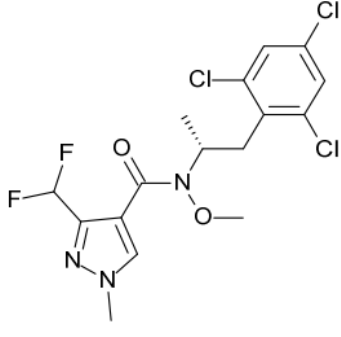
This document summarises all the toxicological data which are relevant for the approval of pydiflumetofen in GB under Regulation EC No 1107/2009 in accordance with the requirements of Regulation EC No 283/2013.

An EU peer-reviewed DAR¹, EFSA Conclusion² and RAC Opinion³ on harmonized classification are available for pydiflumetofen. Therefore, HSE will perform an independent evaluation of the available data but make use of the assessments already available at EU level as appropriate. New information generated and submitted by the applicant following the completion of the EU process, has been fully evaluated by HSE. With regard to the classification of pydiflumetofen, HSE has already produced and published a Technical Report⁴ supporting the Mandatory Classification & Labelling (MCL) of the substance in GB.

Pydiflumetofen (also called SYN545974 or ADEPIDYNTM) is a new broad spectrum fungicide of the chemical group of N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide. The mode of action of the active substance is respiration inhibition at complex II (Succinate-DeHydrogenase) in mitochondria of phytopathogenic fungi, thus SYN545974 belongs to the SDHI fungicide group. There is no cross resistance between compounds belonging to this group and strobilurin (QoI) or triazole (DMI) chemistry.

Pydiflumetofen has a very broad spectrum of disease control across multiple crops. It delivers very good efficacy against leaf spots (such as *Venturia* sp. and *Alternaria* sp.), powdery mildews and *Botrytis*.

Pydiflumetofen contains two enantiomers, both of which are biologically active. The two enantiomers are separately numbered SYN546968 and SYN546969. The technical specification of pydiflumetofen covers an enantiomer ratio of 1 (in all cases expressed as SYN546968/SYN546969, i.e. an enantiomer fraction ratio for SYN546968:SYN546969 of 50:50).

ABSOLUTE	ABSOLUTE
	
<p style="text-align: center;">SYN546968</p> <p>(S)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide</p>	<p style="text-align: center;">SYN546969</p> <p>(R)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide</p>

B.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

The mammalian fate of SYN545974 has been assessed in studies investigating the absorption, distribution, metabolism (qualitative and quantitative) and excretion (ADME) in rats. The excretion and biotransformation of SYN545974 was also investigated in mice. The toxicokinetic profile of SYN545974 following repeat gavage, dietary or capsule dosing in rats, mice, rabbits and dogs was

¹ [Peer review of the pesticide risk assessment of the active substance pydiflumetofen \(wiley.com\)](#)

² [Peer review of the pesticide risk assessment of the active substance pydiflumetofen \(wiley.com\)](#)

³ [\[04.01-ML-014.03\] \(europa.eu\)](#)

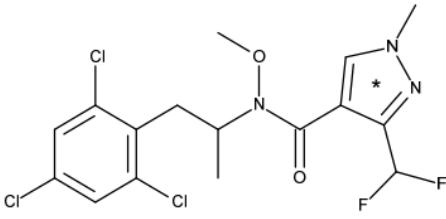
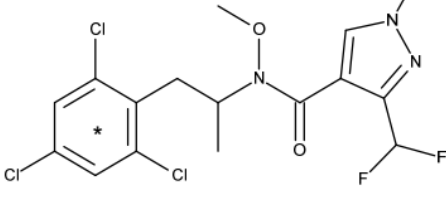
⁴ [Updating the GB mandatory classification and labelling list \(GB MCL List\) \(hse.gov.uk\)](#)

determined. Toxicokinetic data was used to support dose level selection for some toxicity studies based on linear versus non-linear kinetics in rat, mouse, dog and rabbit. In addition, intravenous administration of the test substance (radiolabelled and non-radiolabelled) and measurement of the test substance in blood and/or excreta was used to establish oral bioavailability or oral absorption in rats and mouse.

In rat, preliminary ADME studies using [pyrazole-5-¹⁴C]- and [phenyl-U-¹⁴C]- radiolabelled SYN545974 indicated that SYN545974 was metabolically cleaved between the pyrazole and phenyl moieties. Therefore, subsequent ADME studies used both radiolabels. Bile duct cannulated rats were used in the main ADME study, as the preliminary study showed that greater than 20% of the administered dose was excreted in feces.

The structure of SYN545974 and position of the radiolabels is shown in Table 6.1-1:

Table 6.1-1: Radiolabelled Forms of SYN545974 used in ADME Studies – Structure and Position of the Label

Radiolabel	Structure and position of label
[Pyrazole-5- ¹⁴ C]SYN545974 (* = Position of ¹⁴ C)	
[Phenyl-U- ¹⁴ C]-SYN545974 (* = Position of ¹⁴ C)	

The table below provides an overview of the ADME studies conducted with SYN545974.

Table 6.1-2: Summary of ADME studies conducted

Title	Author/Date	Report
A Preliminary Study of Pharmacokinetics, Absorption, Metabolism and Excretion in Rats Following Single Oral and Intravenous Administration of ¹⁴ C-SYN545974	██████████, ██████████ (2015). Syngenta File No. SYN545974_10188	SGA-64
The Absorption and Excretion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C] SYN545974 Following Single Oral Administration in the Rat	██████████ (2015). Syngenta File No. SYN545974_12048	34214
Tissue Depletion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C] SYN545974 Following Single Oral Administration in the Rat	██████████ (2015a). Syngenta File No. SYN545974_10252	34340
The Pharmacokinetics of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral and Intravenous Administration in the Rat	██████████, ██████████ (2015). Syngenta File No. SYN545974_10250	34107
Biotransformation of [¹⁴ C]-SYN545974 in Rat	██████████, ██████████ (2015). Syngenta File No. SYN545974_10259	34216
Pharmacokinetics of SYN545974 in the Rat Following Multiple Oral and Single Intravenous Administration	██████████, ██████████ (2014). Syngenta File No. SYN545974_10104	33409
Pharmacokinetics of SYN545974 in the Mouse Following Multiple Oral and Single Intravenous Administration	██████████, ██████████ (2014a). Syngenta File No. SYN545974_10124	33408

The Excretion and Biotransformation of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral Administration in the Mouse	██████████, ██████████, ██████████ (2015). Syngenta File No. SYN545974_10257	35415
SYN545974 - Oral (Gavage) Toxicokinetic Study in the Pregnant Rabbit.	██████████ (2015). Syngenta File No. SYN545974_10125	BFI0126

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

Report:	K-CA 5.1.1/01 ██████████ and ██████████ (2015). SYN545974 - A Preliminary Study of Pharmacokinetics, Absorption, Metabolism and Excretion in Rats Following Single Oral and Intravenous Administration of ¹⁴ C-SYN545974. ██████████ ██████████ ██████████ Report No. SGA-64. Issue date 20 May 2015. Unpublished. Syngenta File No. SYN545974_10188.
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Guidelines: Metabolism – rat; OECD 417 (2010); EPA OPPTS 870.7485 (1998); EC 1107/2009 (2009), EU 283/2013 (2013), JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE: the study is considered acceptable.

EXECUTIVE SUMMARY

The pharmacokinetics, absorption, metabolism and excretion of total radioactivity were investigated following a single oral administration of two radiolabelled forms of [¹⁴C]-SYN545974 (5 or 1000 mg/kg) and a single intravenous dose of 1 mg/kg to male and female Han Wistar rats. Pharmacokinetic parameters were calculated from the concentrations of total radioactivity determined in blood and plasma. Excretion samples were obtained over a 7 day period, with expired air collected following oral doses over the first 2 days. After this period, the rats were humanely killed and residual radioactivity was measured in the remaining carcass. Subsequently, the nature and identity of metabolites present in excreta and plasma samples was investigated.

Following intravenous administration, exposure to [pyrazole-5-¹⁴C]-SYN545974 and [phenyl-U-¹⁴C]-SYN545974 was broadly comparable, and irrespective of gender. Oral exposure to both labels was broadly comparable between genders. Exposure to both labels increased sub-proportionally with respect to dose for oral administration. Exposure appeared slightly higher in plasma than blood for [pyrazole-5-¹⁴C]-SYN545974 and [phenyl-U-¹⁴C]-SYN545974, but only at the higher 1000 mg/kg dose.

Mean recoveries of administered radioactivity were >90% for all dose groups with the exception of the 1000 mg/kg female rat dose groups. Mean recoveries for the 1000 mg/kg female rat dose groups were >85%, excretion of radioactivity for these groups was very similar to males and therefore it is believed the incomplete recovery may be in the feces. The major route of elimination was *via* the feces in both males and females and the majority of the administered radioactivity was excreted in urine and feces within the first 72 hours following dosing. There was no notable radioactivity remaining in the carcass of male or female rats from either dose group, indicating excretion was complete by 168 hours after dosing.

The major components (>5% of dose) identified in excreta following oral administration of [pyrazole-5-¹⁴C] and [phenyl-U-¹⁴C]-SYN545974 were:

- 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid
- 2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid
- hexose sugar conjugate of di-hydroxy SYN545974 and 2,4,6-TCP glucuronide (co-eluting)

- 2,4,6-TCP sulphate, tentative⁵ linked 2,4,6-TCP disulphate and oxidative dechlorinated hydroxy SYN545974 sulphate (co-eluting)
- di-hydroxy SYN545974 and hydroxy O,N-demethylated SYN545974 (co-eluting)
- O-demethylated SYN545974, hydroxy N-demethylated SYN545974 and di-hydroxy N-demethanolated SYN545974 (co-eluting)
- oxidative dechlorinated SYN545974
- hydroxy SYN545974
- tentative methyl mercapto oxidative dechlorinated hydroxy SYN545974, tentative hydroxy N-demethanolated SYN545974 and second isomer of oxidative dechlorinated SYN545974 (co-eluting)
- SYN545974

The major circulating components identified following oral administration of [pyrazole-5-¹⁴C] and [phenyl-U-¹⁴C]-SYN545974 were:

- 3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide
- 3-(difluoromethyl)-N-(2-hydroxy-1-methyl-ethyl)-N-methoxy-1-methyl-pyrazole-4-carboxamide glucuronide
- 2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid
- 2,4,6-TCP sulphate
- di-hydroxy SYN545974
- hydroxy SYN545974 and hydroxy-N-demethanolated SYN545974 glucuronide (co-eluting)
- SYN545974

Following oral and intravenous administration of [¹⁴C]-SYN545974, the major route of elimination was *via* the feces in both males and females, with the majority of the administered radioactivity excreted in urine and feces within the first 72 hours following dosing. No notable radioactivity was recorded in expired air.

Oral exposure to both labels was broadly comparable between genders. Exposure to both labels increased with dose for oral administration, but was sub-proportional to dose.

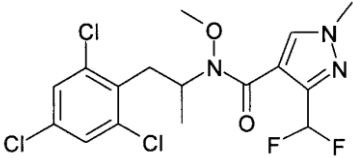
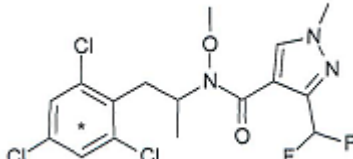
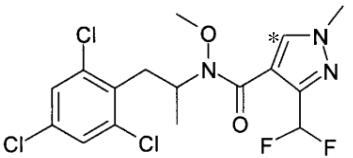
The biotransformation of SYN545974 in rat proceeded by various phase 1 and phase 2 metabolic pathways. Together with oxidation, glucuronidation and sulphation there was cleavage at the amide bond to form pyrazole related metabolites and cleavage of SYN545974 to form 2, 4, 6-trichlorophenol related metabolites.

⁵ Tentative assignment 2,4,6-TCP disulphate based on structural assignment, molecular weight and fragmentation, in the absence of an analytical standard.

MATERIALS AND METHODS

Materials:

Unlabelled test material:

Name :	SYN545974
Structure :	
Source :	Syngenta Crop Protection Münchwilen AG
Physical state :	Off white, powder
Batch reference :	2637-BA/110
Purity:	99.5%
Radiolabelled Test Material:	[Phenyl-U- ¹⁴ C]-SYN545974
Radiochemical purity:	98.7%
Source:	Syngenta Crop Protection LLC
Lot/Batch number:	DAD-XIII-38-5
Structure:	 <p style="text-align: right;">* position of [¹⁴C]-label</p>
Radiolabelled Test Material:	[Pyrazole-5- ¹⁴ C]-SYN545974
Radiochemical purity:	1. 97.8% 2. 97.2%
Source:	Selcia
Lot/Batch number:	1. 5222MFO001-1 2. 2.RDR-XIV-49
Structure:	 <p style="text-align: right;">* position of [¹⁴C]-label</p>

Vehicle: 5 mg/kg oral doses: 5% (v/v) dimethylsulphoxide (DMSO) and 0.5% (v/v) Tween 80 in 0.5% (w/v) aqueous carboxymethylcellulose (CMC). 1000 mg/kg oral doses: 20% (v/v) dimethylsulphoxide (DMSO) and 0.5% (v/v) Tween 80 in 0.5% (w/v) aqueous CMC. The intravenous dose vehicle was 5% (w/v) dimethylsulphoxide (DMSO) in 40% (w/v) aqueous hydroxypropyl-β-cyclodextrin.

Preparation of dosing solutions: Each oral dose preparation was formulated as a homogenous suspension in 0.5% aqueous CMC containing 0.5% Tween 80. The intravenous dose was formulated as a non-irritant solution. Each dose preparation was prepared using radiolabelled and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity.

Test Animals:

Species:	Rat
Strain:	Han Wistar
Age at dosing:	6 - 8 weeks for males; 8 - 11 weeks for females (1 mg/kg IV) 8 - 11 weeks for males; 8 - 11 weeks for females (5 mg/kg Oral) 8 - 11 weeks for males; 8 - 11 weeks for females (1000 mg/kg Oral)
Weight at dosing:	186 - 208 g for males; 181 - 195 g for females (1 mg/kg IV) 218 - 262 g for males; 180 - 211 g for females (5 mg/kg Oral) 219 - 264 g for males; 189 - 216 g for females (1000 mg/kg Oral) On the day of dosing some of the animals were out with the weight range given in the Study Plan (approx. 210 - 250g). The actual weight range was 180 – 264 g. This deviation to the study plan is believed to have no impact on the integrity of the study.
Source:	██████████
Housing:	Animals were housed in groups of up to four (as appropriate) in polypropylene cages, in a thermostatically monitored room ($21 \pm 2^{\circ}\text{C}$) and exposed to 12 hours fluorescent lighting and 12 hours dark per day in the animal unit of ██████████. One the day prior to dosing, animals selected for excretion/balance studies were transferred to individual glass metabolism cages specially designed for the separate collection of urine, feces and expired air under the above environmental conditions for the duration of the study except for dosing. Animals were returned to their individual metabolism cages immediately after completion of this procedure.
Acclimatisation period:	At least 3 days (including acclimatisation to metabolism cages)
Diet:	Pellet diet (RM1 (E) SQC, ██████████) was available <i>ad libitum</i> throughout the holding, acclimatisation and post-dose periods.
Water:	Water, from the domestic water supply <i>ad libitum</i>
Husbandry:	Animals were housed in groups of up to four per sex for the main study.
Identification:	Each rat was given a unique identity number and each was identified by unique tail markings (with indelible ink).
Environmental conditions:	
Temperature:	$21 \pm 2^{\circ}\text{C}$
Humidity:	$55 \pm 10\%$
Photoperiod:	Alternating 12-hour light and dark cycles

Study Design and Methods:

Experimental Dates: Start: 17 April 2012 End: 2 July 2013

Dosing and sample collection:

The target radioactivity in each dose was 5 MBq/kg. The dose volume was 5 mL/kg for the intravenous doses and 10 mL/kg for the oral doses.

Dosing and sampling collection for pharmacokinetic studies for [¹⁴C]-SYN545974

Radiolabelled form	Route	Dose (mg/kg)	Animal numbers		Sampling times (hours after dosing)
			Male	Female	
[pyrazole-5- ¹⁴ C]-SYN545974	IV	1	1M	2F	0.08, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48
	Oral	5	3MR	9FR	0.08 and 0.5*
			4MR	10FR	1 and 4*
			5MR	11FR	2 and 8*
			6MR	12FR	6 and 24*
			7MR	13FR	12 and 30*
			8MR	14FR	48 and 72*
	Oral	1000	15M	21F	0.08 and 0.5*
			16M	22F	1 and 4*
			17M	23F	2 and 8*
			18M	24F	6 and 24*
			19M	25F	12 and 30*
			20M	26F	48 and 72*
[phenyl-U- ¹⁴ C]-SYN545974	IV	1	33M	34F	0.08, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48
	Oral	5	35M	41F	0.08 and 0.5*
			36M	42F	1 and 4*
			37M	43F	2 and 8*
			38M	44F	6 and 24*
			39M	45F	12 and 30*
			40M	46F	48 and 72*
	Oral	1000	47M	53F	0.08 and 0.5*
			48M	54F	1 and 4*
			49M	55F	2 and 8*
			50M	56F	6 and 24*
			51M	57F	12 and 30*
			52M	58F	48 and 72*

* = sample taken as terminal sample

Dosing groups for excretion balance studies for [¹⁴C]-SYN545974

Radiolabelled form	Route	Dose (mg/kg)	Animals Number	
			Male	Female
[pyrazole-5- ¹⁴ C]-SYN545974	IV	1	27M	28F
	Oral	5	29M	30F
	Oral	1000	31M	32F
[phenyl-U- ¹⁴ C]-SYN545974	IV	1	59M	60F
	Oral	5	61M	62F
	Oral	1000	63M	64F

Urine was collected pre-dose and then 0 – 8, 8 – 24 hours post dose then at 24 hour intervals up to 168 hours. Feces were collected pre-dose, then at 24 hour intervals up to 168 hours. At 8 hours each cage was washed with 5 mL of water and the washings collected into the 8 hour urine sample. At 24 hour intervals until animals were terminated at 168 hours cages were rinsed with water and the washes collected into separate pre-weighed containers. At 168 h cages were also rinsed with methanol and the washes collected into separate pre-weighed containers. Expired air was collected at pre-dose, 0 – 24 and 24 – 48 hours post dose. After 168 h, the rats were humanely killed and residual radioactivity was measured in the remaining carcass.

Preparation of representative sample pools for metabolite profiling

Representative sample pools were prepared by combining a fixed percentage by weight of each sample (for urine and feces) at selected time points from each animal. Time points were selected to represent ≥90% of the radioactivity in each sample type. Plasma samples were combined to generate pools

representative of area under the curve (AUC). Pooled plasma samples were prepared by combining a volume proportional to the time between sample collections according to the equation, Volume pooled = $k\Delta t$, where k was determined based on levels of radioactivity in each sample and volume of sample available. Each pool was prepared to provide a single sample per dose per sex.

Analytical Techniques

Radioactivity in all samples (including extracts) was quantified by liquid scintillation counting (direct analysis or following sample oxidation). The nature and identity of metabolites of [^{14}C]-SYN545974 in samples of urine, feces and plasma was investigated by LC-MSⁿ.

Statistics: Not applicable.

RESULTS

Pharmacokinetic Analysis

The concentrations of radioactivity in whole-blood and pharmacokinetic parameters obtained from the composite mean concentration-time profile, following a single intravenous dose of 1 mg/kg and oral doses of 5 and 1000 mg/kg [pyrazole-5- ^{14}C]- and [phenyl-U- ^{14}C]-SYN545974, to male and female rats, are presented in Table 6.1.1-1 and Table 6.1.1-2. A comparison of AUC_{0-t} and C_{max} for all doses and sexes has been presented in Table 6.1.1-3 and Table 6.1.1-4.

Table 6.1.1-1: Blood Concentrations and Pharmacokinetic Parameters Following Single Oral or Intravenous Administration of [pyrazole-5- ^{14}C]-SYN545974 to Rat Expressed as *ng* Equivalents of SYN545974/g

Nominal Time after dosing (h)	IV: 1 mg/kg		Oral : 5 mg/kg		Oral : 1000 mg/kg	
	Male	Female	Male	Female	Male	Female
0.08	352	296	29.8	31.1	1300	1030
0.50	309	179	418	372	3750	3510
1	193	144	278	472	3180	4290
2	85.6	107	246	384	6190	5980
4	71.6	57.7	203	336	7140	6540
6	65.3	74.9	183	262	8220	6280
8	55.6	77.5	207	274	11100	7120
12	46.8	56.2	183	152	13900	10700
24	35.0	28.0	86.7	103	15700	20200
30	28.7	22.6	88.1	74.5	20600	18100
48	19.3	16.6	53.4	59.1	6440	6900
72	NA	NA	28.7	39.4	3330	4300
C_{max} (<i>ng equiv/g</i>)	352	296	418	472	20600	20200
t_{max} (hours)	0.08	0.08	0.5	1	30	24
$t_{1/2}$ (hours)	28	33	26	45	NC	21
$\text{AUC}_{(0-t)}$ (<i>ng equiv.h/g</i>)	2150	2020	6800	7800	724000	724000
$\text{AUC}_{(0-\text{inf})}$ (<i>ng equiv.h/g</i>)	2930	2830	7880	10400	NC	852000
AUC % Extrap	26.7	28.4	13.7	24.9	NC	15.1
F%	NA	NA	63.3	77.2	33.7	35.8
CL (g/h/kg)	342	354	NA	NA	NA	NA
MRT (h)	35	38	NA	NA	NA	NA
V _{ss} (g/kg)	12100	13600	NA	NA	NA	NA

Table 6.1.1-2: Blood Concentrations and Pharmacokinetic Parameters Following Single Oral or Intravenous Administration of [phenyl-U- ^{14}C]-SYN545974 to Rat Expressed as *ng* Equivalents of SYN545974/g

Nominal Time after dosing (h)	IV 1 mg/kg		Oral 5 mg/kg		Oral 1000 mg/kg	
	Male	Female	Male	Female	Male	Female

0.08	298	358	6.84	37.2	742	1570
0.50	249	259	291	557	3090	2370
1	187	222	562	715	3720	3300
2	113	159	458	511	4410	2600
4	96.4	118	255	454	5620	3810
6	76.8	90.6	247	298	4930	2670
8	62.3	89.7	284	351	9330	5940
12	46.7	75.7	184	252	9850	6080
24	28.4	42.5	93.6	133	11200	10100
30	25.3	35.3	96.2	165	8990	7680
48	20.3	25.7	69.8	127	3870	3320
72	NA	NA	50.9	84.3	2380	1710
C _{max} (ng equiv/g)	298	358	562	715	11200	10100
t _{max} (hours)	0.08	0.08	1	1	24	24
t _{1/2} (hours)	51	34	46	43	22	20
AUC _(0-t) (ng equiv.h/g)	2140	2930	8520	12800	449000	353000
AUC _(0-inf) (ng equiv.h/g)	3640	4200	11900	18100	562000	401000
AUC % Extrap	41.1	30.3	28.4	29.0	14.6	12.1
F%	NA	NA	79.6	87.4	21.0	12.0
CL (g/h/kg)	275	238	NA	NA	NA	NA
MRT (h)	59	41	NA	NA	NA	NA
V _{ss} (g/kg)	16200	9650	NA	NA	NA	NA

NC – not calculable due to insufficient points in the terminal phase

NA – not applicable

Table 6.1.1-3: Comparison of AUC_{0-t} and C_{max} for Total Radioactivity in Plasma and Blood Following a Single Oral Administration of [pyrazole-5-¹⁴C]-SYN545974 to Male and Female Rat

Plasma						
Dose (mg/kg)	Fold increase		C _{max} (ng equiv/g)	Fold increase	AUC _(0-t) (ng equiv.h/g)	Fold increase
5		Male	553	-	6070	-
		Female	636	-	6660	-
		M/F Ratio	0.869	-	0.911	-
1000	200	Male	25400	45.9	767000	126
		Female	24200	38.1	826000	124
		M/F Ratio	1.05	1.20	0.929	1.02
Blood						
Dose (mg/kg)	Fold increase		C _{max} (ng equiv/g)	Fold increase	AUC _(0-t) (ng equiv.h/g)	Fold increase
5		Male	418	-	6800	-
		Female	472	-	7800	-
		M/F Ratio	0.886	-	0.872	-
1000	200	Male	20600	49.3	724000	106
		Female	20200	42.8	724000	92.8
		M/F Ratio	1.02	1.15	1	1.14

Table 6.1.1-4: Comparison of AUC_{0-t} and C_{max} for Total Radioactivity in Plasma and Blood Following a Single Oral Administration of [phenyl-U-¹⁴C]-SYN545974 to Male and Female Rat

Plasma						
Dose (mg/kg)	Fold increase		C _{max} (ng equiv/g)	Fold increase	AUC _(0-t) (ng equiv.h/g)	Fold increase
5		Male	950	-	8830	-
		Female	1010	-	10200	-
		M/F Ratio	0.941	-	0.866	-
1000	200	Male	15900	16.7	546000	61.8
		Female	13800	13.7	450000	44.1
		M/F Ratio	1.15	1.22	1.21	1.40
Blood						
Dose (mg/kg)	Fold increase		C _{max} (ng equiv/g)	Fold increase	AUC _(0-t) (ng equiv.h/g)	Fold increase
5		Male	562	-	8520	-
		Female	715	-	12800	-
		M/F Ratio	0.786	-	0.666	-
1000	200	Male	11200	19.9	449000	52.7
		Female	10100	14.1	353000	27.6
		M/F Ratio	1.11	1.41	1.27	1.91

Following intravenous and oral administration of [¹⁴C]-SYN545974, exposure to total radioactivity was broadly comparable for each dose, irrespective of radiolabel position and sex. Following oral administration exposure increased sub-proportionally with respect to dose. Exposure appeared slightly higher in plasma than blood for [pyrazole-5-¹⁴C]-SYN545974 and [phenyl-U-¹⁴C]-SYN545974, but only at the higher 1000 mg/kg dose.

Excretion:

The recovery of radioactivity in excreta and tissues, following administration of a single oral dose of [¹⁴C]-SYN545974 at doses of 5 and 1000 mg/kg are presented in Table 6.1.1-5 and Table 6.1.1-6.

Table 6.1.1-5: Recovery of radioactivity in excreta, expired air and carcass after administration of a single oral dose of [phenyl-U-¹⁴C]-SYN545974 to rats

Time after dosing (h)	IV 1 mg/kg		Oral 5 mg/kg		Oral 1000 mg/kg	
	Male	Female	Male	Female	Male	Female
Urine						
Pre-dose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0-8	12.62	9.43	15.11	7.37	0.87	0.36
8-24	8.41	7.64	5.83	8.55	5.56	4.09
24-48	2.02	2.67	1.85	2.57	5.09	6.67
48-72	0.45	0.69	0.43	0.39	1.28	2.88
72-96	0.17	0.05	0.16	0.09	0.27	0.77
96-120	0.06	0.03	0.08	0.03	0.09	0.22
120-144	0.02	0.04	0.02	0.01	0.02	0.06
144-168	0.02	0.00	0.01	0.01	0.01	0.02
0-168	23.77	20.55	23.49	19.02	13.19	15.07
Feces						
Pre-dose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0-24	45.05	49.08	52.99	56.13	23.27	0.13
24-48	12.23	15.60	14.71	20.87	58.24	39.92
48-72	4.63	6.84	4.49	3.45	8.57	18.02
72-96	1.38	0.96	1.34	0.64	2.12	6.13
96-120	0.73	0.13	0.45	0.08	0.70	3.13
120-144	0.42	0.11	0.23	0.05	0.21	0.31
144-168	0.21	0.03	0.09	0.02	0.06	0.06
0-168	64.65	72.75	74.30	81.24	93.17	67.70
Cage wash						
Pre-dose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8-24	1.39	0.61	2.00	0.49	0.38	0.20
24-48	0.18	0.19	0.30	0.12	0.80	1.65
48-72	0.17	0.09	0.20	0.06	0.16	0.27
72-96	0.20	0.05	0.07	0.02	0.06	0.12
96-120	0.05	0.02	0.02	0.01	0.02	0.06
120-144	0.03	0.01	0.02	<LOD	0.02	0.02
144-168	0.02	<LOD	0.01	<LOD	<LOD	0.01
0-168	2.04	0.97	2.62	0.70	1.44	2.33
Total excreted	90.46	94.27	100.41	100.96	107.80	85.10
Expired Air	0.04	0.04	0.06	0.04	0.04	0.04
Carcass	0.31	0.24	0.32	0.19	0.11	0.14
Total Recovery	90.81	94.55	100.79	101.19	107.95	85.28

Table 6.1.1-6: Recovery of radioactivity in excreta, expired air and carcass after administration of a single oral dose of [pyrazole-5-¹⁴C]-SYN545974 to rats

Time after dosing (h)	IV 1 mg/kg		Oral 5 mg/kg		Oral 1000 mg/kg	
	Male	Female	Male	Female	Male	Female
Urine						
Pre-dose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0-8	8.15	7.19	8.99	11.89	1.11	0.42
8-24	8.14	5.87	8.52	10.27	9.22	7.02
24-48	1.79	2.95	2.34	3.07	10.23	16.84
48-72	0.41	0.73	0.69	0.51	0.98	2.43
72-96	0.11	0.36	0.20	0.17	0.23	0.26
96-120	0.04	0.09	0.06	0.07	0.11	0.14
120-144	0.02	0.03	0.02	0.02	0.04	0.04
144-168	0.01	0.01	0.01	0.01	0.01	0.01
0-168	18.67	17.23	20.83	26.01	21.93	27.16
Feces						
Pre-dose	<LOD	<LOD	0.01	0.01	<LOD	<LOD
0-24	54.73	49.11	53.75	33.05	26.49	2.20
24-48	15.94	21.64	12.99	29.58	30.38	34.28
48-72	2.40	3.99	4.90	4.40	6.67	13.34
72-96	0.77	2.36	1.20	1.13	1.72	3.75
96-120	0.24	0.84	0.47	2.02	1.02	1.09
120-144	0.14	0.22	0.16	0.17	0.28	0.19
144-168	0.07	0.08	0.06	0.02	0.08	0.05
0-168	74.29	78.24	73.54	70.38	66.64	54.90
Cage wash						
Pre-dose	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
8-24	0.83	0.47	1.15	1.21	0.98	1.20
24-48	0.12	1.10	0.70	0.35	0.49	0.86
48-72	0.10	0.18	0.14	0.16	0.22	1.13
72-96	0.03	0.05	0.08	0.05	0.10	0.21
96-120	0.01	0.03	0.05	0.02	0.08	0.10
120-144	0.01	0.01	0.01	0.01	0.02	0.04
144-168	0.01	<LOD	0.01	0.09	0.01	0.04
0-168	1.11	1.84	2.14	1.89	1.90	3.58
Total excreted	94.07	97.31	96.51	98.28	90.47	85.64
Expired Air	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Carcass	0.32	0.22	0.22	0.15	0.13	0.12
Total Recovery	94.39	97.53	96.73	98.43	90.60	85.76

^o=Mean includes results calculated from data less than 30 dpm above background

Mean recoveries of administered radioactivity was >90% for all dose groups with the exception of the 1000 mg/kg female rat dose group. However, the mean recovery for the 1000 mg/kg female rat dose group was >85% and as excretion of radioactivity for this group was very similar to males, it is therefore believed the incomplete recovery may be in the feces. There was no notable radioactivity observed in expired air. The major route of elimination was *via* the feces in both males and females and the majority of the administered radioactivity was excreted in urine and feces within the first 72 hours following dosing. There was no notable radioactivity remaining in the carcass of male or female rats from either dose group, indicating excretion was complete by 168 hours after dosing.

Following oral administration, SYN545974 was well absorbed at 5 mg/kg, with up to 87% of the dose systemically available (based on pharmacokinetic data). This was decreased markedly at 1000 mg/kg with less than 36% absorbed. This was supported by the urine ratio following oral and intravenous administration, suggesting absorption was complete.

Metabolite Identification and Quantification

Composite samples of plasma, urine and feces were prepared for LC-MSⁿ analysis. Extraction efficiency was greater than 64% for plasma and 78% for feces. Accurate mass full scan and product ion spectra were acquired in positive and negative ion ionisation modes for components of interest in plasma, urine and

feces corresponding to notable radiolabelled phase I and phase II metabolites. SYN545974 was metabolised to up to 76 metabolites by rats through phase I and phase II pathways.

SYN545974 was extensively metabolised with the primary routes of metabolism being cleavage of the parent molecule at the benzylic carbon adjacent to the trichlorophenyl moiety and amide hydrolysis. The largest circulating metabolites observed were conjugates of the cleavage product trichlorophenol accounting for up to *ca.* 63% of the total radioactivity AUC. The corresponding half of the molecule was observed as several different metabolites following further oxidation to the carboxylic acid and conjugation. The methyl pyrazole amide was also observed and accounted for up to 24% of the total radioactivity AUC. SYN545974 itself was observed at up to 11% of the total radioactivity AUC. The other circulating metabolites observed were hydroxy SYN545974 and hydroxy N-demethanolated SYN545974 glucuronide.

In excreta, all metabolites >5% were identified and although 22 - 45% of the dose remained unidentified, dependent on the sample, no single component accounted for >5% of the dose. The methyl pyrazole propanoic acid was the single largest metabolite and accounted for 13% of the administered dose at 13%. SYN545974 was the largest single component observed in the 1000 mg/kg dose of up to 34%. Other metabolites observed were products of oxidation, dealkylation and subsequent conjugations of the intact parent molecule, alongside those formed by cleavage of the parent molecule at the carbon adjacent to the trichlorophenyl moiety and amide hydrolysis.

CONCLUSION:

The pharmacokinetics, absorption, metabolism and excretion of total radioactivity were investigated following a single oral administration of two radiolabelled forms of [¹⁴C]-pydiflumetofen (5 or 1000 mg/kg bw) and a single intravenous (iv) dose of 1 mg/kg bw to male and female Han Wistar rats. Pharmacokinetic parameters were calculated from the concentrations of total radioactivity determined in blood and plasma. Excretion samples were obtained over a 7 day period, with expired air collected following oral doses over the first 2 days. After this period, the rats were humanely killed and residual radioactivity was measured in the remaining carcass. Subsequently, the nature and identity of metabolites present in excreta and plasma samples was investigated.

Pharmacokinetic analysis

Following iv administration, systemic exposure to [pyrazole-5-¹⁴C]-pydiflumetofen and [phenyl-U-¹⁴C]-pydiflumetofen was broadly comparable, irrespective of gender. Oral systemic exposure to both labels was broadly comparable between genders. Systemic exposure to both labels increased sub-proportionally with respect to dose for oral administration. Systemic exposure appeared slightly higher in plasma than blood for [pyrazole-5-¹⁴C]-pydiflumetofen and [phenyl-U-¹⁴C]-pydiflumetofen, but only at the higher 1000 mg/kg bw dose.

Absorption

Based on pharmacokinetic data and a comparison of the oral and iv data, after a single oral administration of 5 mg/kg bw pydiflumetofen, up to 80% of the dose in males and 87% of the dose in females was systemically available. At the high dose (1000 mg/kg bw), systemic availability was decreased, with only a maximum 34% available in males and 36% in females. Following iv administration (1 mg/kg bw) and single oral administration, total excreted radioactivity was generally comparable for each dose for both radiolabel positions and sexes.

Metabolism

Pydiflumetofen was extensively metabolised with the primary routes of metabolism being cleavage of the parent molecule at the benzylic carbon adjacent to the trichlorophenyl moiety and amide hydrolysis. The largest circulating metabolites observed were conjugates of the cleavage product trichlorophenol accounting for up to *ca.* 63% of the total radioactivity AUC (Area Under the Curve). The corresponding half of the molecule was observed as several different metabolites following further oxidation to the carboxylic acid and conjugation. The methyl pyrazole amide was also observed and accounted for up to

24% of the total radioactivity AUC. Pydiflumetofen itself was observed at up to 11% of the total radioactivity AUC. The other circulating metabolites observed were hydroxy pydiflumetofen and hydroxy N-demethanolated pydiflumetofen glucuronide.

In excreta, all metabolites >5% were identified and although 22 - 45% of the dose remained unidentified, dependent on the sample, no single component accounted for >5% of the dose. Of the identified components, the most abundant single metabolite was

2-[[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino] propanoic acid, which was present up to 9 and 13% of the administered dose in total excreta at 5 and 1000 mg/kg bw respectively. At 1000 mg/kg bw, unchanged pydiflumetofen was the most abundant component in total excreta (up to 34%). Other metabolites observed were products of oxidation, dealkylation and subsequent conjugations of the intact parent molecule, alongside those formed by cleavage of the parent molecule at the carbon adjacent to the trichlorophenyl moiety and amide hydrolysis.

Excretion

Mean recovery of radioactivity was >90% for all dose groups (iv, 1 mg/kg bw, oral 5 and 1000 mg/kg bw) except for high dose females where mean recovery was > 85% for both radiolabels. Excretion in the 1000 mg/kg bw group was broadly similar between the sexes, with the main difference being lower excretion in the faeces in females than males. Therefore, the missing radioactivity in the high dose female group may have been in the faeces. The major excretion route was via the faeces for both radiolabels in all dose groups. The amount of radioactivity excreted in faeces following iv or the low oral dose was very similar. This indicated that a significant part of the material excreted in faeces following oral administration represented absorbed radioactivity subsequently excreted in bile. No notable radioactivity was available in expired air. Radioactivity was low in the carcass for all groups indicating excretion was essentially complete by 168h post-dose.

Overall, under the conditions of this GLP and OECD TG compliant preliminary ADME study in rats, the major route of elimination of pydiflumetofen after a single oral and iv administration was via the faeces. Excretion was > 20% in faeces indicating investigations in bile duct cannulated rats were needed. Biotransformation involved cleavage of pydiflumetofen to produce phenyl and pyrazole-specific metabolites indicating both radiolabels were required in subsequent studies. Systemic exposure to both radiolabels after oral administration was limited by dose. Absorption and excretion were comparable irrespective of radiolabel or sex.

(██████████ and ██████████, 2015)

Report:	K-CA 5.1.1/02 ██████████ (2015). SYN545974 – The Absorption and Excretion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral Administration in the Rat. ██████████. ██████████ Report No. 34214. Issue date 04 June 2015. Unpublished. Syngenta File No. SYN545974_10248.
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Guidelines: Metabolism – rat; OECD 417 (2010); EPA OPPTS 870.7485 (1998); EC 1107/2009 (2009), EU 283/2013 (2013), JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE: the study is considered acceptable.

EXECUTIVE SUMMARY

The absorption, excretion and tissue distribution of [phenyl-U-¹⁴C] and [pyrazole-5-¹⁴C]-SYN545974 was investigated following oral doses of 5, 100 (females only) and 300 (males only) mg/kg, to groups of 4

male and 4 female rats. Excretion samples were obtained over a 7 day period for non-cannulated animals or 3 days for bile duct cannulated animals. After this period, the rats were humanely killed and residual radioactivity was measured in selected tissues and remaining carcass.

The nature and identity of metabolites present in selected bile, excreta and cage wash samples were also investigated and reported separately, under [REDACTED] Study No. 221595.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 5 mg/kg to intact rats, a mean of 97-99% of the administered dose was eliminated in urine and feces (including cage wash) over seven days. The majority of administered radioactivity (91-96%) was excreted in the first 48 hours. The routes and rates were similar for both radiolabels and for both males and females, with the majority of the dose excreted in the feces (67-76%). Urinary excretion accounted for 18-26% of the dose.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 300 mg/kg to intact male rats, a mean of 101-103% of the administered dose was eliminated in urine and feces (including cage wash) over seven days. The majority of administered radioactivity (98-100%) was excreted in the first 48 hours. The routes and rates were similar for both radiolabels, with 91-92% of the dose excreted in the feces and 6.7-7.8% in the urine.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 100 mg/kg to intact female rats, a mean of 101-102% of the administered dose was eliminated in urine and feces (including cage wash) over seven days. The majority of administered radioactivity (99%) was excreted in the first 48 hours. The routes and rates were similar for both radiolabels, with 84-85% of the dose excreted in the feces and 14-15% in the urine.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 excretion was essentially complete in all animals by 168 h post dose with 0.1% or less remaining in the carcass and gastrointestinal tract.

Seven days after administration of 5, 100 or 300 mg/kg phenyl or pyrazole labelled [¹⁴C]-SYN545974, radioactive residues in the majority of tissues were not detectable. The highest concentrations were in the liver and kidney with the tissue distribution of radioactivity being similar in both sexes for the 5 mg/kg dose. Higher concentrations in these organs are consistent with the biliary and urinary elimination of absorbed radiolabelled compounds.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 5 mg/kg to bile duct cannulated rats, a mean of 97-99% of the administered dose was eliminated in urine, bile and feces (including cage wash) over three days. The routes and rates were similar for both radiolabels and for both males and females, with the exception that urinary excretion in males was approximately 2 fold that of females. The majority of the dose was excreted in the bile (67-81%). Elimination *via* the feces accounted for 10-15% of the dose and urinary excretion accounted for 6.3-13% of the dose.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 300 mg/kg to bile duct cannulated male rats, a mean of 98-100% of the administered dose was eliminated in urine, bile and feces (including cage wash) over three days. The routes and rates were similar for both radiolabels, with 76-79% of the dose excreted in the feces, 15-18% in the bile and 2.5-4.4% in the urine.

Following a single oral administration of phenyl or pyrazole labelled [¹⁴C]-SYN545974 at 100 mg/kg to bile duct cannulated female rats, a mean of 97-99% of the administered dose was eliminated in urine, bile and feces (including cage washes, which derived from urine and feces) over three days. The routes and rates were similar for both radiolabels, with 43-49% of the dose excreted in the feces, 33-41% in the bile and 7-15% in the urine.

Excretion was essentially complete in all bile duct cannulated animals by 72 h post dose with 0.3% or less remaining in carcass gastrointestinal tract.

Irrespective of radiolabel or sex, the routes and rates of elimination were broadly similar. As the dose increased from 5 to 100 (females) or 300 (males) mg/kg, absorption of total radioactivity became dose limited, resulting in higher faecal excretion. Consequently, oral absorption in males at the 300 mg/kg dose (19-24%) was around 4-5 fold less than the 5 mg/kg dose (85-87%), and in females at the 100 mg/kg dose (50-55%) around 2 fold less than the 5 mg/kg dose (87-90%).

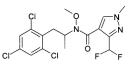
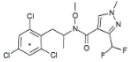
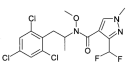
Irrespective of radiolabel, dose or sex, following a single oral administration of [¹⁴C]-SYN545974, the majority of dose related radioactivity was eliminated by 48 hours post dose and excretion was essentially complete by 168 h. Absorption was limited by dose from approximately 85-90% of the 5 mg/kg oral dose to 19-24% at 300 mg/kg in males and 50-55% at 100 mg/kg in females. The majority of the absorbed dose was excreted in feces via bile elimination.

Seven days after administration of [¹⁴C]-SYN545974, radioactive residues in the majority of tissues were not detectable. The highest mean concentrations were in the liver and kidney consistent with the biliary and urinary elimination of absorbed [¹⁴C]-SYN545974.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:

Name :	SYN545974
Structure :	
Source :	Syngenta Crop Protection Münchwilen AG
Physical state :	Off white, powder
Batch reference :	SMU2EP12007
Purity:	98.5%
Radiolabelled Test Material:	[Phenyl-U- ¹⁴ C]-SYN545974
Radiochemical purity:	99.1%
Source:	Syngenta Crop Protection LLC
Lot/Batch number:	RDR-XV-94
Structure:	 * position of [¹⁴ C]-label
Radiolabelled Test Material:	[Pyrazole-5- ¹⁴ C]-SYN545974
Radiochemical purity:	99.2%, 98.8%
Source:	Selcia
Lot/Batch number:	5271GAR001-4, 5283RJP001-2
Structure:	 * * position of [¹⁴ C]-label

Vehicle: 0.5% (w/v) aqueous carboxymethylcellulose (CMC) containing 0.5% Tween 80.

Preparation of dosing solutions: Each dose preparation was formulated as a homogenous suspension in 0.5% aqueous CMC containing 0.5% Tween 80. Each dose preparation was prepared using [¹⁴C]-SYN545974 and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity.

Test Animals:	
Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	Groups 1-4: 8 – 9 weeks Groups 5 & 7: 11 - 13 weeks Groups 6 & 8: 10-12 weeks 235 - 256 g for males; 174 - 188 g for females (Group 1) 207 - 255 g for males; 177 - 195 g for females (Group 2) 236 - 250 g for males; 172 - 182 g for females (Group 3) 220 - 236 g for males; 165 - 195 g for females (Group 4) 307 - 344 g for males; 183 - 240 g for females (Group 5) 282 - 313 g for males; 183 - 211 g for females (Group 6) 309 - 321 g for males; 205 - 228 g for females (Group 7) 314 - 387 g for males; 191 - 209 g for females (Group 8)
Source:	
Housing:	Pre-study: multiply housed by sex in solid bottomed polycarbonate cages with bedding On study: Singly in all-glass metabolism cages
Acclimatisation period:	At least 5 days
Diet:	Rat and Mouse No.1 maintenance diet, <i>Ad libitum</i>
Water:	Tap water <i>ad libitum</i>
Environmental conditions:	Temperature: 18-21°C Humidity: 38-80% Air changes: 10-11 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental Dates: Start: 05 December 2012 End: 24 June 2013

Group Arrangements: Animals were assigned to 8 groups as shown below.

Dosing groups for pharmacokinetic studies for [¹⁴C]-SYN545974

Test Group	Dose (mg/kg)	Radiolabel	Number/sex	Remarks
Group 1 Excretion	5	Phenyl	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.
Group 2 Excretion	5	Pyrazole	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.
Group 3 Excretion	300 (males) or 100 (females)	Phenyl	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.
Group 4 Excretion	300 (males) or 100 (females)	Pyrazole	4 males, 4 females	Excreta collection over 168 hours and selected tissues taken for analysis.
Group 5 Bile duct cannulation	5	Phenyl	4 males, 4 females	Excreta and bile collection over 72 hours.
Group 6 Bile duct cannulation	5	Pyrazole	4 males, 4 females	Excreta and bile collection over 72 hours.
Group 7 Bile duct cannulation	300 (males) or 100 (females)	Phenyl	4 males, 4 females	Excreta and bile collection over 72 hours.
Group 8 Bile duct cannulation	300 (males) or 100 (females)	Pyrazole	4 males, 4 females	Excreta and bile collection over 72 hours.

Dosing and sample collection:

A single oral dose of [¹⁴C]-SYN545974 was administered to each rat by gavage at a target dose rate of 5 mL/kg (groups 1, 2 and 4-8) or 5.5 mL/kg (group 3). Animals in groups 1, 2, 5 and 6 received a dose corresponding to a low dose of 5 mg/kg, and animals in groups 3, 4, 7 and 8 received a dose corresponding to a high dose of 300 mg/kg (males) or 100 mg/kg (females). For each dose, the animals received a target radioactive dose of 5 MBq/kg.

For groups 1-4, urine and feces were collected individually and separately. Urine and feces were frozen immediately upon collection. At the end of each feces collection period, cage wash samples were collected (water).

Animals were humanely killed by CO₂ narcosis at 168 hours post dose. A terminal blood sample was taken and divided between two heparinised tubes, one of which was centrifuged to separate plasma. The following tissues were taken for radioactivity analysis: adrenals, brain, heart, kidneys, liver, lungs, ovaries (females), pancreas, spleen, testes (males), thymus, thyroid, uterus (females), gastrointestinal tract plus contents and residual carcasses together with representative samples of bone mineral (tibia, fibula), fat (renal) and muscle.

For groups 5-8, urine, feces and bile were collected individually and separately. Urine, feces and bile were frozen immediately upon collection. At the end of each excreta collection period, cage wash samples were collected (water).

Animals were humanely killed by CO₂ narcosis. Each terminal blood sample was divided between two heparinised tubes, one of which was centrifuged to separate plasma. The gastrointestinal tract (and contents) and carcass were also retained separately.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Excretion studies:

For groups 1-4, urine was collected at predose and at intervals of 8, 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Feces and cage wash were collected at predose (feces only) and at intervals of 24, 48, 72, 96, 120, 144 and 168 hours after dosing.

To investigate pharmacokinetics, terminal blood samples were collected at 168 hours after dosing.

For groups 3 and 4, bile was collected at intervals of 0-1, 1-2, 2-4, 4-8, 8-12, 12-24, 24-48 and 48-72 hours after dosing. Urine, feces and cage wash were collected at daily intervals until the termination of the study.

To investigate pharmacokinetics, terminal blood samples were collected at 72 hours after dosing.

Metabolite characterisation studies: Metabolite characterisation on selected excreta samples was undertaken in a separate study (██████████ 2015) SYN545974 – Biotransformation in the Rat. ██████████ Report No. 34216. (Syngenta File no. SYN545974_10259).

Statistics: Not applicable.

RESULTS**Excretion (Intact):**

The recovery of radioactivity in excreta and tissues, following administration of a single oral dose of [¹⁴C]-SYN545974 at doses of 5, 100 (females) and 300 mg/kg (males) are presented in Table 6.1.1-7.

Table 6.1.1-7: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [Phenyl-U-¹⁴C]-SYN545974 to rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 1		Group 3	
		5mg/kg		300 mg/kg	100 mg/kg
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-8 h	15	11	2.4	3.4
	8-24 h	4.7	5.1	3.3	9.9
	24-48 h	1.1	1.4	0.8	1.3
	48-72 h	0.3	0.4	0.2	0.3
	72-96 h	0.1	0.2	<0.1	0.1
	96-120 h	<0.1	<0.1	<0.1	<0.1
	120-144 h	<0.1	<0.1	<0.1	<0.1
	144-168 h	<0.1	<0.1	<0.1	<0.1
	<i>Subtotal</i>	22	19	6.7	15
Feces	0-24 h	62	59	83	70
	24-48 h	9.9	14	7.5	12
	48-72 h	1.4	2.4	1.3	1.5
	72-96 h	0.3	0.7	0.3	0.3
	96-120 h	0.1	0.2	0.1	0.1
	120-144 h	0.1	0.1	<0.1	<0.1
	144-168 h	<0.1	<0.1	<0.1	<0.1
	<i>Subtotal</i>	73	76	92	84
Cage wash		3.9	3.7	4.0	2.2
Tissues		0.1	<0.1	<0.1	<0.1
GI tract		<0.1	<0.1	<0.1	°<0.1
GI tract contents		<0.1	<0.1	°<0.1	°<0.1
Carcass		°0.1	°<0.1	°0.1	°0.1
Total Recovery		99	99	103	102

°=Mean includes results calculated from data less than 30 dpm above background

Single low dose: As summarized in the table above, following a single oral dose of 5 mg [Phenyl-U-¹⁴C]-SYN545974/kg, the major route of elimination was via the feces in both males and females, with means of 73 and 76% of the administered radioactivity recovered by seven days post dose, respectively. Urinary excretion accounted for means of 22 and 19% of the administered dose in both males and females, respectively, by the end of the sampling period.

Excretion was fairly rapid with the majority of the administered radioactivity excreted by 48 h post dose (96 and 94% for male and female rats, respectively). Excretion was essentially complete by 168 h post dose with 0.1% remaining in carcass and gastrointestinal tract for both male and female rats

Single high dose: As summarized in the table above, following a single oral dose of 300 mg (males) or 100 mg (females) [¹⁴C]-SYN545974/kg, the major route of elimination was *via* the feces, with 92 and 84% of the administered radioactivity recovered in males and females, respectively, by seven days post dose. Urinary elimination accounted for 6.7 and 15% of the dose in male and female rats, respectively, by the end of the sampling period.

Excretion was fairly rapid with the majority of the administered radioactivity excreted by 48 h post dose (100 and 99% for male and female rats). Excretion was essentially complete by 168 h post dose with 0.1% remaining in carcass and gastrointestinal tract for both sexes.

Table 6.1.1-8: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [Pyrazole-5-¹⁴C]-SYN545974 to rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 2		Group 4	
		5 mg/kg		300 mg/kg	100 mg/kg
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-8 h	16	8.9	2.7	3.9
	8-24 h	6.3	7.6	4.1	8.5
	24-48 h	3.1	1.5	0.8	1.2
	48-72 h	0.8	0.3	0.1	0.2
	72-96 h	0.2	0.1	<0.1	0.1
	96-120 h	0.1	<0.1	<0.1	<0.1
	120-144 h	<0.1	<0.1	<0.1	<0.1
	144-168 h	<0.1	<0.1	<0.1	°<0.1
	<i>Subtotal</i>	26	18	7.8	14
Feces	0-24 h	43	46	83	74
	24-48 h	20	20	6.9	9.8
	48-72 h	3.4	2.9	0.8	1.3
	72-96 h	0.7	0.6	0.2	0.3
	96-120 h	0.2	0.2	0.1	0.1
	120-144 h	0.1	0.1	<0.1	<0.1
	144-168 h	<0.1	<0.1	<0.1	°<0.1
	<i>Subtotal</i>	67	70	91	85
Cage wash		2.8	8.3	2.8	1.6
Tissues		0.1	0.1	<0.1	<0.1
GI tract		<0.1	<0.1	°<0.1	°<0.1
GI tract contents		<0.1	<0.1	<0.1	°<0.1
Carcass		°0.1	°<0.1	°<0.1	°<0.1
Total Recovery		97	97	101	101

°=Mean includes results calculated from data less than 30 dpm above background

Single low dose: As summarized in the table above, following a single oral dose of 5 mg [Pyrazole-5-¹⁴C]-SYN545974/kg, the major route of elimination was via the feces in both males and females, with means of 67 and 70% of the administered radioactivity recovered by seven days post dose, respectively. Urinary excretion accounted for means of 26 and 18% of the administered dose in both males and females, respectively, by the end of the sampling period.

Excretion was fairly rapid with the majority of the administered radioactivity excreted by 48 h post dose (91 and 92% for male and female rats, respectively). Excretion was essentially complete by 168 h post dose with 0.1% or less remaining in carcass and gastrointestinal tract for both male and female rats

Single high dose: As summarized in the table above, following a single oral dose of 300 mg (males) or 100 mg (females) [Pyrazole-5-¹⁴C]-SYN545974/kg, the major route of elimination was *via* the feces, with 91 and 85% of the administered radioactivity recovered in males and females, respectively, by seven days post dose. Urinary elimination accounted for 7.8 and 14% of the dose in male and female rats, respectively, by the end of the sampling period.

Excretion was fairly rapid with the majority of the administered radioactivity excreted by 48 h post dose (100 and 99% for male and female rats). Excretion was essentially complete by 168 h post dose with <0.1% remaining in carcass and gastrointestinal tract for both sexes.

Tissue distribution (Intact):

The concentrations of radioactivity in tissues and organs at 168 hours after administration of a single oral dose of [^{14}C]-SYN545974 at doses of 5, 300 (males only) or 100 (females only) mg/kg are presented in Tables 6.1.1-9 and 6.1.1-10.

Table 6.1.1-9: Distribution of radioactivity in tissues/organs 168 hours after administration of a single oral dose of [Phenyl- ^{14}C]-SYN545974 to rats

Tissue/organ	Group mean tissue residues (μg equivalents of SYN545974/g)			
	Group 1		Group 3	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Adrenals	≤ 0.003	≤ 0.002	≤ 0.2	≤ 0.1
Bone Mineral	≤ 0.001	≤ 0.001	≤ 0.1	≤ 0.1
Brain	≤ 0.001	≤ 0.001	≤ 0.1	≤ 0.1
Fat- renal	≤ 0.002	≤ 0.003	≤ 0.1	≤ 0.1
G.I. Tract	0.005	0.007	0.3	≤ 0.1
G.I. Tract contents	0.007	0.010	≤ 0.4	≤ 0.1
Heart	≤ 0.004	≤ 0.006	≤ 0.2	≤ 0.1
Kidneys	0.035	0.024	0.9	0.2
Liver	0.090	0.048	2.7	0.5
Lungs	0.010	0.018	≤ 0.3	0.1
Muscle	≤ 0.001	≤ 0.001	≤ 0.1	≤ 0.1
Ovaries	NA	≤ 0.003	NA	≤ 0.1
Pancreas	≤ 0.002	≤ 0.002	≤ 0.1	≤ 0.1
Plasma	≤ 0.002	≤ 0.002	≤ 0.1	≤ 0.1
Residual Carcass	≤ 0.003	≤ 0.003	≤ 0.2	≤ 0.1
Spleen	≤ 0.010	0.011	≤ 0.3	≤ 0.1
Testes	≤ 0.001	NA	≤ 0.1	NA
Thymus	≤ 0.001	≤ 0.002	≤ 0.1	≤ 0.1
Thyroid	≤ 0.006	≤ 0.001	≤ 0.5	≤ 0.1
Uterus	NA	≤ 0.001	NA	≤ 0.1
Whole Blood	0.026	0.038	1.0	0.3

\leq = Mean includes results calculated from data less than 30 dpm above background

NA = Not applicable

Single low dose: Seven days following administration of [Phenyl- ^{14}C]-SYN545974 at 5 mg/kg to male and female rats, circulating radioactivity was detected in the blood with levels of 0.026 and 0.038 μg equiv/g in males and females, respectively. Concentrations were not reliably detected in the plasma. The highest tissue concentrations were present in the liver with means of 0.090 and 0.048 μg equiv/g in males and females, respectively. Radioactivity in the male kidneys was also above the blood level with a concentration of 0.035 μg equiv/g. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were not reliably detected.

Single high dose: Seven days following administration of [Phenyl- ^{14}C]-SYN545974 at 300 mg/kg (males) or 100 mg/kg (females), circulating radioactivity was detected in the blood with levels of 1.0 and 0.3 μg equiv/g in males and females, respectively. Concentrations were not reliably detected in the plasma. The highest tissue concentrations were observed in the liver with means of 2.7 and 0.5 μg equiv/g in males and females, respectively. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were not reliably detected.

Table 6.1.1-10: Distribution of radioactivity in tissues/organs 168 hours after administration of a single oral dose of [Pyrazole-5-¹⁴C]-SYN545974 to rats

Tissue/organ	Group mean tissue residues (µg equivalents of SYN545974/g)			
	Group 2		Group 4	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Adrenals	°0.003	°0.003	°0.1	°0.1
Bone Mineral	°0.002	°0.001	°<0.1	°<0.1
Brain	°0.001	°0.001	°<0.1	°<0.1
Fat- renal	°0.001	°0.002	°<0.1	°<0.1
G.I. Tract	0.007	0.007	°0.1	°<0.1
G.I. Tract contents	0.010	0.012	0.2	°<0.1
Heart	°0.005	°0.006	°0.1	°<0.1
Kidneys	0.041	0.020	0.6	0.1
Liver	0.134	0.060	2.4	0.4
Lungs	0.013	0.017	0.2	0.1
Muscle	°0.001	°0.001	°<0.1	°<0.1
Ovaries	NA	°0.003	NA	°<0.1
Pancreas	°0.001	°0.002	°0.1	°<0.1
Plasma	°0.003	°0.001	°0.1	°<0.1
Residual Carcass	°0.007	°0.001	°<0.1	°<0.1
Spleen	0.008	0.016	°0.2	°0.1
Testes	°0.001	NA	°<0.1	NA
Thymus	°0.002	°0.001	°<0.1	°<0.1
Thyroid	°0.002	°0.003	°0.2	°0.1
Uterus	NA	°0.001	NA	°<0.1
Whole Blood	0.018	0.026	0.4	0.2

°=Mean includes results calculated from data less than 30 dpm above background

NA = Not applicable

Single low dose: Seven days following administration of [Pyrazole-5-¹⁴C]-SYN545974 at 5 mg/kg to male and female rats, circulating radioactivity was detected in the blood with levels of 0.018 and 0.026 µg equiv/g in males and females, respectively. Concentrations were not reliably detected in the plasma. The highest tissue concentrations were present in the liver with means of 0.134 and 0.060 µg equiv/g in males and females, respectively. Radioactivity in the male kidneys was also above the blood level with a concentration of 0.041 µg equiv/g. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were not reliably detected.

Single high dose: Seven days following administration of [Pyrazole-5-¹⁴C]-SYN545974 at 300 mg/kg (males) or 100 mg/kg (females), circulating radioactivity was detected in the blood with levels of 0.4 and 0.2 µg equiv/g in males and females, respectively. Concentrations were not reliably detected in the plasma. The highest tissue concentrations were observed in the liver with means of 2.4 and 0.4 µg equiv/g in males and females, respectively. Radioactivity in the male kidneys was also above the blood level with a concentration of 0.6 µg equiv/g. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were not reliably detected.

Absorption (BDC): The percentage absorption of radioactivity over 72 hours after dosing was estimated from the radioactivity recovered from bile duct cannulated rats in urine, bile, cage wash and carcass.

As summarised in the table below, at each dose level, the absorption was similar in males and females, irrespective of radiolabel position. However, the absorption was dose dependent. After a 5 mg/kg oral dose, the % of absorbed dose was 85-90%. At the higher oral doses of 300 mg/kg to male rats and 100 mg/kg to female rats, absorption was markedly lower. In male rats, the oral absorption at the 300 mg/kg dose (19-24%) was around 4-5 fold less than after the 5 mg/kg dose. In female rats, the oral absorption at the 100 mg/kg dose (50-55%) was around 2 fold less than the 5 mg/kg dose. This shows that absorption increases less than proportionally to dose.

Table 6.1.1-11: Absorption of radioactivity after administration of [Phenyl-U-¹⁴C]-SYN545974

	Absorption after oral administration (Percent of radioactive dose)			
	Group 5		Group 7	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	13	6.3	4.4	15
Bile	67	81	18	°33
Cage wash	3.0	°1.2	1.6	4.6
Carcass	0.3	0.2	°0.1	0.1
% Absorbed	85	90	24	55

°=Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-12: Absorption of radioactivity after administration of [Pyrazole-5-¹⁴C]-SYN545974

	Absorption after oral administration (Percent of radioactive dose)			
	Group 6		Group 8	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	12	6.9	2.5	7.0
Bile	72	78	15	41
Cage wash	1.7	1.1	0.8	0.7
Carcass	0.2	°0.1	0.1	°0.1
% Absorbed	87	87	19	50

°=Mean includes results calculated from data less than 30 dpm above background

Excretion (BDC):

The recovery of radioactivity in excreta and bile, following administration of a single oral dose of [¹⁴C]-SYN545974 at doses of 5, 300 (males only) or 100 (females only) mg/kg are presented in Tables 6.1.1-13 and 6.1.1-14.

Table 6.1.1-13: Recovery of radioactivity in excreta and bile after administration of a single oral dose of [Phenyl-U-¹⁴C]-SYN545974 to bile duct cannulated rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 5		Group 7	
		5 mg/kg		300 mg/kg	100 mg/kg
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-8 h	11	3.6	1.1	5.4
	8-24 h	1.1	2.4	2.1	8.9
	24-48 h	0.2	0.3	1.0	0.9
	48-72 h	<0.1	0.1	0.1	0.2
	<i>Subtotal</i>	<i>13</i>	<i>6.3</i>	<i>4.4</i>	<i>15</i>
Feces	0-24 h	14	10	60	39
	24-48 h	0.5	0.2	15	4.2
	48-72 h	°<0.1	<0.1	1.0	0.1
	<i>Subtotal</i>	<i>15</i>	<i>10</i>	<i>76</i>	<i>43</i>
Bile	0-1 h	15	6.4	0.7	°0.5
	1-2 h	19	14	1.2	1.5
	2-4 h	18	23	2.6	5.0
	4-8 h	8.9	22	3.7	5.0
	8-12 h	3.0	8.9	2.4	11
	12-24 h	1.3	5.2	6.7	12
	24-48 h	0.4	0.9	1.9	0.8
	48-72 h	0.1	0.1	0.1	°<0.1
	<i>Subtotal</i>	<i>67</i>	<i>81</i>	<i>18</i>	<i>33</i>
Cage wash		3.0	1.2	1.6	4.6
GI tract		<0.1	°<0.1	°<0.1	°<0.1
GI tract contents		°<0.1	°<0.1	°<0.1	°<0.1
Carcass		0.3	0.2	°0.1	0.1
Total Recovery		98	99	100	97

°=Mean includes results calculated from data less than 30 dpm above background

Single low dose: As summarized in Table 6.1.1-13, following a single oral dose of 5 mg [Phenyl-U-¹⁴C]-SYN545974/kg, the major route of elimination was *via* the bile, with 67 and 81% of the administered radioactivity recovered in males and females, respectively. Faecal elimination accounted for 15 and 10% of the dose, whilst urinary elimination accounted for 13 and 6.3% of the dose, in male and female rats, respectively.

Excretion was fairly rapid and essentially complete by 3 days post dose with 0.3% or less remaining in the carcass and gastrointestinal tract.

Single high dose: As summarized in Table 6.1.1-14, following a single oral dose of 300 mg (males) or 100 mg (females) [Phenyl-U-¹⁴C]-SYN545974/kg, the major route of elimination was *via* the feces, with 76 and 43% of the administered radioactivity recovered in males and females, respectively. Biliary elimination accounted for 18 and 33% of the dose, whilst urinary elimination accounted for 4.4 and 15% of the dose, in male and female rats, respectively.

Excretion was fairly rapid and essentially complete by 3 days post dose with 0.2% or less remaining in the carcass and gastrointestinal tract.

Table 6.1.1-14: Recovery of radioactivity in excreta and bile after administration of a single oral dose of [Pyrazole-5-¹⁴C]-SYN545974 to bile duct cannulated rats

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 6		Group 8	
		5 mg/kg		300 mg/kg	100 mg/kg
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=3)
Urine	0-8 h	11	5.5	1.2	2.1
	8-24 h	1.5	1.3	1.0	4.7
	24-48 h	0.2	0.1	0.2	0.3
	48-72 h	0.1	<0.1	<0.1	<0.1
	<i>Subtotal</i>	<i>12</i>	<i>6.9</i>	<i>2.5</i>	<i>7.0</i>
Feces	0-24 h	13	13	76	46
	24-48 h	0.2	0.5	3.5	2.7
	48-72 h	<0.1	°<0.1	0.1	°<0.1
	<i>Subtotal</i>	<i>13</i>	<i>13</i>	<i>79</i>	<i>49</i>
Bile	0-1 h	11	8.5	0.7	0.8
	1-2 h	19	18	1.0	1.8
	2-4 h	21	24	2.1	3.9
	4-8 h	15	19	3.9	9.6
	8-12 h	3.9	6.0	2.2	9.8
	12-24 h	1.8	3.0	3.7	14
	24-48 h	0.2	0.4	1.5	0.8
	48-72 h	°0.1	°<0.1	<0.1	°<0.1
	<i>Subtotal</i>	<i>72</i>	<i>78</i>	<i>15</i>	<i>41</i>
Cage wash		1.7	1.1	0.8	0.7
GI tract		°<0.1	<0.1	°<0.1	°<0.1
GI tract contents		<0.1	<0.1	°<0.1	°<0.1
Carcass		0.2	°0.1	0.1	°0.1
Total Recovery		99	99	98	98

°=Mean includes results calculated from data less than 30 dpm above background

Single low dose: As summarized in Table 6.1.1-13, following a single oral dose of 5 mg [Pyrazole-5-¹⁴C]-SYN545974/kg, the major route of elimination was *via* the bile, with 72 and 78% of the administered radioactivity recovered in males and females, respectively. Faecal elimination accounted for 13% of the dose for both sexes, whilst urinary elimination accounted for 12 and 6.9% of the dose, in male and female rats, respectively.

Excretion was fairly rapid and essentially complete by 3 days post dose with 0.2% or less remaining in the carcass and gastrointestinal tract.

Single high dose: As summarized in Table 6.1.1-14, following a single oral dose of 300 mg (males) or 100 mg (females) [Pyrazole-5-¹⁴C]-SYN545974/kg, the major route of elimination was *via* the feces, with 79 and 49% of the administered radioactivity recovered in males and females, respectively. Biliary elimination accounted for 15 and 41% of the dose, whilst urinary elimination accounted for 2.5 and 7.0% of the dose, in male and female rats, respectively.

Excretion was fairly rapid and essentially complete by 3 days post dose with 0.2% or less remaining in the carcass and gastrointestinal tract.

CONCLUSION:

The absorption, excretion and tissue distribution of [phenyl-U-¹⁴C] and [pyrazole-5-¹⁴C]-pydiflumetofen was investigated following oral doses of 5, 100 (females only) and 300 (males only) mg/kg bw, to groups

of 4 male and 4 female rats. Excretion samples were obtained over a 7 day period for non-cannulated animals or 3 days for bile duct cannulated animals. After this period, the rats were humanely killed and residual radioactivity was measured in selected tissues and remaining carcass. The nature and identity of metabolites present in selected bile, excreta and cage wash samples were also investigated and reported separately, under [REDACTED] and [REDACTED], 2015.

Absorption

In bile duct cannulated animals, absorption was similar in both sexes irrespective of radiolabel. After single oral administration of 5 mg/kg bw, percentage absorption (sum of material excreted in urine, bile, cage wash and remaining carcass) was **85-90%** in both sexes. In the high dose groups (100 mg/kg bw in females, 300 mg/kg bw in males), oral absorption was decreased to 50-55% in females and 19-24% in males. Irrespective of radiolabel, dose or sex, following a single oral administration of [¹⁴C]-pydiflumetofen, the majority of the radioactivity was eliminated by 48 hours post dose and excretion was essentially complete by 168 h. Absorption was limited by dose. The majority of the absorbed dose was excreted in feces via bile elimination.

Excretion

Mean recovery of radioactivity was > 90% in all non-cannulated dose groups (5, 100 [female] and 300 [male] mg/kg bw). Excretion was generally comparable irrespective of radiolabel and sex. After oral administration of 5 mg/kg bw pydiflumetofen to non-cannulated rats, the main excretion route was via the faeces for both radiolabels in all groups (up to 73 and 76% in males and females respectively). Urinary excretion was relatively low with up to 26% of the administered dose (AD) in males and up to 19% of the AD in females. In the high dose groups, faeces excretion increased up to 92% in males and 85% in females whereas urine excretion decreased for both sexes (up to 8% in males and up to 15% in females). Radioactivity was low in the carcass for all groups indicating excretion was essentially complete by 168 h post-dose.

Mean recovery of pydiflumetofen in bile duct cannulated dose groups was also > 90% at all doses. At 5 mg/kg bw the major route of excretion was via the bile (up to 72 and 81% in males and females respectively), with excretion in faeces representing only up to 15% of the AD in males and up to 13% of the AD dose in females. Urinary excretion was very low with up to 13% of the AD in males and up to 7% of the AD in females. At the high doses, the major route of excretion was via the faeces which accounted for up to 79 and 49% in males (300 mg/kg bw) and females (100 mg/kg bw) respectively, with excretion in bile representing up to 18% of the AD in males and up to 41% of the AD in females. Urinary excretion was extremely low with up to 4.4% of the AD in males and up to 15% of the AD in females. Radioactivity was low in the carcass for all groups indicating excretion was essentially complete by 72 h post-dose.

Tissue Distribution

In non-cannulated animals, the highest tissue radioactivity concentrations were detected in the liver and kidneys for both radiolabels. Plasma concentrations of radioactivity were not reliably detected in any dose group for either radiolabel. After oral administration of 5 (both sexes), 100 (females) and 300 (males) mg/kg bw, concentrations of both radiolabels were higher in the liver than the blood in both sexes. Radioactivity was also higher in the kidneys in males at both dose levels.

Overall, under the conditions of this GLP and OECD TG compliant ADE study in rats with and without bile cannulation, oral absorption was 85-90% at 5 mg/kg bw, but was approximately 2-fold lower at 100 mg/kg bw and 4-fold lower at 300 mg/kg bw indicating it is limited by dose. Once absorbed, radioactivity was highest in the liver and kidneys. The major route of elimination of pydiflumetofen was in the faeces via the bile, which is consistent with the tissue distribution observed. As dose increased, the major route of elimination shifted to the faeces (not via bile) which corresponds with the dose limiting factor on oral absorption.

([REDACTED], 2015)

Report:	K-CA 5.1.1/03 [REDACTED] (2015a). SYN545974- Tissue Depletion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral Administration in the Rat. [REDACTED] [REDACTED]. [REDACTED] Report No. 34340. Issue date 04 June 2015. Unpublished. Syngenta File No.SYN545974_10252.
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Guidelines: Metabolism – rat; OECD 417 (2010); EPA OPPTS 870.7485 (1998); EC 1107/2009 (2009), EU 283/2013 (2013), JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

HSE: the study is considered acceptable.

EXECUTIVE SUMMARY

Four groups of 40 Han Wistar rats (20 per sex) were given a single oral dose of 5, 100 (females only) and 300 (males only) mg/kg [phenyl-U-¹⁴C] or [pyrazole-5-¹⁴C]-SYN545974 to investigate the tissue distribution and depletion of dose-related radioactivity. Following dosing, groups of 4 rats per sex per time point were humanely killed at intervals and the residual radioactivity measured in selected tissues/organs and the remaining carcasses. Where appropriate, terminal phase half-lives of depletion were calculated for individual tissues.

Following single oral administration of [phenyl-U-¹⁴C]-SYN545974 at 5 mg/kg, radioactivity was widely distributed to the tissues in both sexes. Peak mean tissue concentrations were observed at the first sampling time of 2 hours post dose in males and 1 hour post dose in females. The highest mean tissue concentrations in males and females were obtained in the liver (8.6 and 10.9 µg equiv/g), kidneys (2.5 and 3.5 µg equiv/g) and adrenals (0.9 and 5.3 µg equiv/g). In males, all other tissue concentrations were similar or lower than the blood and plasma concentrations (0.7 and 1.5 µg equiv/g or mL, respectively). In females all other tissue concentrations were generally slightly above the blood and plasma concentrations (0.8 and 1.3 µg equiv/g or mL, respectively) at this time point (range 0.9-2.5 µg equiv/g), with the exception of the bone mineral. The liver and kidney concentrations are consistent with both biliary and urinary elimination. Thereafter, all tissue concentrations declined steadily up to 120 hours post dose; with the exceptions of liver and kidney (male only), all tissues were below that of circulating blood or were below the Limit of Detection (LOD). Total radioactivity was still well detected in approximately half of tissues of both sexes at this final time point. The total mean residues in tissues and carcass at 120 hours accounted for 1.6% of the dose in males and 0.5% in females.

Following single oral administration of [pyrazole-5-¹⁴C]-SYN545974 at 5 mg/kg, radioactivity was again widely distributed to the tissues in both sexes. Peak mean tissue concentrations were observed at the first sampling time of 0.5 hours post dose in both sexes. Similar to the phenyl 5 mg/kg dose, the highest mean tissue concentrations were obtained in the liver, kidneys and adrenals at 9.5, 2.3 and 0.9 µg equiv/g, respectively for males and 12.6, 2.8 and 3.1 µg equiv/g respectively for females. In males, the majority of tissues were similar to or lower than the maximum blood and plasma concentrations of total radioactivity (0.4 and 0.7 µg equiv/g or mL, respectively) at this time. In females, the majority of tissues were similar or above the maximum blood and plasma concentrations of total radioactivity (0.5 and 0.9 µg equiv/g or mL, respectively). Thereafter, all tissue concentrations declined steadily up to 96 hours post dose; with the exceptions of liver and kidney (male only), all tissues were below that of circulating blood or were not reliably detected. Total radioactivity was still well detected in approximately a third of tissues of both sexes at this final time point. The total mean residues in tissues and carcass at 96 hours accounted for 3.0% of the dose in males and 2.0% in females.

Following single oral administration of [phenyl-U-¹⁴C]-SYN545974 at 300 mg/kg to males, radioactivity was widely distributed to the tissues. Peak mean tissue concentrations were attained at the first sampling time of 8 hours post dose. The highest mean tissue concentrations were observed in the liver (77.5 µg equiv/g) and kidneys (28.5 µg equiv/g), all other concentrations were similar or below the levels of circulating radioactivity observed in blood/plasma (7.1 and 13.0 µg equiv/g or mL). Thereafter, all tissue

concentrations declined steadily up to 96 hours post dose, where with the exceptions of liver (6.3 µg equiv/g) and kidney (1.7 µg equiv/g), all tissue concentrations were below that of circulating blood/plasma (0.8 and 0.4 µg equiv/g) or were below the LOD. Total radioactivity was still well detected in approximately half of tissues at this final time point. The total mean residues in tissues and carcass at 96 hours accounted for 1.2% of the dose.

Following single oral administration of [phenyl- ^{14}C]-SYN545974 at 100 mg/kg to females, radioactivity was widely distributed to the tissues. Peak mean tissue concentrations were attained at the first sampling time of 8 hours post dose. The highest mean tissue concentration was observed in the liver (40.1 µg equiv/g). High concentrations were also found in the renal fat, adrenals and kidneys (range: 13.5-24.7 µg equiv/g). The majority of tissue concentrations were similar or above the levels observed in blood and plasma (3.7 and 6.2 µg equiv/g or mL, respectively). Thereafter, all tissue concentrations declined steadily up to 96 hours post dose, where with the exceptions of liver (2.7 µg equiv/g) and kidney (0.7 µg equiv/g), all tissues were below that of circulating blood and plasma (0.6 and 0.2 µg equiv/g or mL) or were below the LOD. Total radioactivity was still well detected in approximately a third of tissues at this final time point. The total mean residues in tissues and carcass at 96 hours accounted for 1.2% of the dose.

Following single oral administration of [pyrazole-5- ^{14}C]-SYN545974 at 300 mg/kg to males, radioactivity was widely distributed to the tissues. Peak mean tissue concentrations were attained at the first sampling time of 8 hours post dose. The majority of tissues were similar or above the levels observed in blood and plasma (3.9 and 6.0 µg equiv/g, respectively) at this time with the highest concentrations observed in the liver and kidneys with a mean of 80.9 and 22.4 µg equiv/g, respectively. Thereafter, all tissue concentrations declined steadily up to 96 hours post dose, where with the exceptions of liver and kidney (5.1 and 1.0 µg equiv/g, respectively), all tissues were below that of circulating blood (0.4 µg equiv/g) or were below the LOD. Total radioactivity was only detected in a few tissues at this final time point. The total mean residues in tissues and carcass at 96 hours accounted for 1.2% of the dose.

Following single oral administration of [pyrazole-5- ^{14}C]-SYN545974 at 100 mg/kg to females, radioactivity was widely distributed to the tissues. Peak mean tissue concentrations were attained at the first sampling time of 8 hours post dose. All tissues, except bone mineral, were above the concentration in blood and plasma (2.9 and 4.4 µg equiv/g or mL, respectively), with the highest tissue concentration observed in the liver (41.9 µg equiv/g). Thereafter, all tissue concentrations declined steadily up to 96 hours post dose, where with the exceptions of liver and kidney (3.0 and 0.5 µg equiv/g, respectively), all tissues were below that of circulating blood and plasma (0.5 and 0.2 µg equiv/g or mL, respectively) or below the LOD. Total radioactivity was still well detected in approximately a third of tissues at this final time point. The total mean residues in tissues and carcass at 96 hours accounted for 1.6% of the dose.

Circulating concentrations of total radioactivity were initially more associated with the plasma fraction then generally became increasingly associated with the cellular fraction at later time points.

Throughout the course of the study, irrespective of label, the majority of mean tissue concentrations were, in general, slightly higher in females than in males at the 5 mg/kg dose. This may represent higher partitioning into the tissues of females compared to males. There were no obvious differences noted in relation to radiolabel.

Estimates for tissue depletion half-life appeared similar in male animals and female animals at 5 mg/kg, no significant difference was noted with the site of label.

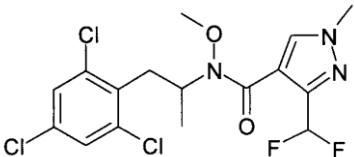
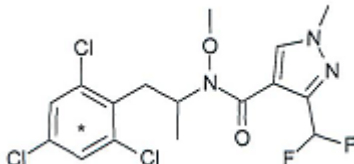
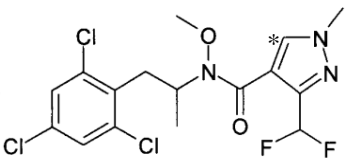
Following a single oral dose of [^{14}C]-SYN545974 to male (5 and 300 mg/kg) and female rats (5 and 100 mg/kg), the tissue distribution and depletion of radioactivity was similar, irrespective of dose, label or sex. Radioactivity was widely distributed, with the highest concentrations of radioactivity observed in the liver and kidney at all sampling time points, consistent with the excretion profile of [^{14}C]-SYN545974. The depletion of radioactivity from tissues broadly reflected that observed in

blood, suggesting accumulation in tissues is unlikely. At termination, total tissue and carcass residues accounted for $\leq 3.0\%$ of the administered dose.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:

Name :	SYN545974
Structure :	
Source :	Syngenta Crop Protection Münchwilen AG
Physical state :	Off white, powder
Batch reference :	SMU2EP12007
Purity:	98.5%
Radiolabelled Test Material:	[Phenyl-U- ^{14}C]-SYN545974
Radiochemical purity:	99.1%
Source:	Syngenta Crop Protection LLC
Lot/Batch number:	RDR-XV-94
Structure:	 <p style="text-align: right;">* position of [^{14}C]-label</p>
Radiolabelled Test Material:	[Pyrazole-5- ^{14}C]-SYN545974
Radiochemical purity:	98.8%
Source:	Selcia
Lot/Batch number:	5283RJP001-2
Structure:	 <p style="text-align: right;">* position of [^{14}C]-label</p>

Vehicle: 0.5% (w/v) aqueous carboxymethylcellulose (CMC) containing 0.5% Tween 80.

Preparation of dosing solutions: Each dose preparation was formulated as a homogenous suspension in 0.5% aqueous CMC containing 0.5% Tween 80. Each dose preparation was prepared using [^{14}C]-SYN545974 and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity.

Test Animals:	
Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	217 - 312 g for males; 183 - 214 g for females (Group 1) 263 - 343 g for males; 211 - 235 g for females (Group 2) 293 - 351 g for males; 189 - 247 g for females (Group 3) 284 - 321 g for males; 199 - 223 g for females (Group 4)
Source:	██████████
Housing:	Pre-study: Multiply housed by sex in solid bottomed polycarbonate cages with bedding On study: Multiply by sex in polycarbonate cages with raised wire mesh grids.
Acclimatisation period:	At least 5 days
Diet:	Rat and Mouse No.1 maintenance diet, ██████████ ██████████ Ad libitum
Water:	Tap water ad libitum
Environmental conditions:	Temperature: 18-21°C Humidity: 38 - 58% Air changes: 10-11 changes/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

Experimental dates: Start: 26 February 2013 End: 22 August 2013

Group Arrangements: Animals were assigned to 4 groups as shown in the table below.

Dosing groups for pharmacokinetic studies for [¹⁴C]-SYN545974

Test Group	Dose (mg/kg)	Radiolabel	Number/sex	Remarks
Group 1 Tissue distribution	5	Phenyl	20 males, 20 females	Tissue collection (sub-groups of 4 males and 4 females terminated at 1 h (females only), 2 h (males only), 12 h, 24 h, 72 h, and 120 h)
Group 2 Tissue distribution	5	Pyrazole	20 males, 20 females	Tissue collection (sub-groups of 4 males and 4 females terminated at 0.5 h, 12 h, 24 h, 48 h, and 96 h)
Group 3 Tissue distribution	300 (males) or 100 (females)	Phenyl	20 males, 20 females	Tissue collection (sub-groups of 4 males and 4 females terminated at 8 h, 24 h, 48 h, 72 h, and 96 h)
Group 4 Tissue distribution	300 (males) or 100 (females)	Pyrazole	20 males, 20 females	Tissue collection (sub-groups of 4 males and 4 females terminated at 8 h, 24 h, 48 h, 72 h, and 96 h)

Dosing and sample collection:

A single oral dose of [¹⁴C]-SYN545974 was administered to each rat by gavage at a target dose rate of 5 mL/kg. Animals in groups 1 and 2 received a dose corresponding to a low dose of 5 mg/kg, and animals in groups 3 and 4 received a dose corresponding to a high dose of 300 mg/kg (males) or 100 mg/kg (females). For each dose, the animals received a target radioactive dose of 5 MBq/kg.

Animals were humanely killed by CO₂ narcosis at various time points after dosing. A terminal blood sample was taken and divided between two heparinised tubes, one of which was centrifuged to separate plasma. The following tissues were taken for radioactivity analysis: adrenals, brain, heart, kidneys, liver, lungs, ovaries (females), pancreas, spleen, testes (males), thymus, thyroid, uterus (females), gastrointestinal tract plus contents and residual carcasses together with representative samples of bone (tibia and fibula), renal fat and muscle.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Statistics: Not applicable.

RESULTS

Pharmacokinetic Studies:

Preliminary experiment: Not applicable

Tissue distribution:

The concentrations of radioactivity in tissues and organs at various time points after single oral administration of [¹⁴C]-SYN545974 at nominal doses of 5, 100 (females) and 300 mg/kg (males) are presented in Tables 6.1.1-15 and 6.1.1-16.

Table 6.1.1-15: Distribution of radioactivity in tissues/organs 2, 12, 24, 72 and 120 hours after administration of [Phenyl-U-¹⁴C]-SYN545974 to male rats at a dose of 5 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	2 h	12 h	24 h	72 h	120 h
Adrenals	0.943	0.169	0.048	°0.010	°0.005
Bone Mineral	0.172	0.038	0.012	0.005	°0.002
Brain	0.135	0.027	0.009	°0.002	°0.002
Fat-Renal	0.420	0.164	0.060	0.011	0.006
Heart	0.560	0.110	0.037	0.011	0.008
Kidneys	2.454	0.525	0.236	0.083	0.061
Liver	8.557	4.019	1.656	0.442	0.203
Lungs	0.733	0.216	0.096	0.035	0.025
Muscle	0.299	0.060	0.018	°0.008	°0.004
Pancreas	0.710	0.126	0.041	0.007	°0.004
Plasma	1.461	0.262	0.076	0.017	0.009
Spleen	0.364	0.084	0.035	0.016	0.017
Testes	0.239	0.065	0.021	°0.003	°0.002
Thymus	0.349	0.060	0.023	°0.006	°0.004
Thyroid	0.558	0.140	°0.046	°0.003	°0.014
Whole blood	0.669	0.162	0.078	0.049	0.038

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-16: Distribution of radioactivity in tissues/organs 1, 12, 24, 72 and 120 hours after administration of [Phenyl-U-¹⁴C]-SYN545974 to female rats at a dose of 5 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	1 h	12 h	24 h	72 h	120 h
Adrenals	5.292	0.336	0.073	°0.012	°0.006
Bone Mineral	0.326	0.044	0.013	°0.003	°0.001
Brain	1.059	0.053	0.014	°0.003	°0.001
Fat-Renal	1.786	0.850	0.214	0.018	0.006
Heart	2.322	0.164	0.045	0.012	0.007
Kidneys	3.502	0.381	0.147	0.043	0.036
Liver	10.882	2.032	0.844	0.168	0.082
Lungs	2.195	0.353	0.160	0.076	0.039
Muscle	1.065	0.097	0.029	°0.003	°0.002
Ovaries	1.674	0.237	0.060	°0.007	°0.005
Pancreas	2.320	0.240	0.047	0.006	°0.003
Plasma	1.296	0.168	0.055	0.011	°0.005
Spleen	1.148	0.115	0.046	0.018	0.020
Thymus	1.171	0.106	0.023	°0.005	°0.003
Thyroid	2.535	0.163	°0.066	°0.005	°0.009
Uterus	0.876	0.130	0.059	°0.005	°0.002
Whole blood	0.811	0.155	0.092	0.076	0.051

° = Mean includes results calculated from data less than 30 dpm above background

As summarised above, following a single oral administration of [Phenyl-U-¹⁴C]-SYN545974 at target dose of 5 mg/kg, highest concentrations of radioactivity were observed at the first sampling time (2 hours (males) and 1 hours (females)). In male rats, the C_{max} blood and plasma concentrations of total radioactivity (0.669 and 1.461 µg equiv/g or mL, respectively) were observed at 2 hours post dose. The highest tissue concentrations were present in the liver (8.557 µg equiv/g), kidneys (2.454 µg equiv/g) and adrenals, (0.943 µg equiv/g). All other tissue concentrations were similar or lower than the blood concentration. Following C_{max} the concentration of total radioactivity in all tissues declined, steadily to 120 concentrations in blood and plasma were 0.038 and 0.009 µg equiv/g or mL, respectively. At this final time point only the liver and kidney concentrations (0.203 and 0.061 µg equiv/g, respectively) were above that of circulating radioactivity. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were below the Limit of Detection (LOD). In female rats, the maximum blood and plasma concentrations of total radioactivity (0.811 and 1.296 µg equiv/g or mL, respectively) were observed at 1 hour post dose. All tissue concentrations were above the blood concentration at this time point (range 0.876-10.882 µg equiv/g), with the exception of the bone mineral. The highest tissue concentrations were present in the liver (10.882 µg equiv/g), adrenals (5.292 µg equiv/g) and kidneys (3.502 µg equiv/g). Following C_{max} the concentration of total radioactivity in all tissues declined, steadily to 120 hours post dose, where the concentration of circulating radioactivity in blood was 0.051 µg equiv/g with the plasma concentration <LOD. The highest tissue concentration was observed in the liver with a mean of 0.082 µg equiv/g with concentrations in the remaining tissues below that of circulating blood or <LOD.

Table 6.1.1-17: Distribution of radioactivity in tissues/organs 0.5, 12, 24, 48 and 96 hours after administration of [Pyrazole-5-¹⁴C]-SYN545974 to male rats at a dose of 5 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	0.5 h	12 h	24 h	48 h	96 h
Adrenals	0.905	0.239	0.043	°0.020	°0.010
Bone Mineral	0.091	0.031	0.017	0.008	°0.003
Brain	0.090	0.027	0.010	0.004	°0.002
Fat-Renal	0.447	0.113	0.041	0.011	°0.003
Heart	0.395	0.094	0.033	0.017	0.009
Kidneys	2.281	0.393	0.154	0.085	0.057
Liver	9.457	3.887	1.681	0.786	0.318
Lungs	0.508	0.159	0.060	0.037	0.018
Muscle	0.166	0.063	0.026	0.010	°0.004
Pancreas	0.549	0.112	0.037	0.010	°0.005
Plasma	0.686	0.153	0.063	0.025	0.013
Spleen	0.389	0.073	0.034	0.017	0.011
Testes	0.078	0.058	0.022	0.008	°0.003
Thymus	0.274	0.060	0.022	0.009	°0.004
Thyroid	0.373	0.099	0.042	°0.025	°0.012
Whole blood	0.358	0.114	0.061	0.042	0.034

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-18: Distribution of radioactivity in tissues/organs 0.5, 12, 24, 48 and 96 hours after administration of [Pyrazole-5-¹⁴C]-SYN545974 to female rats at a dose of 5 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	0.5 h	12 h	24 h	48 h	96 h
Adrenals	3.083	0.280	0.051	°0.020	°0.009
Bone Mineral	0.196	0.049	0.009	°0.005	°0.005
Brain	0.607	0.040	0.008	°0.002	°0.002
Fat-Renal	0.571	0.605	0.160	0.040	°0.004
Heart	1.486	0.146	0.032	0.014	0.010
Kidneys	2.792	0.395	0.113	0.046	0.034
Liver	12.608	2.564	0.855	0.314	0.199
Lungs	1.284	0.269	0.117	0.074	0.035
Muscle	0.545	0.095	0.018	0.008	°0.005
Ovaries	1.003	0.210	0.052	°0.014	°0.005
Pancreas	1.606	0.203	0.058	0.014	°0.005
Plasma	0.888	0.134	0.042	0.015	°0.009
Spleen	0.749	0.103	0.034	0.017	0.011
Thymus	1.267	0.092	0.020	0.006	°0.004
Thyroid	1.602	0.141	°0.030	°0.005	°0.003
Uterus	0.485	0.191	0.055	0.016	°0.004
Whole blood	0.528	0.123	0.071	0.051	0.042

° = Mean includes results calculated from data less than 30 dpm above background

As summarised in Tables 5.1.1-17 and 5.1.1-18, following a single oral administration of [Pyrazole-5-¹⁴C]-SYN545974 at target dose of 5 mg/kg, highest concentrations of radioactivity were observed at the first sampling time (0.5 hours in both sexes). In male rats, the maximum blood and plasma concentrations of total radioactivity (0.358 and 0.686 µg equiv/g or mL, respectively) were observed at 0.5 hours post dose. The highest tissue concentrations were observed in the liver and kidneys with means of 9.457 and 2.281 µg equiv/g, respectively. The majority of tissues were similar to the concentrations of circulating radioactivity at this time (range 0.373-0.905 µg equiv/g). All other tissue concentrations (bone mineral, brain, muscle, testes and thymus) were lower than the blood concentration. Following C_{max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where concentrations in blood and plasma were 0.034 and 0.013 µg equiv/g, respectively. The highest tissue concentrations were observed in the liver and kidneys with means of 0.318 and 0.057 µg equiv/g, respectively. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were < LOD. In female rats, the maximum blood and plasma concentrations of total radioactivity (0.528 and 0.888 µg equiv/g or mL, respectively) were observed at 0.5 hours post dose. The majority of tissues were similar or above the concentrations of circulating radioactivity with the highest tissue concentrations observed in the liver, adrenals and kidneys with means of 12.608, 3.083 and 2.792 µg equiv/g, respectively. Following C_{max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where circulating radioactivity was detected in the blood at 0.042 µg equiv/g, but plasma concentration was <LOD. At this final time point all concentrations, except liver (0.199 µg equiv/g) were below that in blood or <LOD.

Table 6.1.1-19: Distribution of radioactivity in tissues/organs 8, 24, 48, 72 and 96 hours after administration of [Phenyl-U-¹⁴C]-SYN545974 to male rats at a dose of 300 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	8 h	24 h	48 h	72 h	96 h
Adrenals	15.5	°1.1	°0.6	°0.6	°0.3
Bone Mineral	1.9	0.4	°0.2	°0.1	°0.1
Brain	2.5	0.2	°0.1	°0.1	°0.1
Fat-Renal	11.4	3.4	1.0	0.4	0.3
Heart	7.3	0.9	0.4	0.2	0.3
Kidneys	28.5	6.5	2.7	1.5	1.7
Liver	77.5	31.8	14.3	6.9	6.3
Lungs	9.8	2.0	1.2	0.7	0.7
Muscle	3.9	0.6	0.3	°<0.1	°0.1
Pancreas	10.1	1.7	0.4	°0.2	°0.2
Plasma	13.0	2.6	0.8	0.3	0.4
Spleen	4.7	0.8	0.5	0.3	0.4
Testes	3.4	0.5	°0.2	°<0.1	°0.1
Thymus	4.2	0.5	°0.2	°0.1	°0.1
Thyroid	6.1	°0.5	°0.4	°<0.1	°0.3
Whole blood	7.1	1.8	1.1	0.9	0.8

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-20: Distribution of radioactivity in tissues/organs 8, 24, 48, 72 and 96 hours after administration of [Phenyl-U-¹⁴C]-SYN545974 to female rats at a dose of 100 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	8 h	24 h	48 h	72 h	96 h
Adrenals	20.2	1.1	°0.2	°0.2	°0.1
Bone Mineral	1.3	0.2	°0.1	°0.1	°<0.1
Brain	4.1	0.2	°0.1	°<0.1	°<0.1
Fat-Renal	24.7	6.6	1.2	0.3	°0.3
Heart	7.6	0.7	0.2	0.2	0.1
Kidneys	13.5	2.9	1.2	0.7	0.7
Liver	40.1	14.6	6.6	4.2	2.7
Lungs	8.5	1.7	0.8	0.5	0.5
Muscle	3.5	0.5	0.2	°0.1	°0.1
Ovaries	10.6	1.2	°0.3	°0.1	°0.1
Pancreas	11.6	0.8	0.2	°0.1	°0.1
Plasma	6.2	1.2	0.4	0.2	0.2
Spleen	4.8	0.5	0.3	0.2	0.2
Thymus	5.2	°0.3	°0.1	°0.1	°0.1
Thyroid	8.9	°0.6	°0.1	°0.3	°0.1
Uterus	4.7	0.9	0.2	°0.1	°0.1
Whole blood	3.7	1.0	0.6	0.6	0.6

° = Mean includes results calculated from data less than 30 dpm above background

As summarised in Tables 6.1.1-19 and 6.1.1-20, following a single oral administration of [Phenyl-U-¹⁴C]-SYN545974 at target dose of 300 mg/kg (males) or 100 mg/kg (females), highest concentrations of radioactivity were observed at the first sampling time (8 hours post dose in both sexes). In male rats, the maximum blood and plasma concentrations of total radioactivity (7.1 and 13.0 µg equiv/g or mL, respectively) were observed at 8 hours post dose. Approximately half of the tissues (adrenals, renal fat, heart, kidneys, liver, lungs and pancreas) were similar or above the levels of circulating radioactivity at this time with the highest concentrations observed in the liver and kidneys with means of 77.5 and 28.5 µg equiv/g, respectively. All other tissue concentrations were lower than the blood concentration. Following C_{max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where concentrations in blood and plasma were 0.8 and 0.4 µg equiv/g or mL, respectively. The highest tissue concentrations were observed in the liver and kidneys with means of 6.3 and 1.7 µg equiv/g, respectively. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were < LOD. In female rats, the maximum blood and plasma concentrations of total radioactivity (3.7 and 6.2 µg equiv/g or mL, respectively) were observed at 8 hours post dose. The majority of tissues were similar or above the levels of circulating radioactivity at this time with the highest mean concentrations

observed in the liver, renal fat, adrenals and kidneys with concentrations of 40.1, 24.7, 20.2 and 13.5 µg equiv/g, respectively. Following C_{\max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where concentrations in blood and plasma were 0.6 and 0.2 µg equiv/g or mL, respectively. At this final time point all concentrations, except liver and kidneys (2.7 and 0.7 µg equiv/g, respectively) were below that in blood or <LOD.

Table 6.1.1-21: Distribution of radioactivity in tissues/organs 8, 24, 48, 72 and 96 hours after administration of [Pyrazole-5- 14 C]-SYN545974 to male rats at a dose of 300 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	8 h	24 h	48 h	72 h	96 h
Adrenals	21.5	°2.7	°0.1	°0.1	°0.1
Bone Mineral	2.1	0.3	°0.5	°<0.1	°<0.1
Brain	3.4	0.4	°0.1	°<0.1	°<0.1
Fat-Renal	19.3	2.1	0.4	°<0.1	°0.1
Heart	7.7	1.1	0.3	°0.1	°0.1
Kidneys	22.4	5.6	1.7	0.9	1.0
Liver	80.9	38.7	15.1	7.1	5.1
Lungs	9.7	1.7	0.6	0.4	0.3
Muscle	4.2	0.7	°0.1	°<0.1	°<0.1
Pancreas	13.3	1.1	°0.1	°0.1	°0.1
Plasma	6.0	2.1	0.5	°0.2	°0.3
Spleen	5.4	1.0	°0.2	°0.1	°0.1
Testes	3.6	0.7	°0.1	°<0.1	°<0.1
Thymus	6.4	0.8	°0.1	°<0.1	°<0.1
Thyroid	9.5	°1.4	°0.9	°<0.1	°0.4
Whole blood	3.9	1.7	0.8	0.4	0.4

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-22: Distribution of radioactivity in tissues/organs 8, 24, 48, 72 and 96 hours after administration of [Pyrazole-5- 14 C]-SYN545974 to female rats at a dose of 100 mg/kg

Time after dosing	Group mean tissue residues (µg equiv/g or mL)				
	8 h	24 h	48 h	72 h	96 h
Adrenals	16.7	1.1	°0.1	°1.3	°0.2
Bone Mineral	1.3	0.1	°0.2	°<0.1	°<0.1
Brain	3.3	0.2	°<0.1	°<0.1	°<0.1
Fat-Renal	17.9	7.5	1.0	0.2	°0.1
Heart	6.8	0.6	0.1	0.1	0.1
Kidneys	13.5	2.0	0.6	0.4	0.5
Liver	41.9	14.8	6.1	3.1	3.0
Lungs	7.7	1.3	0.5	0.3	0.3
Muscle	3.3	0.5	°0.1	°<0.1	°0.1
Ovaries	10.5	1.3	°0.2	°0.1	°0.1
Pancreas	16.7	0.8	°0.1	°0.1	°0.2
Plasma	4.4	0.8	0.2	0.1	0.2
Spleen	4.4	0.5	0.2	0.1	0.2
Thymus	7.1	0.4	°0.1	°<0.1	°0.1
Thyroid	7.9	°0.4	°<0.1	°0.6	°0.7
Uterus	5.5	1.3	0.2	°0.1	0.1
Whole blood	2.9	0.9	0.5	0.4	0.5

° = Mean includes results calculated from data less than 30 dpm above background

As summarised in Tables 6.1.1-21 and 6.1.1-22, following a single oral administration of [Pyrazole-5- 14 C]-SYN545974 at target dose of 300 mg/kg (males) or 100 mg/kg (females), highest concentrations of radioactivity were observed at the first sampling time (8 hours post dose in both sexes). In male rats, the maximum blood and plasma concentrations of total radioactivity (3.9 and 6.0 µg equiv/g or mL, respectively) were observed at 8 hours post dose. The majority of tissues were similar or above the levels of circulating radioactivity at this time with the highest mean concentrations observed in the liver and kidneys of 80.9 and 22.4 µg equiv/g, respectively. Following C_{\max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where circulating radioactivity was detected in the blood at 0.4 µg equiv/g, but was < LOD in plasma. The highest tissue concentrations were observed in

the liver and kidneys with means of 5.1 and 1.0 µg equiv/g, respectively. Concentrations of radioactivity in the remaining tissues were below that of circulating blood or were < LOD. In female rats, the maximum blood and plasma concentrations of total radioactivity (2.9 and 4.4 µg equiv/g or mL, respectively) were observed at 8 hours post dose. All tissues were similar or above the levels of circulating radioactivity at this time with the highest mean concentration observed in the liver with a mean of 41.9 µg equiv/g. Following C_{\max} the concentration of total radioactivity in all tissues declined steadily to 96 hours post dose, where concentrations in blood and plasma were 0.5 and 0.2 µg equiv/g or mL, respectively. At this final time point all concentrations, except liver and kidneys (3.0 and 0.5 µg equiv/g, respectively) were below that in blood or < LOD.

Tissue elimination:

The half-lives of elimination from tissues and organs following single oral administration of [^{14}C]-SYN545974 at doses of 5, 100 (females) and 300 mg/kg (males) are presented in Tables 6.1.1-23 and 6.1.1-24.

Table 6.1.1-23: Elimination of radioactivity from rat tissues/organs after single administration of [Phenyl- ^{14}C]-SYN545974 to rats at a dose of 5, 300 or 100 mg/kg

	Values are expressed as $T_{1/2\text{el}}$ (h). Each value is a mean of 4 rats			
	5 mg/kg		300 mg/kg	100 mg/kg
Tissue	Male	Female	Male	Female
Adrenals	5.17	9.74	NC	15.5
Bone Mineral	22.8	15.7	8.64	39.3*
Brain	26.5*	12.4	7.27	17.1
Fat-Renal	29.0	18.6	25.3	19.4*
Heart	43.3	35.8	40.5*	56.6
Kidneys	49.0	46.8	36.8*	35.4
Liver	31.7	28.6	40.9	37.5
Lungs	49.9	47.1	45.4	40.0
Muscle	30.5	11.9	10.5	22.7
Ovaries	NA	4.80	NA	13.4
Pancreas	22.9	21.1	43.7*	20.6
Plasma	31.1	16.3	25.6	48.0
Spleen	88.6*	78.2*	66.9*	60.5*
Testes	25.2	NA	24.8*	NA
Thymus	28.5	30.1	23.6	30.1
Thyroid	5.10	3.21	NC	NC
Uterus	NA	12.8	NA	27.6
Whole Blood	91.3*	115*	138*	125*

NA = Not Applicable

* = The coefficient of determination was less than 0.800 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area

NC = Not calculable, an apparent terminal phase could not be characterised.

Table 6.1.1-24: Elimination of radioactivity from rat tissues/organs after single administration of [Pyrazole-5-¹⁴C]-SYN545974 to rats at a dose of 5, 300 or 100 mg/kg

	Values are expressed as T _{1/2} el (h). Each value is a mean of 4 rats			
	5 mg/kg		300 mg/kg	100 mg/kg
Tissue	Male	Female	Male	Female
Adrenals	6.32	25.2	NC	20.4*
Bone Mineral	15.6	50.6*	19.6*	20.7*
Brain	13.2	16.4	6.69	NC
Fat-Renal	10.8	11.4	7.31	14.3
Heart	41.9	46.1	15.0	32.5*
Kidneys	53.5	44.8	28.6	35.4*
Liver	30.8	37.1	31.0	30.5
Lungs	41.8	41.2	48.0	93.3*
Muscle	27.7	40.2	7.48	11.5
Ovaries	NA	18.7	NA	5.99
Pancreas	24.4	18.5	5.00	14.2*
Plasma	34.1	33.8	17.0	30.8*
Spleen	47.8	46.5	7.42	61.1*
Testes	21.1	NA	6.46	NA
Thymus	31.0	27.8	6.29	23.4*
Thyroid	9.94	3.13	NC	37.8*
Uterus	NA	16.4	NA	59.5*
Whole Blood	88.4*	103*	37.2	86.8*

NA = Not Applicable

* = The coefficient of determination was less than 0.800 and/or the extrapolation of the AUC to infinity represented more than 20% of the total area

NC = Not calculable, an apparent terminal phase could not be characterised.

The depletion profile of radioactivity from all tissues appeared to mirror that observed in blood/plasma, with no plateau in any tissue. In general the half life was broadly similar to that observed in plasma. The tissue depletion half life of total radioactivity in each tissue was generally similar following an increasing dose to the phenyl males and pyrazole females. The half lives were generally longer in the phenyl females with an increased dose, and shorter in the pyrazole males following an increased dose. Estimates for tissue depletion half-life appeared similar in male animals and female animals at 5 mg/kg, no significant difference was noted with the site of label.

CONCLUSION:

Four groups of 40 Han Wistar rats (20 per sex) were given a single oral dose of 5, 100 (females only) and 300 (males only) mg/kg bw [phenyl-U-¹⁴C] or [pyrazole-5-¹⁴C]-pydiflumetofen to investigate the tissue distribution and depletion of radioactivity. Following dosing, groups of 4 rats per sex per time point were humanely killed at intervals and the residual radioactivity measured in selected tissues/organs and the remaining carcasses. Where appropriate, terminal phase half-lives of depletion were calculated for individual tissues.

Tissue Distribution

Following a single oral administration of 5 mg/kg bw pydiflumetofen radiolabelled with either [phenyl-U-¹⁴C] or [pyrazole-5-¹⁴C], the highest total radioactivity was observed in the liver, kidneys and adrenals for both sexes. Tissue concentrations depleted steadily and reflected those of blood up to the final sampling time (120 h [phenyl-U-¹⁴C] or 96h [pyrazole-5-¹⁴C]) at which only the kidneys (males only) and

Following a single oral administration of 100 mg/kg bw (females) or 300 mg/kg bw (males) pydiflumetofen radiolabelled with either [phenyl- U - ^{14}C] or [pyrazole-5- ^{14}C], the highest total radioactivity was observed in the liver and kidneys in both sexes, as well as in the renal fat and adrenals in females. Tissue concentrations depleted steadily and reflected those of blood up to the final sampling time (96 h) at which only the liver and kidneys (both sexes) were above circulating total concentration.

Tissue depletion generally followed the same pattern of elimination observed in blood/plasma. No plateaus were observed in any tissue. Half-life of total radioactivity was generally similar in all tissues after exposure to the phenyl radiolabel in males and the pyrazole radiolabel in females. Half-life of total radioactivity was generally longer with increasing dose in females after phenyl exposure, whereas it was generally shorter with increasing dose in males after pyrazole exposure. Half-life in all tissues was largely similar at 5 mg/kg bw irrespective of sex or radiolabel.

Overall, under the conditions of this GLP and OECD TG compliant tissue depletion study in rats, tissue distribution and depletion was largely similar irrespective of dose, sex or radiolabel. In general, the majority of mean tissue concentrations were higher in females compared to males at 5 mg/kg bw, which may represent higher partitioning of the substance and/or its metabolites into the tissues of females. However, tissue depletion half-life was similar between the sexes at 5 mg/kg bw. Radioactivity was widely distributed with the highest tissue concentrations in the liver and kidneys at all time points and doses, which corresponds with the excretion profile determined in the absorption and excretion study (█, 2015). The depletion of radioactivity broadly reflected that observed in blood, with no tissues plateauing, suggesting accumulation in tissues is unlikely. At termination, total tissue and carcass residues accounted for < 3.0% of the AD.

([REDACTED], 2015a)

Report: (2015). SYN545974 – The Pharmacokinetics of [Phenyl-U-¹⁴C] and [Pyrazole-5-¹⁴C]-SYN545974 Following Single Oral and Intravenous Administration in the Rat. [REDACTED] [REDACTED]
[REDACTED] Report No. 34107. Issue date 04 June 2015. Unpublished. Syngenta File No. SYN545974 10250.

Guidelines: Metabolism – rat; OECD 417 (2010); EPA OPPTS 870.7485 (1998); EC 1107/2009 (2009), EU 283/2013 (2013), JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study is considered acceptable.

Single oral doses of 5, 100 (females only) and 300 (males only) mg/kg and a single intravenous dose of 1 mg/kg [phenyl-U-14C] or [pyrazole-5-14C]-SYN545974 were administered to groups of 4 male and 4 female animals. Blood samples were obtained over a 4 (oral) or 2 (intravenous) day period to determine the pharmacokinetics of total radioactivity in blood (following oral and IV administration) and plasma (following oral administration). Oral bioavailability was determined by comparing the dose normalised exposures following oral and intravenous administration of [14C]-SYN545974. The nature and identity

of metabolites present in the plasma samples from the oral dose groups was investigated and reported separately, under [REDACTED] Study No. 221595.

Following a single oral administration of phenyl or pyrazole labelled [14C]-SYN545974 (5, 100 or 300 mg/kg) absorption was rapid with peak blood and plasma concentrations observed up to 8 hours post dose. Thereafter, concentrations initially decreased, before slowly declining to a plateau at the last timepoints. Following intravenous administration of phenyl or pyrazole labelled [14C]-SYN545974 (1 mg/kg) peak blood concentrations declined steadily following the first sampling time.

In the oral dose groups, in the earlier timepoints (5 mg/kg: 0.25-12 h, 100 mg/kg: 0.25-30 h and 300 mg/kg: 0.25-24 h) plasma concentrations of total radioactivity were approximately twice those in blood at the majority of time points, with blood to plasma ratios of *ca* 0.6, suggesting that total radioactivity remained predominantly in plasma rather than the cellular component of whole blood. The blood to plasma ratio appeared to increase at later timepoints, becoming either evenly distributed or greater in the cellular fraction.

Overall, systemic exposure to [phenyl-U-14C] and [pyrazole-5-14C]-SYN545974 (based on C_{max} and AUC(0-t) estimates) increased in a sub-proportional manner between the low and high dose levels in whole blood and plasma for both males and females. For both [phenyl-U-14C]-SYN545974 and [pyrazole-5-14C]-SYN545974 administration, overall total systemic exposure was comparable between whole blood and plasma within the same dose levels.

Absolute oral bioavailability (F) of [phenyl-U-14C]-SYN545974 ranged from 28 to 55% in males and 34 to 55% in females, across the respective dose ranges. Absolute oral bioavailability of [pyrazole-5-14C]-SYN545974 (F) was between 27 to 52% in males and 26 to 48% in females.

There was no difference in systemic exposure between sexes following intravenous administration for both [phenyl-U-14C]-SYN545974 and [pyrazole-5-14C]-SYN545974.

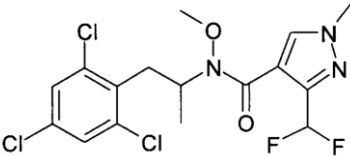
Following single oral doses of 5, 100 (females only) and 300 mg/kg (males only) phenyl or pyrazole labelled [14C]-SYN545974 to male and female rats, the mean peak blood and plasma concentrations were observed between 0.5-8 hours post dose. Systemic exposure increased in a sub-proportional manner between the low and high dose levels in whole blood and plasma for both males and females.

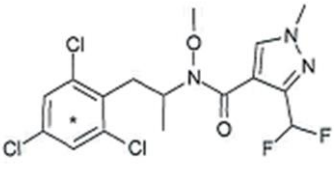
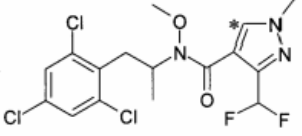
At the 5 mg/kg oral dose, absolute oral bioavailability (F) in blood ranged from 48-55%, for males and females irrespective of radiolabel position. However, after the 100 or 300 mg/kg oral dose, bioavailability was decreased with F estimates of between 26-34%, for males and females, respectively. This data indicates that the sub-proportional increase in exposure was limited by absorption at the higher dose.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:

Name :	SYN545974
Structure :	
Source :	Syngenta Crop Protection Münchwilen AG
Physical state :	Off white, powder
Batch reference :	SMU2EP12007
Purity:	98.5%

Radiolabelled Test Material:	[Phenyl-U- ¹⁴ C]-SYN545974
Radiochemical purity:	99.1%
Source:	Syngenta Crop Protection LLC
Lot/Batch number:	RDR-XV-94
Structure:	 <p>* position of [¹⁴C]-label</p>
Radiolabelled Test Material:	[Pyrazole-5- ¹⁴ C]-SYN545974
Radiochemical purity:	99.2%
Source:	Selcia
Lot/Batch number:	5271GAR001-4
Structure:	 <p>* position of [¹⁴C]-label</p>

Vehicle: Oral doses: 0.5% (w/v) aqueous carboxymethylcellulose (CMC) containing 0.5% Tween 80. Intravenous dose: dimethylsulfoxide (DMSO): 10% aqueous hydroxypropyl-β-cyclodextrin (HPβC) (5:95, (v/v)).

Preparation of dosing solutions: For oral doses, radiolabelled SYN545974 was homogenously suspended in 0.5% aqueous CMC containing 0.5% Tween 80. The intravenous dose was a radiolabelled SYN545974 solution in DMSO: 10% aqueous HPβC (5:95). Each oral dose preparation was prepared using [¹⁴C]-SYN545974 and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity. The intravenous preparations were prepared using [¹⁴C]-SYN545974.

Test Animals:	
Species:	Rat
Strain:	Han Wistar
Age/weight at dosing:	Groups 1-4: 8-9 weeks Groups 5-6: 7-8 weeks (males); 7-10 weeks (females) 222 - 270 g for males; 160 - 196 g for females (Group 1) 226 - 272 g for males; 159 - 205 g for females (Group 2) 214 - 254 g for males; 160 - 195 g for females (Group 3) 204 - 257 g for males; 153 - 186 g for females (Group 4) 248 - 276 g for males; 179 - 195 g for females (Group 5) 236 - 264 g for males; 180 - 194 g for females (Group 6)
Source:	
Housing:	Pre-study: multiply housed by sex in solid bottomed polycarbonate cages with wood shavings On study: Groups 1-4 housed multiply by sex in polycarbonate and stainless steel cages with raised wire mesh floors. Groups 5-6 housed in pairs by sex in polycarbonate and stainless steel cages with raised wire mesh floors
Acclimatisation period:	At least 5 days
Diet:	Rat and Mouse No.1 maintenance diet, <i>Ad libitum</i> .
Water:	Tap water <i>ad libitum</i>

Environmental conditions:	Temperature: 20 °C Humidity: 41-79% Photoperiod: 12 hours light / 12 hours dark
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Study Design and Methods:

Experimental dates: Start: 06 December 2012; End: 18 April 2013

Group Arrangements: Animals were assigned to 6 groups as shown in the table below.

Dosing groups for pharmacokinetic studies for [14C]-SYN545974

Test Group	Dose (mg/kg)	Radiolabel	Number/sex	Remarks
Group 1 (oral) Blood and plasma kinetics	5	Phenyl	24 males, 24 females	Composite blood collections (1 serial/1 terminal from each rat) taken over a time course of up to 4 days.
Group 2 (oral) Blood and plasma kinetics	5	Pyrazole	24 males, 24 females	Composite blood collections (1 serial/1 terminal from each rat) taken over a time course of up to 4 days.
Group 3 (oral) Blood and plasma kinetics	300 (males) or 100 (females)	Phenyl	24 males, 24 females	Composite blood collections (1 serial/1 terminal from each rat) taken over a time course of up to 4 days.
Group 4 (oral) Blood and plasma kinetics	300 (males) or 100 (females)	Pyrazole	24 males, 24 females	Composite blood collections (1 serial/1 terminal from each rat) taken over a time course of up to 4 days.
Group 5 (intravenous) Blood kinetics	1	Phenyl	4 males, 4 females	Serial blood collections taken from each rat over a time course of 2 days.
Group 6 (intravenous) Blood kinetics	1	Pyrazole	4 males, 4 females	Serial blood collections taken from each rat over a time course of 2 days.

Dosing and sample collection: A single oral dose of [14C]-SYN545974 was administered to each rat by gavage at a dose rate of 5 mL/kg (groups 1, 2 and 4) or 5.5 mL/kg (group 3). Animals in groups 1 and 2 received a dose corresponding to a nominal low dose of 5 mg/kg, and animals in groups 3 and 4 received a dose corresponding to a nominal high dose of 300 mg/kg (males) or 100 mg/kg (females). For each oral dose, the animals received a target radioactive dose of 5 MBq/kg. The intravenous dose was administered as a slow bolus over a period of *ca* 30 seconds at a target dose volume of 5 mL/kg. Animals in groups 5 and 6 received a dose corresponding to a nominal low dose of 1 mg/kg and target radioactive dose of 5 MBq/kg.

For the oral groups, blood samples were removed from each rat at 2 time points per group of 4 male and 4 female animals. The first sample was taken by venepuncture of a tail vein. The last time point from each group was taken as terminal sample. Blood was collected into heparinised tubes. A sample of blood was retained for whole blood analysis and plasma was then separated from the remaining blood sample by centrifugation. Blood cells were discarded.

For the intravenous dose group, blood samples were serially collected from tail vein of each rat (into blood tubes containing lithium heparin anticoagulant) at a defined interval following dosing.

All animals were humanely killed by CO₂ narcosis. All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Pharmacokinetic studies:

To investigate pharmacokinetics, blood samples were collected at the following intervals:

Table 6.1.1-25: Blood collection times for pharmacokinetic studies for [14C]-SYN545974

Groups	Nominal dose	Animal numbers		Sampling times (hours after dosing)
		Male	Female	
1 (Phenyl)	5 mg/kg Oral	1-4	5-8	0.25 and 1 ^A
		9-12	13-16	0.5 and 2 ^A
		17-20	21-24	4 and 12 ^A
		25-28	29-32	8 and 24 ^A
		33-36	37-40	30 and 72 ^A
		41-44	45-48	48 and 96 ^A
2 (Pyrazole)	5 mg/kg Oral	49-52	53-56	0.25 and 1 ^A
		57-60	61-64	0.5 and 2 ^A
		65-68	69-72	4 and 12 ^A
		73-76	77-80	8 and 24 ^A
		81-84	85-88	30 and 72 ^A
		89-92	93-96	48 and 96 ^A
3 (Phenyl)	300 mg/kg (male) or 100 mg/kg (female) Oral	145-148	149-152	0.25 and 1 ^A
		153-156	157-160	0.5 and 2 ^A
		161-164	165-168	4 and 12 ^A
		169-172	173-176	8 and 24 ^A
		177-180	181-184	30 and 72 ^A
		185-188	189-192	48 and 96 ^A
4 (Pyrazole)	300 mg/kg (male) or 100 mg/kg (female) Oral	97-100	101-104	0.25 and 1 ^A
		105-108	109-112	0.5 and 2 ^A
		113-116	117-120	4 and 12 ^A
		121-124	125-128	8 and 24 ^A
		129-132	133-136	30 and 72 ^A
		137-140	141-144	48 and 96 ^A
5 (Phenyl)	1 mg/kg Intravenous	193-196	197-200	0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48
6 (Pyrazole)	1 mg/kg Intravenous	201-204	205-208	0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30 and 48

A = sample taken as terminal sample

Metabolite characterisation studies: Metabolite characterisation on selected plasma samples was undertaken in a separate study (██████████ 2015) SYN545974 – Biotransformation in the Rat. ██████████

Report No. 34216. (Syngenta File no. SYN545974_10259).

Statistics: Not applicable.

RESULTS

Pharmacokinetic Studies:

Blood kinetics: The concentration of total radioactivity in plasma and whole blood following a single oral administration of [14C]-SYN545974 at nominal doses of 5, 100 (females) and 300 mg/kg (males) are presented in Tables 6.1.1-26 to 6.1.1-29.

The concentration of total radioactivity in plasma and whole blood following a single intravenous dose of [14C]-SYN545974 at a dose of 1 mg/kg in males and females are presented in Table 6.1.1-30. A comparison of the pharmacokinetics parameters in plasma and whole blood following a single oral or intravenous dose are presented in Tables 6.1.1-31 to 6.1.1-35.

Table 6.1.1-26: Concentrations of radioactivity in whole blood over a time course after oral

administration of [Phenyl-U-¹⁴C]-SYN545974

Sampling time	Group mean blood concentrations (µg equivalents of [¹⁴ C]-SYN545974/g)			
	Group 1		Group 3	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.25 h	0.17	0.15	1.5	0.7
0.5 h	0.38	0.24	1.9	1.6
1 h	0.48	0.72	3.3	1.9
2 h	0.63	0.62	4.5	2.4
4 h	0.27	0.30	6.8	2.0
8 h	0.16	0.19	8.1	3.8
12 h	0.13	0.13	6.1	3.2
24 h	0.06	0.07	2.1	0.9
30 h	0.05	0.06	1.5	0.7
48 h	0.04	0.05	1.3	0.4
72 h	0.03	0.05	1.2	0.3
96 h	0.03	0.03	1.2	0.3

Table 6.1.1-27: Concentrations of radioactivity in plasma over a time course after oral administration of [Phenyl-U-¹⁴C]-SYN545974

Sampling time	Group mean plasma concentrations (µg equivalents of [¹⁴ C]-SYN545974/mL)			
	Group 1		Group 3	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.25 h	0.32	0.27	3.3	1.2
0.5 h	0.79	0.46	4.0	2.6
1 h	0.87	1.17	6.5	3.3
2 h	1.13	0.97	9.2	3.9
4 h	0.43	0.42	12.5	3.3
8 h	0.22	0.23	13.0	5.8
12 h	0.20	0.18	11.2	4.9
24 h	0.06	0.06	3.1	1.2
30 h	0.05	0.04	1.7	0.7
48 h	0.02	0.01	1.3	0.3
72 h	0.02	0.01	1.2	0.2
96 h	0.01	0.01	0.9	0.1

Table 6.1.1-28: Concentrations of radioactivity in whole blood over a time course after oral administration of [Pyrazole-5-¹⁴C]-SYN545974

Sampling time	Group mean blood concentrations (µg equivalents of [¹⁴ C]-SYN545974/g)			
	Group 2		Group 4	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.25 h	0.10	0.14	0.6	0.4
0.5 h	0.26	0.45	1.1	1.0
1 h	0.19	0.26	1.8	1.4
2 h	0.27	0.31	2.4	2.0
4 h	0.19	0.20	3.9	1.8

8 h	0.13	0.17	4.7	2.1
12 h	0.12	0.12	4.7	1.6
24 h	0.07	0.07	2.1	0.5
30 h	0.06	0.05	2.1	0.5
48 h	0.04	0.03	0.8	°0.3
72 h	0.02	0.03	1.1	0.2
96 h	0.02	0.02	0.7	0.3

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-29: Concentrations of radioactivity in plasma over a time course after oral administration of [Pyrazole-5-14C]-SYN545974

Sampling time	Group mean plasma concentrations (µg equivalents of [¹⁴ C]-SYN545974/mL)			
	Group 2		Group 4	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.25 h	0.21	0.25	1.2	0.8
0.5 h	0.47	0.67	2.0	1.7
1 h	0.39	0.45	3.5	2.5
2 h	0.49	0.49	4.7	3.1
4 h	0.29	0.28	5.8	2.5
8 h	0.20	0.21	7.1	3.0
12 h	0.16	0.14	6.6	2.3
24 h	0.07	0.06	2.7	0.6
30 h	0.06	0.03	2.4	0.4
48 h	0.02	0.01	°0.6	°0.1
72 h	0.01	0.01	0.9	°0.1
96 h	0.01	°<0.01	°0.3	°0.1

° = Mean includes results calculated from data less than 30 dpm above background

Table 6.1.1-30: Concentrations of radioactivity in whole blood over a time course after intravenous administration of [¹⁴C]-SYN545974

Sampling time	Group mean blood concentrations (µg equivalents of [¹⁴ C]-SYN545974/g)			
	Group 5 (phenyl)		Group 6 (pyrazole)	
	1 mg/kg		1 mg/kg	
	Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
0.25 h	0.370	0.291	0.295	0.251
0.5 h	0.297	0.233	0.199	0.188
1 h	0.266	0.194	0.127	0.130
2 h	0.136	0.133	0.076	0.088
4 h	0.079	0.081	0.056	0.062
6 h	0.059	0.071	0.051	0.054
8 h	0.047	0.064	0.044	0.043
12 h	0.042	0.040	0.034	0.036
24 h	0.020	0.024	0.022	0.025
30 h	0.016	0.019	0.017	0.019
48 h	0.012	0.017	0.013	0.012

Table 6.1.1-31: A comparison of pharmacokinetic parameters in blood following oral administration of [Phenyl-U-14C]-SYN545974

	Pharmacokinetic parameters			
	Group 1		Group 3	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male	Female	Male	Female
C _{max} (µg equiv/g)	0.63	0.72	8.1	3.8
C _{max} /D	0.130	0.147	0.0288	0.0396
t _{max} (hours) ¹	2	1	8	8
t _{1/2} (hours)	116*	82.1*	163*	160*
AUC(0-t) (µg equiv.h/g)	7.19	8.16	218	89.7
AUC(0-t)/D	1.48	1.66	0.778	0.936
AUC(0-inf) (µg equiv.h/g)	12.2*	11.7*	488*	165*
AUC(0-inf)/D	2.51*	2.38*	1.74*	1.72*
AUC % Extrapolation	41.1*	30.3*	55.3*	45.6*
F(%) ²	55.0	55.0	27.9	33.7

* = 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.

¹ = Median reported for t_{max}

² = F% was calculated using AUC(0-48h) for Groups 1 to 4.

Table 6.1.1-32: A comparison of pharmacokinetic parameters in plasma following oral administration of [Phenyl-U-14C]-SYN545974

	Pharmacokinetic parameters			
	Group 1		Group 3	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male	Female	Male	Female
C _{max} (µg equiv/mL)	1.13	1.17	13.0	5.8
C _{max} /D	0.233	0.239	0.0465	0.0607
t _{max} (hours) ¹	2	1	8	8
t _{1/2} (hours)	56.6	149*	85.3*	42.2
AUC(0-t) (µg equiv.h/mL)	8.75	7.89	316	113
AUC(0-t)/D	1.80	1.61	1.13	1.18
AUC(0-inf) (µg equiv.h/mL)	9.77	10.0*	433*	121
AUC(0-inf)/D	2.01	2.04*	1.55*	126
AUC % Extrapolation	10.5	21.4*	27.0*	6.29

* = 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.

¹ = Median reported for t_{max}

Table 6.1.1-33: A comparison of pharmacokinetic parameters in blood following oral administration of [Pyrazole-5-14C]-SYN545974

	Pharmacokinetic parameters			
	Group 2		Group 4	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male	Female	Male	Female
C _{max} (µg equiv/g)	0.27	0.45	4.7	2.1

C _{max} /D	0.0531	0.0874	0.0171	0.0246
t _{max} (hours) ¹	2	0.5	8	8
t _{1/2} (hours)	75.3*	68.5*	196*	NC
AUC(0-t) (µg equiv.h/g)	5.60	5.86	167	54.0
AUC(0-t)/D	1.10	1.15	0.605	0.632
AUC(0-inf) (µg equiv.h/g)	8.05*	7.84*	358*	NC
AUC(0-inf)/D	1.58*	1.53*	1.30*	NC
AUC % Extrapolation	30.4*	25.2*	53.3*	NC
F(%) ²	51.8	48.3	27.0	26.4

* = 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.

¹ = Median reported for t_{max}

² = F% was calculated using AUC(0-48h) for Groups 1 to 4.

NC = Not calculable, terminal elimination phase was not appropriately characterised.

Table 6.1.1-34: A comparison of pharmacokinetic parameters in plasma following oral administration of [Pyrazole-5-14C]-SYN545974

	Pharmacokinetic parameters			
	Group 2		Group 4	
	5 mg/kg		300 mg/kg	100 mg/kg
	Male	Female	Male	Female
C _{max} (µg equiv/mL)	0.49	0.67	7.1	3.1
C _{max} /D	0.0969	0.131	0.0259	0.0365
t _{max} (hours) ¹	2	0.5	8	2
t _{1/2} (hours)	56.6*	30.4*	18.6*	10.6
AUC(0-t) (µg equiv.h/mL)	6.43	5.37	195	55.9
AUC(0-t)/D	1.26	1.05	0.705	0.653
AUC(0-inf) (µg equiv.h/mL)	7.45*	5.81*	197*	56.2
AUC(0-inf)/D	1.47*	1.13*	0.712*	0.658
AUC % Extrapolation	13.7*	7.56*	1.02*	0.678

* = 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.

¹ = Median reported for t_{max}

Table 6.1.1-35: A comparison of pharmacokinetic parameters in blood following intravenous administration of [14C]-SYN545974

	Pharmacokinetic parameters			
	Group 5 (phenyl)		Group 6 (pyrazole)	
	1 mg/kg		1 mg/kg	
	Male	Female	Male	Female
C ₀ (µg equiv/g)	0.463	0.366	0.439	0.341
t _{1/2} (hours)	39.4*	182*	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-inf) (µg equiv.h/g)	2.60*	5.96*	1.93	1.91
AUC(0-inf)/D	2.84*	6.90*	2.10	2.24
AUC % Extrapolation	26.8*	50.7*	18.9	15.8
MRT(0-inf) (h)	37.3*	235*	38.7	32.7
CL (mL/h/kg)	355*	216*	475	447
V _d (mL/kg)	19950*	27300*	17300	13400

* = 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.

CONCLUSION:

Single oral doses of 5, 100 (females only) and 300 (males only) mg/kg bw and a single iv dose of 1 mg/kg bw [phenyl-U-¹⁴C] or [pyrazole-5-¹⁴C]-pydiflumetofen were administered to groups of 4 male and 4 female rats. Blood samples were obtained over a 4 (oral) or 2 (iv) day period to determine the pharmacokinetics of total radioactivity in blood (following oral and iv administration) and plasma (following oral administration). Oral bioavailability (F) was determined by comparing the dose normalised exposures following oral and iv administration of [¹⁴C]-pydiflumetofen. The nature and identity of metabolites present in the plasma samples from the oral dose groups was investigated and reported separately, under [REDACTED] and [REDACTED], 2015.

After a single oral dose of pydiflumetofen radiolabelled with either [Phenyl-U-¹⁴C] or [Pyrazole-5-¹⁴C], oral absorption into the blood/plasma was rapid. At a dose of 5 mg/kg bw, peak concentration was reached after 1-2 hours post-exposure in females and males respectively. At higher doses (300 mg/kg bw in males, 100 mg/kg bw in females) peak concentration was reached after 8 hours in both sexes. From 9 hours, blood/plasma concentrations steadily fell before plateauing at later timepoints.

After oral administration, in the earlier timepoints (5 mg/kg bw: 0.25-12 h, 100 mg/kg bw: 0.25-30 h and 300 mg/kg bw: 0.25-24 h), plasma concentrations of total radioactivity were approximately twice those in blood at the majority of timepoints, with blood to plasma ratios of *ca* 0.6, suggesting that total radioactivity remained predominantly in plasma rather than the cellular component of whole blood. The blood to plasma ratio appeared to increase at later timepoints, becoming either evenly distributed or greater in the cellular fraction. After iv administration of 1 mg/kg bw radiolabeled pydiflumetofen, mean concentration in whole blood steadily decreased from 0.25 up to 96 hours. No notable differences were observed between the sexes or radiolabels.

Overall, systemic exposure to [phenyl-U-¹⁴C] and [pyrazole-5-¹⁴C]-pydiflumetofen (based on C_{max} and AUC(0-t) estimates) increased in a sub-proportional manner between the low and high dose levels in whole blood and plasma for both males and females.

Oral bioavailability (F) in blood ranged between 52-55% in males and 48-55% in females at the low dose of 5 mg/kg bw irrespective of radiolabel position. In the higher dose groups, oral bioavailability (F) was 27-28% in males and 26-34% in females in consistency with the observed sub-proportional increase in systemic exposure. These findings are consistent with those of the preliminary study ([REDACTED] and [REDACTED], 2015).

Overall under the conditions of this GLP and OECD TG compliant pharmacokinetic study in rats, at the low oral dose of 5 mg/kg bw, **oral bioavailability** (F) in blood ranged from **48-55%**, for males and females irrespective of radiolabel position. However, after the 100 or 300 mg/kg bw oral dose, bioavailability was decreased with F estimates of between 26-34%, for males and females, respectively. These data indicate that the sub-proportional increase in exposure was limited by absorption at higher doses.

([REDACTED] and [REDACTED], 2015)

Report:	K-CA 5.1.1/05 [REDACTED] (2015). SYN545974 - Biotransformation of [¹⁴ C]-SYN545974 in Rat, [REDACTED] Report No. 34216, issue date 31 July 2015. Unpublished. Syngenta File No. SYN545974-10259.
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Guidelines: Metabolism – rat; OECD 417 (2010); OPPTS 870.7485; 67/548/EEC B.36, Regulation (EC) No 1107/2009, (EU) No 283/2013, 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

The toxicokinetic fate of SYN545974 in rats following administration of a single or multiple oral doses was investigated in previous studies (██████████, (2015) (Syngenta File No. SYN545974_10248) and ██████████ and ██████████ (2015) (Syngenta File No. SYN545974_10250) samples from which were used to investigate the biotransformation of SYN545974 in rats.

The nature and identity of metabolites present in samples of urine, plasma, feces, bile and cage wash obtained from male and female rats following oral administration of [phenyl-U-14C]-SYN545974 and [pyrazole-5-14C]-SYN545974 were investigated. Metabolism of [phenyl-U-14C]-SYN545974 and [pyrazole-5-14C]-SYN545974 was compared following single oral administration at either 5 mg/kg (male and female rats) or 300 mg/kg (male rats) and 100 mg/kg (female rats).

Metabolites were identified using a combination of comparative chromatography (combined liquid chromatography and mass spectrometry (LCMSⁿ)) with standard reference compounds, accurate mass measurement and fragmentation.

Following oral administration of SYN545974, the molecule was extensively metabolised with the majority of the dose excreted in feces via biliary elimination, with urine as a minor route. In total, up to 84.8% of the administered dose was identified, with all individual metabolites accounting for greater than 4.8% dose identified. Across all individual samples up to 35 unknown metabolites were observed, with no individual component accounting for >4.8% dose.

In the excreta from the 5 mg/kg oral dose group, unchanged parent accounted for less than 3.9% of the dose in excreta. However, at the higher doses (100 mg/kg or 300 mg/kg), unchanged SYN545974 was the major component recovered at up to 48.2% of the administered dose. In the feces of the bile duct cannulated rats, very little of the total dose was recovered compared to non-cannulated animals from the 5 mg/kg dose group with the majority of the dose recovered in bile. Whereas, at the higher doses significantly more of the administered dose was recovered in feces, with the majority excreted as unchanged parent. This data shows that as the dose increased the fraction of dose absorbed decreased.

The high % of dose absorbed at 5 mg/kg and the relatively low levels of unchanged parent circulating also suggest that SYN545974 undergoes extensive first pass metabolism.

Following absorption, only two metabolites (2,4,6 TCP sulphate and SYN548263) individually accounted for >10% of the administered dose. Numerous other metabolites were detected as cleavage products and also those that retained both the phenyl and pyrazole ring moieties. The intact metabolites detected were qualitatively and quantitatively similar between the two labelled dose forms. The cleavage of the molecule occurred following hydroxylation of SYN545974 on the carbon adjacent to the trichlorophenyl ring to give SYN547948. This then cleaved to yield the 2,4,6 trichlorophenol (2,4,6-TCP) and SYN548263. 2,4,6-TCP was then sulphated, which accounted for the largest % of dose excreted at up to 14.9% of the administered dose. Other metabolites that retained the phenyl ring were hydroxyl 2,4,6-TCP (HTCP) sulphate and 2,4,6-TCP methanol (TCPM) glucuronide. SYN548263 was further metabolised by demethoxylation to SYN548264 and by N-demethylation. Amide hydrolysis of the pyrazole half molecules gave the pyrazole amide, SYN508272. Reduction of SYN548263 yielded the alcohol SYN548265. SYN548265 was further metabolised via demethylation and glucuronidation.

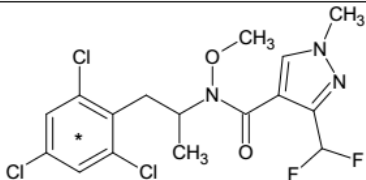
Numerous metabolites were observed that retained both the phenyl and pyrazole moieties. These primary metabolic routes for these metabolites included demethoxylation to SYN545547, N-dealkylation to SYN547891, single and di-hydroxylation, O-demethylation to SYN547890, oxidative dechlorination to SYN547894 and reductive dechlorination to SYN547893. The majority of these metabolites were also mono and di-hydroxylated and in many cases conjugated with glucuronide or in the case of SYN547894, glutathione.

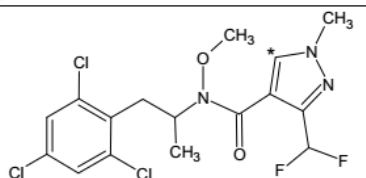
Following a single oral administration of SYN545974, the majority of the absorbed dose underwent extensive first pass metabolism and was excreted in feces via biliary elimination with urine as a minor route. Based on the amount of unchanged parent in bile and feces, absorption was complete in the 5 mg/kg dose group, however in the higher dose group animals (100 and 300 mg/kg) up to 63% of the dose was unabsorbed. In general, the major metabolites present were qualitatively and quantitatively similar irrespective of dose and sex. Numerous metabolites were detected as cleavage products and also those that retained both the phenyl and pyrazole ring moieties. Only two metabolites (2,4,6-TCP sulphate and SYN548263) individually accounted for >10% of the administered dose. 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 hydroxyl SYN545547.
- Hydroxylation and demethylation to hydroxy SYN547891 and other desmethyl hydroxy metabolites.
- Cleavage of SYN545974 to give the pyrazole metabolites SYN548265, SYN548263, SYN548264, desmethyl SYN548263 and SYN508272 and the phenyl metabolite 2,4,6 TCP.
- Dechlorination to SYN547893.
- Dechlorination and hydroxylation to SYN547894 and other dechlorinated hydroxy and dechlorinated dihydroxy metabolites.
- Glutathione conjugation followed by metabolism of the conjugate to give dechlorinated hydroxy thiomethyl SYN545974 and dechlorinated dihydroxy thiomethyl SYN545974.
- Glucuronic acid conjugation and some sulphate conjugation

MATERIALS AND METHODS

Materials:

[phenyl-U- ¹⁴ C]-SYN545974	
Name/Company Code:	[phenyl-U- ¹⁴ C]-SYN545974
Use:	Fungicide
IUPAC Name:	1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichloro-(U- ¹⁴ C)-phenyl) ethyl]
Molecular Formula:	C ₁₆ H ₁₆ Cl ₃ F ₂ N ₃ O ₂
Molecular Weight:	426.7 (unlabelled)
Batch:	RDR-XV-94
Radiochemical Purity	99.1%
Structure (*position of ¹⁴ C label):	

[pyrazole-5- ¹⁴ C]-SYN545974	
Name/Company Code:	[pyrazole-5- ¹⁴ C]-SYN545974
Use:	Fungicide
IUPAC Name:	3-Difluoromethyl-1-methyl-1H-[5- ¹⁴ C]pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]amide
Molecular Formula:	C ₁₆ H ₁₆ Cl ₃ F ₂ N ₃ O ₂
Molecular Weight:	426.7 (unlabelled)
Batch:	5271GAR001-4 and 5283RJP001-2
Radiochemical Purity:	99.2 and 98.8%
Structure (*position of ¹⁴ C label):	

Vehicle: The dose vehicle for dose preparations was 0.5% (w/v) aqueous carboxymethylcellulose (CMC) containing 0.5% Tween 80.

Preparation of dosing suspensions: Each dose preparation was formulated as a homogenous suspension of 0.5% aqueous CMC containing 0.5% Tween 80. Each dose preparation was prepared using [14C]-SYN545974 and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity.

Test Animals:	
Species:	Rat
Strain:	Han Wistar
Source:	

Full details of the test animals were reported in separate studies [REDACTED], (2015a) (*Syngenta File No. SYN545974_10248*) and [REDACTED], (2015b) (*Syngenta File No. SYN545974_10250*).

Study Design and Methods:

Study dates: Start: 07 February 2013; End: 31 July 2015

Origin of Samples: Representative samples of excreta and bile collected following single oral dosing and selected plasma samples collected from the pharmacokinetic study were pooled for metabolite isolation and quantification as noted in the table below:

Origin of samples for metabolite characterisation

Study	Test Group	Position of radiolabel	Dose level (mg/kg)
SYN545974_10248	Absorption and Excretion study	phenyl	5
	Absorption and Excretion study	pyrazole	5
	Absorption and Excretion study	phenyl	300
	Absorption and Excretion study	phenyl	100
	Absorption and Excretion study	pyrazole	300
	Absorption and Excretion study	pyrazole	100
SYN545974_10250	Pharmacokinetic study	phenyl	5
	Pharmacokinetic study	pyrazole	5
	Pharmacokinetic study	phenyl	300
	Pharmacokinetic study	phenyl	100
	Pharmacokinetic study	pyrazole	300
	Pharmacokinetic study	pyrazole	100

Dosing and sample collection: In the above studies, a single oral dose of [phenyl-U-14C]-SYN545974 or [pyrazole-5-14C]-SYN545974 formulated as a homogenous suspension in 0.5% aqueous CMC containing 0.5% Tween 80 was administered to each rat by gavage in a dose volume of 5-5.5 mL/kg or 5.5 mL/kg to give a dose of 5, 100 or 300 mg/kg, respectively. Urine, feces, and bile as appropriate were collected separately. Blood was collected as appropriate and centrifuged to separate plasma. Urine, bile feces and plasma were frozen immediately upon collection.

All excreta and plasma samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Further details of dosing and sample collections were reported in separate studies [REDACTED], (2015a) (*Syngenta File No. SYN545974_10248*) and [REDACTED], (2015b) (*Syngenta File No. SYN545974_10250*).

Metabolite characterisation studies: Representative samples of urine, feces and bile from each group were pooled for metabolite identification and quantification.

For the absorption and excretion experiments, urine collected over 0-72 h and 0-48 h for intact and bile-

duct cannulated rats, feces collected over 0-96 h and 0-48 h for intact and bile-duct cannulated rats and bile collected over 0-48 h and 0-24 h for the low dose (5 mg/kg) and high dose (100 or 300 mg/kg), respectively, were pooled for each group. Two exceptions to this were for bile duct cannulated male rats administered phenyl 300 mg/kg where the urine and feces collected over 0-72 hours was pooled. The representative pooled samples account for >95% of the recovered dose. For plasma, timepoint pools were prepared by combining an equivalent volume of plasma from each animal at the same timepoint for each dose and sex. Timepoint pools were combined to generate pools representative of area under the curve (AUC) for metabolite profiling by combining a calculated volume of each sample at selected timepoints to generate a single sample for AUC determination.

Urine and bile samples were centrifuged to remove particulates and analysed directly by HPLC-MSⁿ. Feces samples were extracted with acetonitrile. The non bile duct cannulated feces pools of the 5 mg/kg dose rate were further extracted with acetonitrile:water 3:1 v/v followed by acetonitrile:water 1:1 v/v.

These extracts were then combined with the initial acetonitrile extract. The acetonitrile and aqueous acetonitrile extracts were partitioned against 3 equal volumes of hexane to clean the extract by removing fatty material. The radioactive content in the hexane fraction obtained from the 5 mg/kg dose group (with the exception of the bile duct cannulated for the phenyl label) was negligible and no further work was conducted on these fractions. All other hexane fractions were partitioned twice against an equal volume of acetonitrile. The radioactive content in the partitioned hexane fraction was negligible and further work was carried out on this fraction. The combined aqueous fraction was reduced to incipient dryness under a gentle stream of nitrogen and reconstituted in a suitable volume of acetonitrile:water (*ca* 1:9 to 1:4 v/v) to facilitate LC-MSⁿ analysis. Plasma samples were extracted with acetonitrile and the extract was reduced to incipient dryness and reconstituted in a suitable volume of acetonitrile:water (1:9 v/v) to facilitate LC-MSⁿ analysis. Cage wash samples were centrifuged to remove particulates. The supernatants reduced to incipient dryness under a gentle stream of nitrogen and reconstituted in a suitable volume of acetonitrile:water (1:9 v/v) to facilitate LC-MSⁿ analysis.

Metabolites were identified by radio-HPLC-MS using a combination of comparative chromatography with authentic reference standards, accurate mass measurement and MSⁿ fragmentation. Metabolites were quantified by radiochemical detection.

Statistics: Not applicable.

RESULTS

Metabolite characterization studies: Representative pooled samples of urine, feces, bile, cage wash and plasma were analysed to determine the metabolite profile. The metabolites of SYN545974 identified in the rat are shown in Table 6.1.1-36.

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat

Metabolite Name	Structure
SYN545974	
SYN547890	
SYN545547	
Hydroxy SYN545547	
Hydroxylated Metabolites	<p>SYN547897</p> <p>SYN547948</p> <p>Hydroxy SYN545974</p>

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat (continued)

Metabolite Name	Structure
Dihydroxylated Metabolites	<p>Dihydroxy SYN545974</p>
N-Desmethyl SYN547890	
Hydroxy and Desmethyl Metabolites	<p>Hydroxy SYN547891</p> <p>Desmethyl hydroxy SYN545974</p> <p>Representative structure shown, a number of isomers were detected. The exact positions of demethylation and hydroxylation were not determined</p>
Glucuronide Conjugates	<p>Hydroxy SYN545974 glucuronide</p> <p>Dihydroxy SYN545974 glucuronide</p> <p>Desmethyl SYN545974 glucuronide</p> <p>Representative structure shown, the positions of demethylation were not determined. The removal of the methoxy group and addition of a hydroxy in an alternative position is also possible.</p>

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat (continued)

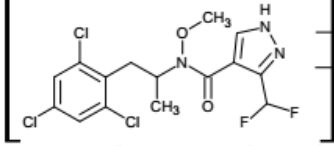
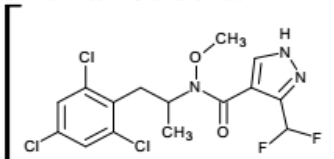
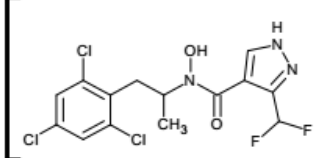
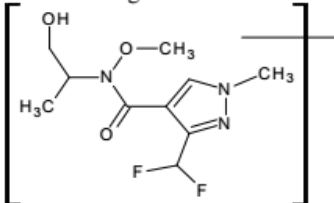
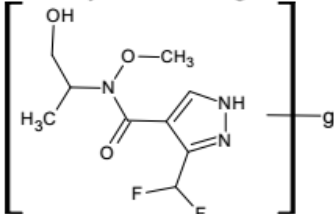
Metabolite Name	Structure
Glucuronide Conjugates (continued)	<p>Desmethyl hydroxy SYN545974 glucuronide</p>  <p>Representative structure shown, the positions of demethylation, hydroxylation and conjugation were not determined</p> <p>SYN547891 Glucuronide</p>  <p>Didesmethyl SYN545974 glucuronide</p>  <p>Representative structure shown, the positions of demethylation were not determined. The removal of the methoxy group and addition of a hydroxy in an alternative position is also possible.</p> <p>SYN548265 glucuronide</p>  <p>Desmethyl SYN548265 glucuronide</p>  <p>Other isomers of Desmethyl SYN548265 are possible however the position of demethylation was not determined.</p>

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat (continued)

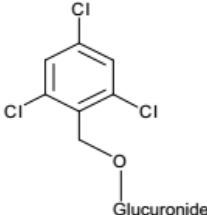
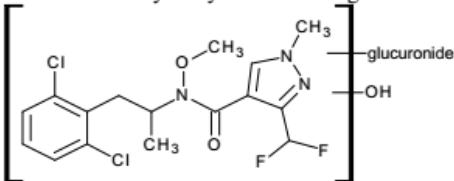
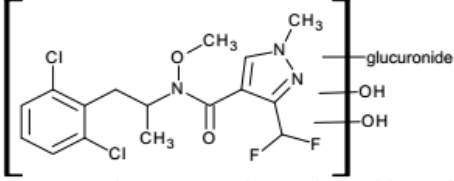
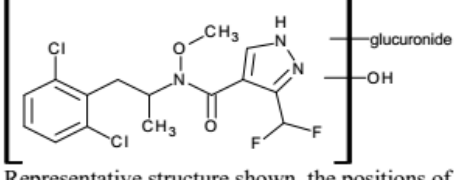
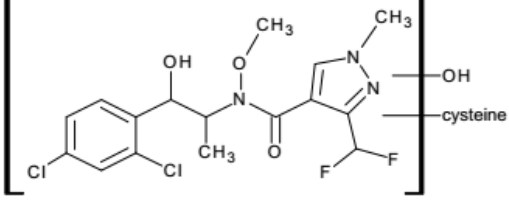
Metabolite Name	Structure
Glucuronide Conjugates (continued)	<p>TCPM glucuronide</p>  <p>Dechlorinated hydroxy SYN545974 glucuronide</p>  <p>Representative structure shown, the positions of dechlorination and hydroxylation and conjugation were not determined.</p> <p>Dechlorinated dihydroxy SYN545974 glucuronide</p>  <p>Representative structure shown, the positions of dechlorination, hydroxylation and conjugation were not determined.</p> <p>Dechlorinated hydroxy desmethyl SYN545974 glucuronide</p>  <p>Representative structure shown, the positions of dechlorination, demethylation and hydroxylation and conjugation were not determined.</p>
Cysteine Conjugates	<p>Dechlorinated hydroxy SYN545974 cysteine</p>  <p>Representative structure shown, the positions of dechlorination, hydroxylation and conjugation were not determined.</p>

Table 6.1.1–36: Metabolites of [^{14}C]- SYN545974 found in the rat continued)

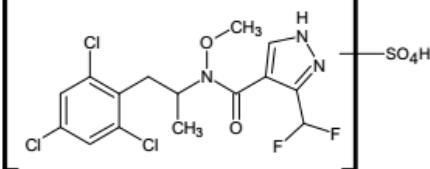
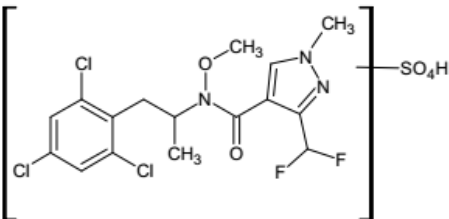
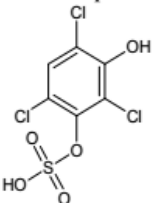
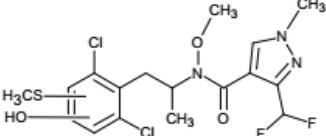
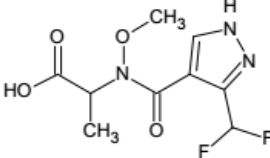
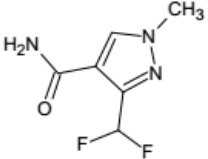
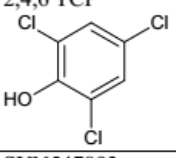
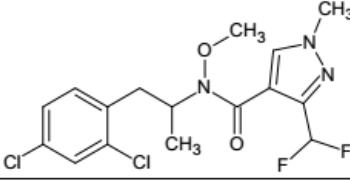
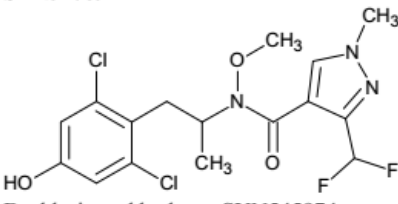
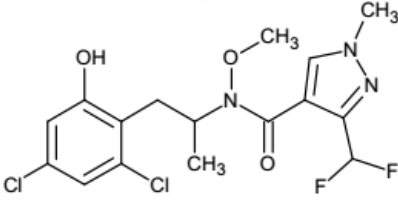
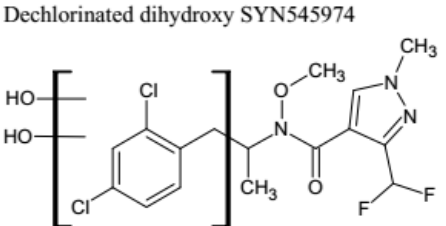
Metabolite Name	Structure
Sulphate Conjugates	<p>Desmethyl hydroxy SYN545974 sulphate</p>  <p>Representative structure shown, the positions of demethylation, hydroxylation and conjugation were not determined. Hydroxy SYN545974 sulphate.</p>  <p>2,4,6 TCP sulphate</p>  <p>HTCP sulphate</p>
Thiomethyl Conjugates	<p>Dechlorinated hydroxy thiomethyl SYN545974</p> 

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat (continued)

Metabolite Name	Structure
Thiomethyl Conjugates (continued)	<p>Dechlorinated dihydroxy thiomethyl SYN545974</p> <p>Dechlorinated hydroxy thiomethyl SYN545974 glucuronide</p> <p>Representative structure shown for the thiomethyl conjugates, the exact positions of dechlorination, hydroxylation and conjugation were not determined.</p>
Pyrazole Half Molecule Metabolites	<p>SYN548265</p> <p>SYN548263</p> <p>SYN548264</p>

Table 6.1.1–36: Metabolites of [^{14}C] - SYN545974 found in the rat (continued)

Metabolite Name	Structure
Pyrazole Half Molecule Metabolites (Continued)	<p>Desmethyl SYN548263</p>  <p>Representative structure shown, the positions of demethylation were not determined</p> <p>SYN508272</p> 
Phenyl Half Molecule Metabolites	<p>2,4,6 TCP</p> 
Dechlorinated Metabolites	<p>SYN547893</p> 
Dechlorinated and Hydroxylated Metabolites	<p>SYN547894</p>  <p>Dechlorinated hydroxy SYN545974</p>  <p>Dechlorinated dihydroxy SYN545974</p>  <p>Representative structure shown, the exact position of dechlorination and hydroxylation could not be determined</p>

The metabolite profiles in urine, feces, bile and plasma following administration of a single oral dose of [14C]-SYN545974 at doses of 5, 100 or 300 mg/kg are presented in Tables 5.1.1-37 to 5.1.1-48.

Table 6.1.1–37: Metabolite profile in excreta of rats following a single oral dose of 300 mg or 100 mg [Phenyl-¹⁴C]-SYN545974/kg

Compound	Percent of administered dose					
	Male (300 mg/kg)			Female (100 mg/kg)		
	Urine 0-72	Feces 0-96	Total excreta	Urine 0-72	Feces 0-96	Total excreta
SYN545974	0.1	44.3	44.4	0.1	31.1	31.2
HTCP sulphate	0.1	ND	0.1	0.1	ND	0.1
Desmethyl hydroxy SYN545974 sulphate	0.1	ND	0.1	ND	ND	ND
TCPM glucuronide	0.4	ND	0.4	0.3	ND	0.3
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	0.1	ND	0.1
N-desmethyl SYN547890	ND	2.7	2.7	ND	ND	ND
Dihydroxy SYN545974	<0.1	2.5	2.5	0.5	3.5	4.0
Dechlorinated dihydroxy thiomethyl SYN545974	ND	ND	ND	ND	0.8	0.8
Desmethyl hydroxy SYN545974	ND	ND	ND	ND	1.0	1.0
Desmethyl hydroxy SYN545974 sulphate	0.1	ND	0.1	0.1	ND	0.1
Desmethyl hydroxy SYN545974	ND	1.6	1.6	ND	1.1	1.1
Desmethyl hydroxy SYN545974	ND	1.3	1.3	ND	ND	ND
2,4,6-TCP sulphate	2.5	ND	2.5	4.6	ND	4.6
Desmethyl hydroxy SYN545974 glucuronide	<0.1	ND	<0.1	0.2	ND	0.2
Dechlorinated dihydroxy SYN545974	ND	0.5	0.5	ND	0.6	0.6
Hydroxy SYN545974	ND	ND	ND	ND	1.5	1.5
Dihydroxy SYN545974	ND	0.5	0.5	ND	1.1	1.1
SYN547891 glucuronide	ND	ND	ND	0.4	ND	ND
Hydroxy SYN547891	ND	1.3	1.3	ND	0.6	0.6
Hydroxy SYN545547	ND	1.0	1.0	0.1	1.1	1.2
2,4,6-TCP	3.1	ND	3.1	6.2	ND	6.2
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	0.1	ND	0.1
SYN547894	ND	0.9	0.9	ND	1.7	1.7
Dihydroxy SYN545974	ND	ND	ND	0.1	ND	0.1
Dechlorinated hydroxy SYN545974	ND	0.4	0.4	ND	ND	ND
Hydroxy SYN545974	ND	1.5	1.5	ND	1.0	1.0
SYN547897	ND	5.3	5.3	0.5	10.5	11.0
Dechlorinated hydroxy thiomethyl SYN545974	ND	1.0	1.0	ND	2.1	2.1

(Continued on following page) ND

Not detected

NA Not applicable

(A) A total of 4 unidentified components with no individual component accounting for > 0.1 %Dose

(B) A total of 11 unidentified components one of which accounted for 3.6 % dose and another 1.5 % dose with no other individual component accounting for > 0.9 %Dose

(C) A total of 21 unidentified components with no individual component accounting for > 0.1 %Dose

(D) A total of 9 unidentified components one of which accounted for 2.6 % dose and another 1.2 % dose with no other individual component accounting for > 0.5 %Dose

Table 6.1.1–37: Metabolite profile in excreta of rats following a single oral dose of 300 mg or 100 mg [Phenyl-U¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose					
	Male (300 mg/kg)			Female (100 mg/kg)		
	Urine 0-72	Feces 0-96	Total excreta	Urine 0-72	Feces 0-96	Total excreta
SYN547890	ND	1.1	1.1	ND	5.3	5.3
SYN545547	ND	ND	ND	ND	0.5	0.5
Unidentified components	0.1 (A)	10.5 (B)	10.6	1.7 (C)	6.1 (D)	7.8
Post extraction solids	NA	5.2	5.2	NA	6.3	6.3
Total identified	6.4	65.9	72.3	13.4	63.5	76.9
Total unidentified	0.1	10.5	10.6	1.7	6.1	7.8
Total accounted for	6.5	81.6	88.1	15.1	75.9	91.0
Losses/Gains	0.1	10.1	10.2	-0.2	8.3	8.1
Total	6.6	91.7	98.3	14.9	84.2	99.1
LOQ	0.1	1.1	NA	0.3	1.4	NA

ND Not detected

NA Not applicable

(A) A total of 4 unidentified components with no individual component accounting for > 0.1 %Dose

(B) A total of 11 unidentified components one of which accounted for 3.6 % dose and another 1.5 % dose with no other individual component accounting for > 0.9 %Dose

(C) A total of 21 unidentified components with no individual component accounting for > 0.1 %Dose

(D) A total of 9 unidentified components one of which accounted for 2.6 % dose and another 1.2 % dose with no other individual component accounting for > 0.5 %Dose

Table 6.1.1–38: Metabolite profile in excreta of rats following a single oral dose of 5 mg [Phenyl-¹⁴C] - SYN545974/kg

Compound	Percent of administered dose					
	Male			Female		
	Urine 0-72	Feces 0-96	Total excreta	Urine 0-72	Feces 0-96	Total excreta
SYN545974	ND	2.2	2.2	ND	3.9	3.9
TCPM glucuronide	1.7	ND	1.7	ND	ND	ND
Dihydroxy SYN545974	ND	4.4	4.4	0.2	2.0	2.2
Dechlorinated dihydroxy thiomethyl SYN545974	ND	ND	ND	ND	1.9	1.9
Desmethyl hydroxy SYN545974	ND	4.0	4.0	ND	1.5	1.5
Desmethyl hydroxy SYN545974	ND	4.1	4.1	ND	3.5	3.5
Desmethyl hydroxy SYN545974 sulphate	0.1	ND	0.1	ND	ND	ND
2,4,6-TCP sulphate	14.9	ND	14.9	7.8	ND	7.8
SYN547948	ND	2.7	2.7	ND	ND	ND
Hydroxy SYN545974	ND	ND	ND	ND	3.2	3.2
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
Hydroxy SYN545974	ND	ND	ND	ND	1.2	1.2
SYN547897	ND	5.2	5.2	0.1	8.2	8.3
Hydroxy SYN545974 sulphate	ND	ND	ND	0.2	ND	0.2
Dechlorinated hydroxy thiomethyl SYN545974	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 (A)	27.4 (B)	28.2	3.2 (C)	17.0 (D)	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

ND Not detected

NA Not applicable

(A) A total of 6 unidentified components with no individual component accounting for > 0.3 %Dose

(B) A total of 19 unidentified components one of which accounted for 3.2 % dose and another 2.7 % dose with no other individual component accounting for > 2.0 %Dose

(C) A total of 27 unidentified components with no individual component accounting for > 0.4 %Dose

(D) A total of 14 unidentified components one of which accounted for 2.7 % dose and another 2.5 % dose with no other individual component accounting for > 1.9 %Dose

Table 6.1.1–39: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 300 mg or 100 mg [Phenyl-U¹⁴C] - SYN545974/kg

Compound	Percent of administered dose									
	Male (300 mg/kg)				Female (100 mg/kg)				Female (100 mg/kg)	
	Urine	Feces	Bile	Total excreta	Urine	Feces	Bile (1)	Total excreta	Bile (2)	Total excreta
	0-72	0-72	0-48		0-48	0-48	0-48		0-48	
SYN545974	ND	63.1	0.2	63.3	ND	35.6	ND	35.6	ND	35.6
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	ND	ND	0.6	0.6
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.9 (A)	0.9	1.1	ND	2.4 (A)	3.5	2.2 (A)	3.3
HTCP sulphate	0.1	ND	ND	0.1	ND	ND	ND	ND	ND	ND
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND	ND	ND
Dechlorinated hydroxy desmethyl SYN545974 glucuronide	ND	ND	0.6	0.6	ND	ND	ND	ND	ND	ND
Dihydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	3.1	3.1
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.1	ND	ND	0.1	ND	0.1
Hydroxy SYN545974 glucuronide	ND	ND	2.7	2.7	1.5	ND	3.4	4.9	ND	1.5
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	2.2	2.2
Hydroxy SYN545974 glucuronide	0.1	ND	ND	0.1	ND	ND	ND	ND	4.3	4.3
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.2	ND	ND	0.2	ND	0.2
TCPM glucuronide	0.1	ND	ND	0.1	ND	ND	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	0.3	ND	0.9 (D)	1.2	1.4 (D)	1.7
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	0.3	0.3	ND	ND	ND	ND	1.6 (E)	1.6
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	1.7	1.7	ND	ND
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND	ND	ND

(Continued on following page)

ND Not detected (1) 053F and 055 F NA

Not applicable (2) 054F and 056 F

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) unresolved from desmethyl SYN545974 glucuronide

(E) Unresolved from dechlorinated hydroxy SYN545974 glucuronide

(F) A total of 5 unidentified components with no individual component accounting for > 0.1 % Dose

(G) A total of 7 unidentified components with no individual component accounting for > 0.5 % Dose

(H) A total of 20 unidentified components with no individual component accounting for > 0.3 % Dose

(I) A total of 12 unidentified components with no individual component accounting for > 0.9 % Dose

(J) A total of 5 unidentified components with no individual component accounting for > 0.7 % Dose

(K) A total of 9 unidentified components one of which accounted for 1.6% with no other individual component accounting for > 1.0% Dose

(L) A total of 12 unidentified components with no individual component accounting for > 1.0 % Dose

Table 6.1.1–39: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 300 mg or 100 mg [Phenyl-U¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose									
	Male (300 mg/kg)				Female (100 mg/kg)				Female (100 mg/kg)	
	Urine	Feces	Bile	Total excreta	Urine	Feces	Bile (1)	Total excreta	Bile (2)	Total excreta
	0-72	0-72	0-48		0-48	0-48	0-48		0-48	
Dihydroxy SYN545974	ND	ND	ND	ND	0.3	ND	ND	0.3	ND	0.3
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	<0.1	<0.1	0.3	0.3
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	0.2	0.2	ND	ND	0.4	0.4	0.6	0.6
Hydroxy SYN545974 glucuronide	ND	ND	2.2	2.2	0.1	ND	4.7	4.8	6.2	6.3
Desmethyl hydroxy SYN545974 glucuronide	0.1	ND	ND	0.1	ND	ND	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	0.2	ND	1.3	1.5	1.2	1.4
2,4,6-TCP sulphate	2.6	ND	ND	2.6	2.9	ND	ND	2.9	ND	2.9
Hydroxy SYN545974 glucuronide	ND	ND	0.2	0.2	ND	ND	0.2	0.2	ND	ND
Dechlorinated hydroxy thiomethyl SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND	ND	ND
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	0.4 (B)	0.4	ND	ND	0.4 (B)	0.4	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	0.6	0.6
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.5 (C)	0.5	ND	ND	0.8 (C)	0.8	0.7 (C)	0.7
Didesmethyl SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND	ND	ND
Didesmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	0.7	0.7
Desmethyl SYN545974 glucuronide	ND	ND	0.9	0.9	0.4	ND	1.5	1.9	1.9	2.3
SYN547891 glucuronide	0.1	ND	1.9	2.0	1.1	ND	4.8	5.9	7.2	8.3
Hydroxy SYN545547	ND	ND	ND	ND	0.2	ND	ND	0.2	ND	0.2

(Continued on following page)

ND Not detected (1) 053F and 055 F

NA Not applicable (2) 054F and 056 F

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) unresolved from desmethyl SYN545974 glucuronide

(E) Unresolved from dechlorinated hydroxy SYN545974 glucuronide

(F) A total of 5 unidentified components with no individual component accounting for > 0.1 % Dose

(G) A total of 7 unidentified components with no individual component accounting for > 0.5 % Dose

(H) A total of 20 unidentified components with no individual component accounting for > 0.3 % Dose

(I) A total of 12 unidentified components with no individual component accounting for > 0.9 % Dose

(J) A total of 5 unidentified components with no individual component accounting for > 0.7 % Dose

(K) A total of 9 unidentified components one of which accounted for 1.6% with no other individual component accounting for > 1.0% Dose

(L) A total of 12 unidentified components with no individual component accounting for > 1.0 % Dose

Table 6.1.1–39: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 300 mg or 100 mg [Phenyl-U¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose									
	Male (300 mg/kg)				Female (100 mg/kg)				Female (100 mg/kg)	
	Urine	Feces	Bile	Total excreta	Urine	Feces	Bile ⁽¹⁾	Total excreta	Bile ⁽²⁾	Total excreta
	0-72	0-72	0-48		0-48	0-48	0-48		0-48	
2,4,6-TCP	0.9	ND	ND	0.9	3.1	ND	ND	3.1	ND	3.1
SYN547894	ND	ND	ND	ND	0.1	ND	ND	0.1	ND	0.1
Desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.3	0.3	ND	ND
Desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	ND	ND	0.3	0.3
Hydroxy SYN545974 sulphate	ND	ND	ND	ND	0.2	ND	ND	0.2	ND	0.2
SYN547897	0.1	ND	ND	0.1	0.9	ND	ND	0.9	ND	0.9
SYN547890	ND	ND	ND	ND	0.1	0.9	ND	1.0	ND	1.0
SYN545547	ND	0.7	ND	0.7	ND	0.4	ND	0.4	ND	0.4
SYN547893	ND	0.3	ND	0.3	ND	ND	ND	ND	ND	ND
Unidentified components	0.1 (F)	2.1 (G)	3.8 (H)	6.0	2.3 (I)	1.8 (J)	4.6 (K)	8.7	4.1 (L)	8.2
Post extraction solids	NA	0.6	NA	0.6	NA	1.2	NA	1.2	NA	1.2
Total identified	4.1	64.1	13.9	82.1	12.8	36.9	22.8	72.5	35.1	84.8
Total unidentified	0.1	2.1	3.8	6.0	2.3	1.8	4.6	8.7	4.1	8.2
Total accounted for	4.2	66.8	17.7	88.7	15.1	39.9	27.4	82.4	39.2	94.2
Losses/Gains	0.2	7.5	-0.1	7.6	0.0	3.3	0.0	3.3	0.0	3.3
Total	4.4	74.3	17.6	96.3	15.1	43.2	27.4	85.7	39.2	97.5
LOQ	0.1	0.1	0.9	NA	0.3	0.1	0.7	NA	0.6	NA

ND Not detected (1) 053F and 055 F

NA Not applicable (2) 054F and 056 F

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) unresolved from desmethyl SYN545974 glucuronide

(E) Unresolved from dechlorinated hydroxy SYN545974 glucuronide

(F) A total of 5 unidentified components with no individual component accounting for > 0.1 % Dose

(G) A total of 7 unidentified components with no individual component accounting for > 0.5 % Dose

(H) A total of 20 unidentified components with no individual component accounting for > 0.3 % Dose

(I) A total of 12 unidentified components with no individual component accounting for > 0.9 % Dose

(J) A total of 5 unidentified components with no individual component accounting for > 0.7 % Dose

(K) A total of 9 unidentified components one of which accounted for 1.6% with no other individual component accounting for > 1.0% Dose

(L) A total of 12 unidentified components with no individual component accounting for > 1.0 % Dose

Table 6.1.1–40: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 5 mg [Phenyl-U14C]-SYN545974/kg

Compound	Percent of administered dose							
	Male (5 mg/kg)				Female (5 mg/kg)			
	Urine	Feces	Bile	Total excreta	Urine	Feces	Bile	Total excreta
	0-72	0-48	0-24		0-72	0-48	0-48	
SYN545974	ND	7.3	ND	7.3	ND	5.9	ND	5.9
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.9	ND	ND	0.9
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	4.6	4.6	ND	ND	3.0 (A)	3.0
HTCP sulphate	<0.1	ND	ND	<0.1	ND	ND	ND	ND
Dechlorinated hydroxy desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	2.5	2.5
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	1.0	1.0	ND	ND	ND	ND
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	4.4	4.4	ND	ND	ND	ND
Dechlorinated hydroxy desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	7.4	7.4
Hydroxy SYN545974 glucuronide	ND	ND	9.0(B)	9.0	2.0	ND	6.8	8.8
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.2	ND	ND	0.2
TCPM glucuronide	0.5	ND	ND	0.5	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.4	ND	ND	0.4
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	3.6	3.6	ND	ND	8.6	8.6
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	1.0	1.0	ND	ND	ND	ND
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	1.2	1.2
Hydroxy SYN545974 glucuronide	ND	ND	10.8	10.8	1.1	ND	3.9	5.0
Hydroxy SYN545974 glucuronide	ND	ND	ND	ND	0.4	ND	ND	0.4

(Continued on following page)

ND Not detected

NA Not applicable

(A) Unresolved from dihydroxy glucuronide SYN545974

(B) Unresolved from desmethyl hydroxy glucuronide

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) A total of 14 unidentified components with no individual component accounting for > 0.1 % Dose

(E) One unidentified component detected

(F) A total of 25 unidentified components one of which accounted for 3.6% Dose and another 2.0% Dose with no other individual component accounting for > 1.1% Dose

(G) A total of 26 unidentified components one of which accounted for 2.8% Dose and another 1.7% Dose with no other individual component accounting for > 1.0% Dose

(H) A total of 5 unidentified components with no individual component accounting for > 0.2 % Dose 1

(I) A total of 20 unidentified components one of which accounted for 3.5% Dose and another 2.4% Dose with no other individual component accounting for > 1.8% Dose

Table 6.1.1-40: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 5 mg [Phenyl-U14C]-SYN545974/kg (continued)

Compound	Percent of administered dose							
	Male (5 mg/kg)				Female (5 mg/kg)			
	Urine	Feces	Bile	Total excreta	Urine	Feces	Bile	Total excreta
	0-72	0-48	0-24		0-72	0-48	0-48	
Desmethyl hydroxy SYN545974 sulphate	<0.1	ND	ND	<0.1	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	0.4	0.4	ND	ND	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.9	0.9
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	2.1	2.1	ND	ND	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	6.0 (C)	6.0
2,4,6-TCP sulphate	5.6	ND	ND	5.6	1.0	ND	ND	1.0
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	3.0	3.0	ND	ND	ND	ND
Desmethyl SYN545974 glucuronide	ND	ND	1.5	1.5	1.0	ND	6.3	7.3
Desmethyl hydroxy SYN545974 sulphate	<0.1	ND	ND	<0.1	ND	ND	ND	ND
SYN547891 glucuronide	ND	ND	4.6	4.6	3.1	ND	9.7	12.8
2,4,6-TCP	5.8	ND	ND	5.8	0.7	ND	ND	0.7
Hydroxy SYN545974 sulphate	ND	ND	ND	ND	0.2	ND	ND	0.2
SYN547897	ND	ND	ND	ND	0.5	ND	ND	0.5
SYN547890	ND	0.7	ND	0.7	ND	1.2	ND	1.2
Unidentified components	0.4 (D)	4.0 (E)	19.5 (F)	23.9	10.0 (G)	0.7 (H)	24.8 (I)	35.5
Post extraction solids	NA	1.2	NA	1.2	NA	1.6	NA	1.6
Total identified	11.9	8.0	46.4	66.3	11.5	7.1	56.3	74.9
Total unidentified	0.4	4.0	19.5	23.9	10.0	0.7	24.8	35.5
Total accounted for	12.3	13.2	65.9	91.4	21.5	9.4	81.1	112.0
Losses/Gains	0.3	1.8	0.2	2.3	0.1	0.5	0.1	0.7
Total	12.6	15.0	66.1	93.7	21.6	9.9	81.2	112.7
LOQ	0.1	0.5	2.3	NA	0.2	0.3	5.3	NA

ND Not detected NA Not applicable

(A) Unresolved from dihydroxy glucuronide SYN545974

(B) Unresolved from desmethyl hydroxy glucuronide

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) A total of 14 unidentified components with no individual component accounting for > 0.1 % Dose

(E) One unidentified component detected

(F) A total of 25 unidentified components one of which accounted for 3.6% Dose and another 2.0% Dose with no other individual component accounting for > 1.1% Dose

(G) A total of 26 unidentified components one of which accounted for 2.8% Dose and another 1.7% Dose with no other individual component accounting for > 1.0% Dose

(H) A total of 5 unidentified components with no individual component accounting for > 0.2 % Dose 1

(I) A total of 20 unidentified components one of which accounted for 3.5% Dose and another 2.4% Dose with no other individual component accounting for > 1.8% Dose

Table 6.1.1–41: Metabolite profile in excreta of rats following a single oral dose of 100 mg or 300 mg [Pyrazole-5-¹⁴C]-SYN545974/kg

Compound	Percent of administered dose					
	Male (300 mg/kg)			Female (100 mg/kg)		
	Urine	Feces	Total excreta	Urine	Feces	Total excreta
	0-72	0-96		0-72	0-96	
SYN545974	ND	48.2	48.2	ND	31.2	31.2
SYN548264	0.5	ND	0.5	0.5	ND	0.5
Desmethyl SYN548265 glucuronide	0.1	ND	0.1	0.2	ND	0.2
Desmethyl SYN548263	0.3	ND	0.3	0.5	ND	0.5
SYN548265 glucuronide	0.4	ND	0.4	0.8	ND	0.8
SYN548263	2.1	5.8	7.9	2.9	1.4	4.3
SYN548265	ND	ND	ND	0.1	ND	0.1
Desmethyl hydroxy SYN545974	ND	0.6	0.6	ND	0.9	0.9
Hydroxy SYN545974 glucuronide	ND	ND	ND	0.1	ND	0.1
N-Desmethyl SYN547890	ND	0.8	0.8	ND	ND	ND
Dihydroxy SYN545974	ND	2.3	2.3	0.1	2.1	2.2
Dechlorinated dihydroxy thiomethyl SYN545974	ND	ND	ND	ND	0.4	0.4
Desmethyl hydroxy SYN545974	ND	0.5	0.5	ND	0.6	0.6
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	0.1	ND	0.1
Desmethyl hydroxy SYN545974	ND	1.2	1.2	ND	1.6	1.6
Dechlorinated dihydroxy SYN545974	ND	0.6	0.6	ND	0.6	0.6
Hydroxy SYN545974	ND	1.1	1.1	ND	0.8	0.8
Dihydroxy SYN545974	ND	ND	ND	ND	0.9	0.9
SYN547891 glucuronide	ND	ND	ND	0.2	ND	0.2
Hydroxy SYN547891	ND	1.1	1.1	ND	0.6	0.6
Hydroxy SYN545547	ND	ND	ND	0.1	ND	ND
Hydroxy SYN545547	ND	0.7	0.7	ND	0.9	0.9
Dechlorinated dihydroxy SYN545974	ND	ND	ND	ND	0.4	0.4
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	<0.1	ND	<0.1
SYN547894	ND	ND	ND	ND	1.4	1.4
Dihydroxy SYN545974	ND	1.1	1.1	ND	ND	ND
SYN547897	ND	3.9	3.9	0.2	7.5	7.7
Dechlorinated hydroxy thiomethyl SYN545974	ND	1.0	1.0	ND	2.5	2.5
SYN547890	ND	2.5	2.5	<0.1	4.3	4.3
SYN545547	ND	0.3	0.3	ND	0.6	0.6

(Continued on following page)

ND Not detected

NA Not applicable

(A) A total of 15 unidentified components with no individual component accounting for > 0.9 %Dose

(B) A total of 10 unidentified components one of which accounted for 2.0 % dose and another 1.7 % dose with no other individual component accounting for > 0.6 %Dose

(C) A total of 21 unidentified components one of which accounted for 1.8 % dose with no other individual component accounting for > 0.4 % Dose

(D) A total of 9 unidentified components one of which accounted for 2.6 % dose and another 1.9 % dose with no other individual component accounting for > 0.9 %Dose

Table 6.1.1–41: Metabolite profile in excreta of rats following a single oral dose of 100 mg or 300 mg [Pyrazole-5-¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose					
	Male (300 mg/kg)			Female (100 mg/kg)		
	Urine	Feces	Total excreta	Urine	Feces	Total excreta
	0-72	0-96		0-72	0-96	
Unidentified components	1.7 (A)	7.7 (B)	9.4	3.1 (C)	8.2 (D)	11.3
Post extraction solids	NA	5.2	5.2	NA	5.8	5.8
Total identified	3.4	71.7	75.1	5.8	58.7	64.5
Total unidentified	1.7	7.7	9.4	3.1	8.2	11.3
Total accounted for	5.1	84.6	89.7	8.9	72.7	81.6
Losses/Gains	0.2	5.9	6.1	0.2	6.8	7.0
Total	5.3	90.5	95.8	9.1	79.5	88.6
LOQ	0.1	1.2	NA	0.2	1.5	NA

ND Not detected

NA Not applicable

(A) A total of 15 unidentified components with no individual component accounting for > 0.9 %Dose

(B) A total of 10 unidentified components one of which accounted for 2.0 % dose and another 1.7 % dose with no other individual component accounting for > 0.6 %Dose

(C) A total of 21 unidentified components one of which accounted for 1.8 % dose with no other individual component accounting for > 0.4 % Dose

(D) A total of 9 unidentified components one of which accounted for 2.6 % dose and another 1.9 % dose with no other individual component accounting for > 0.9 %Dose

Table 6.1.1–42: Metabolite profile in excreta of rats following a single oral dose of 5 mg [Pyrazole-5-¹⁴C]-SYN545974/kg

Compound	Percent of administered dose					
	Male			Female		
	Urine 0-72	Feces 0-96	Total excreta	Urine 0-72	Feces 0-96	Total excreta
SYN545974	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 SYN545974	ND	6.0	6.0	ND	1.4	1.4
Dechlorinated dihydroxy thiomethyl SYN545974	ND	ND	ND	ND	1.0	1.0
Desmethyl hydroxy SYN545974	ND	2.1	2.1	ND	ND	ND
Desmethyl hydroxy SYN545974 sulphate	0.5	ND	0.5	ND	ND	ND
Desmethyl hydroxy SYN545974	ND	ND	ND	ND	2.7	2.7
Dechlorinated dihydroxy SYN545974	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 SYN545547	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 SYN545974	ND	1.4	1.4	ND	5.9	5.9
SYN547890	ND	2.5	2.5	ND	4.4	4.4
SYN545547	ND	0.7	0.7	ND	ND	ND
Unidentified components	10.3 (A)	21.7 (B)	32.0	10.0 (C)	24.8 (D)	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

ND Not detected

NA Not applicable

(A) A total of 24 unidentified components one of which accounted for 4.4 % dose and another 1.1 % dose with no other individual component accounting for > 0.5 %Dose

(B) A total of 23 unidentified components with no individual component accounting for > 1.8 %Dose

(C) A total of 35 unidentified components one of which accounted for 2.8% with no other individual component accounting for > 0.6 %Dose

(D) A total of 31 unidentified components one of which accounted for 2.3 % dose and another two accounted for 1.8% dose with no other individual component accounting for > 1.3 % Dose

Table 6.1.1–43: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 100 or 300 mg [Pyrazole-5-¹⁴C]-SYN545974/kg

Compound	Percent of administered dose							
	Male (300 mg/kg)				Female (100 mg/kg)			
	Urine	Feces	Bile	Total	Urine	Feces	Bile	Total
	0-48	0-48	0-24	excreta	0-48	0-48	0-24	excreta
SYN545974	ND	24.5	ND	24.5	ND	32.6	ND	32.6
SYN548264	0.4	ND	0.1	0.5	0.4	ND	ND	0.4
desmethyl SYN548265 glucuronide	<0.1	ND	ND	<0.1	0.1	ND	0.1	0.2
Desmethyl SYN548263	0.1	ND	ND	0.1	ND	ND	ND	ND
SYN548265 glucuronide	0.2	ND	0.3	0.5	0.7	ND	1.6	2.3
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	0.4	0.4
SYN548263	1.3	ND	0.2	1.5	3.4	1.7	0.1	5.2
SYN548265	ND	ND	ND	ND	0.1	ND	ND	0.1
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.9 (A)	0.9	ND	ND	2.3 (A)	2.3
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	ND	ND
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.2	0.2	ND	ND	0.1	0.1
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	0.2	0.2	ND	ND	ND	ND
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	3.5 (E)	3.5
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.3	0.3	ND	ND		
Hydroxy SYN545974 glucuronide	ND	ND	2.1	2.1	0.2	ND	4.6	4.8
Dihydroxy SYN545974 glucuronide	ND	ND	0.1 (B)	0.1	ND	ND	ND	ND
Desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.7 (B)	0.7
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	0.5 (C)	0.5	ND	ND	2.0 (C)	2.0
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	ND	ND

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(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from hydroxy SYN545974 glucuronide

(C) Unresolved from dechlorinated hydroxy SYN545974 glucuronide

(D) Unresolved from didesmethyl SYN545974 glucuronide

(E) Unresolved components

(F) A total of 8 unidentified components with no individual component accounting for > 0.4 % Dose

(G) A total of 10 unidentified components accounting for 1.5-4.8% Dose

(H) A total of 30 unidentified components with no individual component accounting for > 0.4 % Dose

(I) A total of 21 unidentified components with no individual component accounting for > 0.5 % Dose

(J) A total of 15 unidentified components accounting for 0.7-1.4% Dose

(K) A total of 24 unidentified components with no individual component accounting for > 1.0 % Dose

Table 6.1.1–43: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 100 or 300 mg [Pyrazole-5-¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose							
	Male (300 mg/kg)				Female (100 mg/kg)			
	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.3	0.3
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	0.1 (A)	0.1	ND	ND	0.3	0.3
Hydroxy SYN545974 glucuronide	ND	ND	2.6	2.6	0.1	ND	5.3	5.4
Hydroxy SYN545974 glucuronide	ND	ND	0.2	0.2	ND	ND	1.2	1.2
Hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.3	0.3
Dechlorinated hydroxy thiomethyl SYN545974 glucuronide	ND	ND	0.1	0.1	ND	ND	0.4	0.4
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.4	0.4
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.3 (D)	0.3	ND	ND	1.1 (D)	1.1
Didesmethyl SYN545974 glucuronide	ND	ND	0.2	0.2	ND	ND	0.7	0.7
Desmethyl SYN545974 glucuronide	ND	ND	0.5	0.5	ND	ND	2.6	2.6
SYN547891 glucuronide	ND	ND	1.0	1.0	0.2	ND	5.7	5.9
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	ND	<0.1	ND	ND	<0.1
Desmethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.8	0.8
SYN547897	ND	ND	ND	ND	<0.1	ND	ND	<0.1
Dechlorinated hydroxy thiomethyl SYN545974	ND	2.9	ND	2.9	ND	ND	ND	ND
SYN547893	ND	2.0	ND	2.0	ND	ND	ND	ND
Unidentified components	0.6 (F)	27.2 (G)	5.0 (H)	32.8	1.3 (I)	14.7 (J)	6.6 (K)	22.6
Post extraction solids	NA	0.4	NA	0.4	NA	0.7	NA	0.7
Total identified	2.0	29.4	10.3	41.7	5.2	34.3	34.5	74.0
Total unidentified	0.6	27.2	5.0	32.8	1.3	14.7	6.6	22.6
Total accounted for	2.6	57.0	15.3	74.9	6.5	49.7	41.1	97.3
Losses/Gains	-0.1	22.2	-0.2	21.9	0.5	-0.7	0.0	-0.2
Total	2.5	79.2	15.1	96.8	7.0	49.0	41.1	97.1
LOQ	0.1	0.6	0.8	NA	0.1	0.4	1.0	NA

ND Not detected

NA Not applicable

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from hydroxy SYN545974 glucuronide

(C) Unresolved from dechlorinated hydroxy SYN545974 glucuronide

(D) Unresolved from didesmethyl SYN545974 glucuronide

(E) Unresolved components

(F) A total of 8 unidentified components with no individual component accounting for > 0.4 % Dose

(G) A total of 10 unidentified components accounting for 1.5-4.8% Dose

(H) A total of 30 unidentified components with no individual component accounting for > 0.4 % Dose

(I) A total of 21 unidentified components with no individual component accounting for > 0.5 % Dose

(J) A total of 15 unidentified components accounting for 0.7-1.4%Dose

(K) A total of 24 unidentified components with no individual component accounting for > 1.0 % Dose

Table 6.1.1–44: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 5 mg [Pyrazole-5-14C]-SYN545974/kg

Compound	Percent of administered dose							
	Male (5 mg/kg)				Female (5 mg/kg)			
	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta
SYN545974	ND	7.9	ND	7.9	ND	6.1	ND	6.1
SYN548264	1.6	ND	0.9	2.5	0.7	ND	ND	0.7
Desmethyl SYN548265 glucuronide	0.2	ND	ND	0.2	0.1	ND	ND	0.1
SYN548265 glucuronide	ND	ND	3.6	3.6	ND	ND	3.1	3.1
Desmethyl SYN548263	0.9	ND	ND	0.9	0.4	ND	ND	0.4
SYN548265 glucuronide	1.1	ND	ND	1.1	0.6	ND	ND	0.6
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	1.0	1.0	ND	ND	ND	ND
SYN548263	6.5	0.4	1.7	8.6	2.4	0.8	ND	3.2
SYN548265	ND	ND	ND	ND	0.1	ND	ND	0.1
Dihydroxy SYN545974 glucuronide	ND	ND	4.0 (A)	4.0	ND	ND	3.0 (A)	3.9
Dechlorinated dihydroxy SYN545974 glucuronide	ND	ND	1.9	1.9	ND	ND	ND	ND
Dechlorinated hydroxy SYN545974 glucuronide	ND	ND	5.5	5.5	ND	ND	6.4	6.4
Hydroxy SYN545974 glucuronide	ND	ND	10.1 (B)	10.1	0.1	ND	8.5	8.6
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	3.6	3.6	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	4.6	4.6
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	0.9	0.9	ND	ND	ND	ND
Dechlorinated hydroxy SYN545974 cysteine	ND	ND	2.9 (A)	2.9	ND	ND	ND	ND
Hydroxy SYN545974 glucuronide	ND	ND	10.8	10.8	ND	ND	7.6	7.6
Hydroxy SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	0.8	0.8
Dechlorinated hydroxy thiomethyl SYN545974 glucuronide	ND	ND	ND	ND	ND	ND	1.0	1.0
Desmethyl hydroxy SYN545974 glucuronide	ND	ND	1.2 (C)	1.2	ND	ND	1.4	1.4

(Continued on following page)

ND Not detected

NA Not applicable

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) A total of 8 unidentified components with no individual component accounting for > 1.0 %Dose

(E) A total of 3 unidentified components with no individual component accounting for > 0.3 %Dose

(F) A total of 16 unidentified components with no individual component accounting for > 1.7 %Dose

(G) A total of 21 unidentified components with no individual component accounting for > 0.1 %Dose

(H) A total of 7 unidentified components with no individual component accounting for > 0.2 %Dose

(I) A total of 9 unidentified components one of which accounted for 2.9%Dose and another 2.3% with no other individual component accounting for > 1.9%Dose

Table 6.1.1–44: Metabolite profile in excreta of bile duct cannulated rats following a single oral dose of 5 mg [Pyrazole-5-¹⁴C]-SYN545974/kg (continued)

Compound	Percent of administered dose							
	Male (5 mg/kg)				Female (5 mg/kg)			
	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta	Urine 0-48	Feces 0-48	Bile 0-24	Total excreta
Didesmethyl SYN545974 glucuronide	ND	ND	1.6	1.6	ND	ND	6.4	6.4
Desmethyl SYN545974 glucuronide	ND	ND	2.1	2.1	ND	ND	4.7	4.7
SYN547891 glucuronide	ND	ND	6.0	6.0	0.3	ND	14.0	14.3
Desmethyl hydroxy SYN545974 sulphate	ND	ND	ND	ND	0.1	ND	ND	0.1
SYN547897	ND	0.2	ND	0.2	0.1	0.2	ND	0.3
Dechlorinated hydroxy thiomethyl SYN545974	ND	0.2	ND	0.2	ND	0.2	ND	0.2
SYN547890	ND	0.8	ND	0.8	ND	1.1	ND	1.1
Unidentified components	2.0 ^(D)	0.5 ^(E)	13.7 ^(F)	16.2	1.8 ^(G)	1.0 ^(H)	15.1 ^(I)	17.9
Post extraction solids	NA	1.3	NA	1.3	NA	1.8	NA	1.8
Total identified	10.3	9.5	57.8	77.6	4.9	8.4	62.4	75.7
Total unidentified	2.0	0.5	13.7	16.2	1.8	1.0	15.1	17.9
Total accounted for	12.3	11.3	71.5	95.1	6.7	11.2	77.5	95.4
Losses/Gains	0.0	1.8	0.0	1.8	0.1	2.0	0.2	2.3
Total	12.3	13.1	71.5	96.9	6.8	13.2	77.7	97.7
LOQ	0.2	0.3	2.4	NA	0.2	0.3	3.5	NA

ND Not detected

NA Not applicable

(A) Unresolved from dihydroxy SYN545974 glucuronide

(B) Unresolved from desmethyl hydroxy SYN545974 glucuronide

(C) Unresolved from didesmethyl SYN545974 glucuronide

(D) A total of 8 unidentified components with no individual component accounting for > 1.0 %Dose

(E) A total of 3 unidentified components with no individual component accounting for > 0.3 %Dose

(F) A total of 16 unidentified components with no individual component accounting for > 1.7 %Dose

(G) A total of 21 unidentified components with no individual component accounting for > 0.1 %Dose

(H) A total of 7 unidentified components with no individual component accounting for > 0.2 %Dose

(I) A total of 9 unidentified components one of which accounted for 2.9% Dose and another 2.3% with no other individual component accounting for > 1.9% Dose

Table 6.1.1-45: Metabolite profile in plasma of rats following a single oral dose of [Phenyl U-¹⁴C]-SYN545974 (Expressed as µg Equivalents of SYN545974.h/g of Plasma)

Compound	Concentration (µg equiv.h/g)			
	Male		Female	
	300 mg/kg	5 mg/kg	100 mg/kg	5 mg/kg
	0-96 h	0-96 h	0-96 h	0-96 h
SYN545974	4	0.16	6	0.22
Dihydroxy SYN545974 glucuronide	3	ND	1	0.11
HTCP sulphate	15	0.53	10	0.73
TCPM glucuronide	11	0.29	3	0.07
Dechlorinated dihydroxy SYN545974 glucuronide	3	ND	1	ND
Dechlorinated dihydroxy SYN545974 glucuronide	3	0.20	ND	0.09
Hydroxy SYN545974 glucuronide	10	0.09	1	0.13
2,4,6-TCP sulphate	140	3.60	36	3.23
SYN547948	ND	ND	4	0.20
Desmethyl hydroxy SYN545974 sulphate	6	ND	ND	ND
SYN547891 glucuronide	6	0.24	2	0.28
2,4,6-TCP	7	0.37	6	0.41
SYN547897	5	0.07	3	0.34
Unidentified components	15 (A)	0.99 (B)	10 (C)	0.87 (D)
Additional extracts	<LOQ	NA	<LOQ	NA
Post extraction solids	58	1.65	14	1.03
Total identified	213	5.55	73	5.81
Total unidentified	15	0.99	10	0.87
Total accounted for	286	8.19	97	7.71
Losses/Gains	30	0.56	16	0.18
Total	316	8.75	113	7.89
LOQ	4	0.07	1	0.07

ND Not detected NA Not applicable

(A) A total of 5 unidentified components with no individual component accounting for > 5 µg equiv.h/g

(B) A total of 8 unidentified components with no individual component accounting for > 0.22 µg equiv.h/g

(C) A total of 10 unidentified components with no individual component accounting for > 2 µg equiv.h/g

(D) A total of 11 unidentified components with no individual component accounting for > 0.16 µg equiv.h/g

Table 6.1.1–46: Metabolite profile in plasma of rats following a single oral dose of [Phenyl U-¹⁴C]-SYN545974 (expressed as percentage of total AUC)

Compound	% AUC			
	Male		Female	
	300 mg/kg	5 mg/kg	100 mg/kg	5 mg/kg
	0-96 h	0-96 h	0-96 h	0-96 h
SYN545974	1.3	1.9	5.0	2.8
Dihydroxy SYN545974 glucuronide	0.9	ND	1.0	1.4
HTCP sulphate	4.8	6.1	9.2	9.3
TCPM glucuronide	3.4	3.4	2.4	0.9
Dechlorinated dihydroxy SYN545974 glucuronide	0.8	ND	0.9	ND
Dechlorinated dihydroxy SYN545974 glucuronide	1.1	2.2	ND	1.2
Hydroxy SYN545974 glucuronide	3.1	1.0	1.0	1.6
2,4,6-TCP sulphate	44.1	41.1	32.2	41.0
SYN547948	ND	ND	3.6	2.5
Desmethyl hydroxy SYN545974 sulphate	1.8	ND	ND	ND
SYN547891 glucuronide	1.8	2.7	1.8	3.6
2,4,6-TCP	2.4	4.3	5.3	5.2
SYN547897	1.5	0.8	2.5	4.3
Unidentified components	4.7 (A)	11.3 (B)	8.3 (C)	10.9 (D)
Additional extracts	<LOQ	NA	<LOQ	NA
Post extraction solids	18.3	18.8	12.5	13.1
Total identified	67.0	63.5	64.9	73.8
Total unidentified	4.7	11.3	8.3	10.9
Total accounted for	90.0	93.6	85.7	97.8
Losses/Gains	10.0	6.4	14.3	2.2
Total	100.0	100.0	100.0	100.0
LOQ	1.2	0.8	1.2	0.9

ND Not detected

NA Not applicable

(A) A total of 5 unidentified components with no individual component accounting for > 1.7 %AUC

(B) A total of 8 unidentified components with no individual component accounting for > 2.5 %AUC

(C) A total of 10 unidentified components with no individual component accounting for > 1.9 %AUC

(D) A total of 11 unidentified components with no individual component accounting for > 2.0 %AUC

Table 6.1.1–47: Metabolite profile in plasma of rats following a single oral dose of [Pyrazole-5-¹⁴C] - SYN545974 (Expressed as µg Equivalents of SYN545974.h/g of Plasma)

Compound	Concentration (µg equiv.h/g)			
	Male		Female	
	300 mg/kg	5 mg/kg	100 mg/kg	5 mg/kg
	0-96 h	0-96 h	0-96 h	0-96 h
SYN545974	4	0.03	2.7	0.28
SYN508272	25	0.61	8.3	0.79
SYN548264	5	0.17	1.1	0.11
SYN548265 glucuronide	5	0.19	3.5	0.36
SYN548263	13	0.50	3.9	0.43
Dihydroxy SYN545974 glucuronide	1	0.07	0.7	0.07
Dechlorinated dihydroxy SYN545974 glucuronide	3	0.12	0.8	ND
Hydroxy SYN545974 glucuronide	6	0.15	1.0	ND
SYN547948	2	0.09	2.0	0.18
SYN547891 glucuronide	4	0.25	1.1	0.42
SYN547897	3	0.11	1.9	0.22
Additional extracts	<LOQ	NA	<LOQ	NA
Unidentified components	51 (A)	2.39 (B)	11.8 (C)	2.13 (D)
Post extraction solids	50	1.58	8.9	0.92
Total identified	71	2.29	27.0	2.86
Total unidentified	51	2.39	11.8	2.13
Total accounted for	172	6.26	47.7	5.91
Losses/Gains	23	0.17	8.2	-0.54
Total	195	6.43	55.9	5.37
LOQ	4	0.07	1.1	0.07

ND Not detected

NA Not applicable

(A) A total of 24 unidentified components with no individual component accounting for > 6 µg equiv.h/g

(B) A total of 34 unidentified components with no individual component accounting for > 0.22 µg equiv.h/g

(C) A total of 15 unidentified components with no individual component accounting for > 1.8 µg equiv.h/g

(D) A total of 29 unidentified components with no individual component accounting for > 0.19 µg equiv.h/g

Table 6.1.1–48: Metabolite profile in plasma of rats following a single oral dose of [Pyrazole-5-¹⁴C] - SYN545974 (expressed as percentage of total AUC)

Compound	% AUC			
	Male		Female	
	300 mg/kg	5 mg/kg	100 mg/kg	5 mg/kg
	0-96 h	0-96 h	0-96 h	0-96 h
SYN545974	1.9	0.5	4.9	5.2
SYN508272	13.0	9.5	14.8	14.7
SYN548264	2.5	2.6	1.9	2.0
SYN548265 glucuronide	2.5	2.9	6.2	6.7
SYN548263	6.9	7.7	7.0	8.1
Dihydroxy SYN545974 glucuronide	0.7	1.1	1.2	1.4
Dechlorinated dihydroxy SYN545974 glucuronide	1.6	1.8	1.4	ND
Hydroxy SYN545974 glucuronide	3.2	2.3	1.7	ND
SYN547948	1.2	1.4	3.5	3.3
SYN547891 glucuronide	2.3	3.9	2.0	7.8
SYN547897	1.8	1.7	3.5	4.0
Additional extracts	<LOQ	NA	<LOQ	NA
Unidentified components	27.1 (A)	36.9 (B)	21.0 (C)	39.6 (D)
Post extraction solids	25.6	24.6	16.0	17.2
Total identified	37.6	35.4	48.1	53.2
Total unidentified	27.1	36.9	21.0	39.6
Total accounted for	90.3	96.9	85.1	110.0
Losses/Gains	9.7	3.1	14.9	-10.0
Total	100.0	100.0	100.0	100.0
LOQ	1.9	1.0	2.0	1.2

ND Not detected

NA Not applicable

(A) A total of 24 unidentified components with no individual component accounting for > 3.3 %AUC

(B) A total of 34 unidentified components with no individual component accounting for > 3.4 %AUC

(C) A total of 15 unidentified components with no individual component accounting for > 3.2 %AUC

(D) A total of 29 unidentified components with no individual component accounting for > 3.6 %AUC

SYN545974 metabolites in urine: Urine was the minor excretion route for SYN545974 and its related products with 4.4-26.1% dose recovered. Metabolites detected in urine were numerous and generally comprised a low percentage of the administered dose. Unchanged SYN545974 was a minor component in urine at ≤0.1% of the dose. The major urinary metabolites were qualitatively similar between males and females and irrespective of dose, with no significant quantitative differences observed in urine from male compared with female rats. At least 41 metabolites were detected with the majority of metabolites identified as products following cleavage of the SYN545974 molecule. In the urine from BDC rats the profile was generally the same as that from the intact rats. Therefore, the data below represents the metabolites in urine from the intact rats.

Only three metabolites accounted for >5% of the dose in urine. These were the phenyl specific metabolites 2,4,6-TCP (TCP) and TCP sulphate at up to 6.6% and 14.9% of the administered dose and the corresponding pyrazole specific metabolite, SYN548263, at up to 8.9% of the administered dose.

There were a few other half molecules that accounted for between 1.4 and 2.2% of the dose. These were the phenyl specific metabolite, 2,4,6-trichlorophenyl methanol (TCPM) glucuronide and the pyrazole specific metabolites desmethyl SYN548263, SYN548264 and SYN548265 glucuronide. All other metabolites accounted for <1% of the dose and included products following further hydroxylation of both pyrazole and phenyl specific metabolites. Metabolites containing both the phenyl and pyrazole rings were also excreted in urine each accounting for <1% of the dose. These included products following demethylation, hydroxylation, dechlorination and corresponding glucuronide and sulphate conjugates.

Up to 27 and 35 components were not identified in urine from rats administered phenyl and pyrazole labelled SYN545974, respectively. Four of these components individually accounted for 1.1-4.4% dose, while no other unidentified component accounted for greater than 1% dose.

SYN545974 metabolites in bile: The major metabolites present in bile were qualitatively and quantitatively similar across all samples. As with urine, unchanged SYN545974 was either not detected or detected as a minor component (0.2% dose) in bile. Metabolites detected in bile were numerous, with at least 52 metabolites observed and generally comprised of glucuronide and cysteine conjugated metabolites.

No phenyl specific metabolites of SYN545974 were detected in bile. However, the pyrazole specific metabolites SYN548264, SYN548263, SYN548265 glucuronide and desmethyl SYN548265 glucuronide were observed, with SYN548265 glucuronide the largest of these accounting for $\leq 3.6\%$ dose. SYN548264 and SYN548263 were the only non-conjugated metabolites observed in bile.

The largest metabolites observed in bile were whole molecules products of parent. Those that accounted for >5% of the administered dose included SYN547891 glucuronide ($\leq 14\%$ of the dose), several hydroxy SYN545974 glucuronides ($\leq 10.8\%$ of the dose), dechlorinated hydroxy SYN545974 cysteine ($\leq 8.6\%$ of the dose), and a dechlorinated hydroxy desmethyl SYN545974 glucuronide ($\leq 7.4\%$ of the dose). Numerous other products following demethylation, hydroxylation, dechlorination and glucuronide and glutathione conjugation were observed accounting for up to 5% of the dose metabolites

Up to 25 components were not identified in bile from rats administered phenyl labelled SYN545974. Thirteen of these components each accounted for 1.1-3.6% dose, while no other unidentified component accounted for greater than 1% dose. Up to 30 components were not identified in bile from rats administered pyrazole labelled SYN545974. Thirteen of the unidentified components detected accounted for 1.0-2.9% dose, while no other unidentified component accounted for greater than 1% dose.

SYN545974 metabolites in feces: In general, the major metabolites present in feces were qualitatively similar across all samples with some minor quantitative differences observed in feces from male compared with female rats. At least 42 metabolites were detected in faecal samples from non-cannulated rats, with at least 17 metabolites detected in faecal samples from BDC rats.

In the non-cannulated animals, unchanged SYN545974 was a relatively minor component in feces at the 5 mg/kg dose accounting for only up to 3.9% dose. However, after the 100 mg/kg (females) and 300 mg/kg (males) doses, unchanged SYN545974 was the major component accounting for up to 48.2% dose. This was also similar to the unchanged SYN545974 observed in the feces from the BDC rats at up to 7.9% dose following a 5 mg/kg dose and 63.1% dose following a 100 or 300 mg/kg dose.

Metabolites detected in non-cannulated rats were mostly identified as demethylated and/or hydroxylated and/or dechlorinated SYN545974. Only one label specific metabolite was observed and this was the pyrazole containing metabolite SYN548263 at up to 5.8% dose. SYN547897 accounted for the largest % administered dose in feces at up to 10.5%. SYN547894 was detected in nearly all samples and accounted for 0.9-3.9% dose. Only three other metabolites accounted for >5% of the dose and these were SYN547890 (up to 5.9%), a dihydroxy SYN545974 (up to 6.0%) and dechlorinated hydroxy thiomethyl SYN545974 (up to 6.3%).

Up to 19 and 31 components were not identified in faecal sample from non-cannulated rats administered phenyl and pyrazole labelled SYN545974, respectively. Up to 14 unidentified components in each sample accounted for 1.1-3.6% dose, while no other unidentified component accounted for greater than 0.9% dose.

In BDC rats, the metabolites observed were relatively minor. The metabolites observed were SYN548263, SYN545547, dechlorinated hydroxy thiomethyl SYN545974, SYN547897, SYN547890 and SYN547893, with no single metabolite accounting for >1.7% of the dose.

SYN545974 metabolites in plasma: In general the major metabolites present in plasma were qualitatively similar irrespective of dose and sex. The major metabolites detected in plasma were cleavage products and therefore the profiles for the ¹⁴C-metabolites were different between the rats administered the phenyl and pyrazole labelled dose. In general, the intact metabolites detected were qualitatively and quantitatively similar between the two labelled dose forms. Unchanged SYN545974 was detected in all plasma samples and accounted for 0.5- 5.2% of the total radioactivity AUC (TRA).

At least 22 and 45 metabolites were detected on radiochromatograms for plasma samples following oral administration of phenyl and pyrazole labelled [¹⁴C]-SYN545974 to rats, respectively. As with the urine, these were mostly identified as cleaved metabolites and glucuronide and sulphate conjugates.

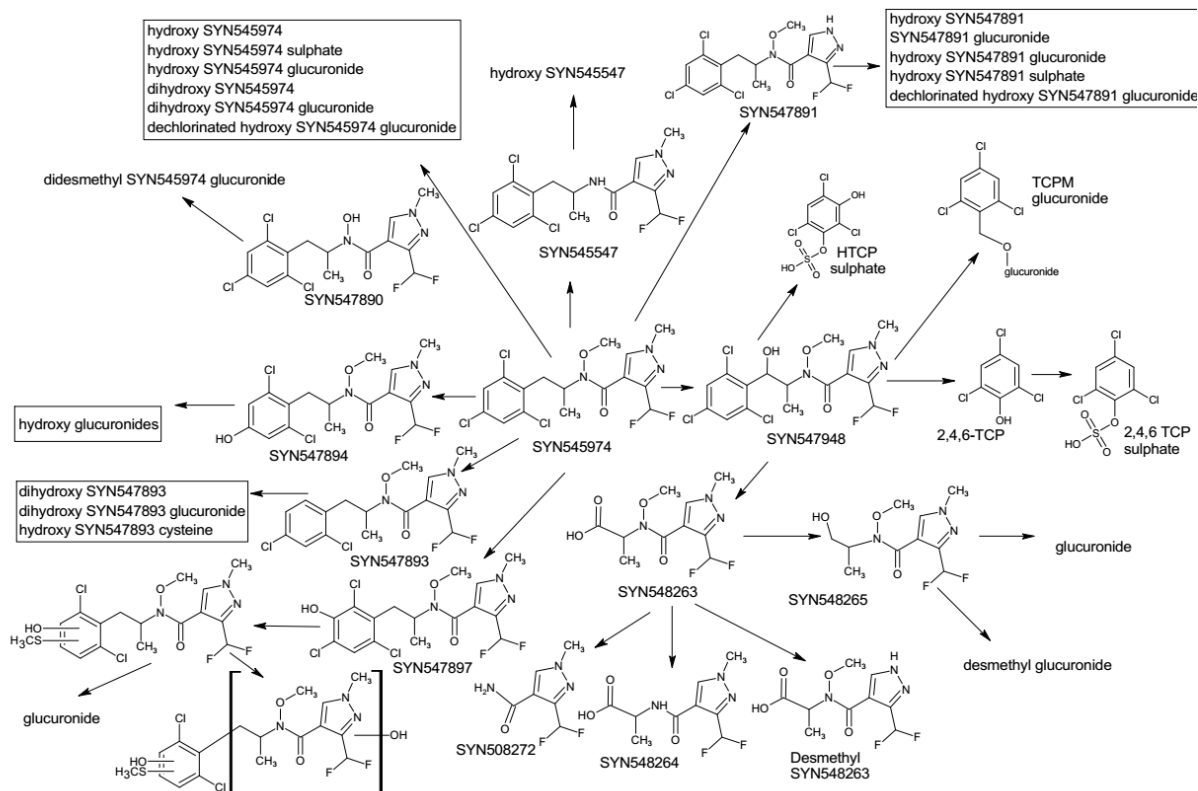
The major phenyl specific component detected was 2, 4, 6-TCP sulphate which accounted for up 44.1% TRA. No other component represented greater than 10% TRA. However, HTCP sulphate was close at up to 9.3% TRA in females (6.1% TRA in males). The major pyrazole specific metabolite detected was SYN508272 accounting for up to 14.8% TRA with its precursor SYN548263 accounting for up to 8.1% TRA.

Other circulating metabolites were phenyl or pyrazole specific half molecules in addition to intact metabolites following demethylation, hydroxylation, dechlorination and glucuronidation. The precursor to the cleavage of the molecule, SYN547948, was observed at up to 3.6% TRA.

Up to 11 components were not identified in plasma from rats administered phenyl labelled SYN545974. None of these components individually accounted for greater than 2.5% TRA. Up to 34 components were not identified in plasma from rats administered pyrazole labelled SYN545974. None of these components individually accounted for greater than 3.6% TRA.

SYN545974 metabolites in cage wash: The components identified in cage wash were also identified in urine and/or feces samples from rats administered SYN545974. No single metabolite in cage wash accounted for >2.2% dose (SYN545547).

Figure 6.1.1–1: Biotransformation Pathways Based on Identified Metabolites of SYN545974



CONCLUSION:

The toxicokinetic fate of pydiflumetofen in rats following administration of single oral doses was investigated in previous studies (██████, (2015) and ██████ and ██████ (2015). Samples of plasma and excreta from these studies were taken to investigate the biotransformation of pydiflumetofen in rats.

Samples of urine, plasma, faeces, bile and cage wash were obtained from male and female rats following oral administration of [phenyl-¹⁴C]-pydiflumetofen and [pyrazole-5-¹⁴C]-pydiflumetofen at 5 mg/kg bw (male and female rats), 300 mg/kg bw (male rats) and 100 mg/kg bw (female rats).

Pydiflumetofen was extensively metabolised primarily by first pass metabolism after a single oral dose radiolabelled with either [Phenyl-¹⁴C] or [Pyrazole-5-¹⁴C]. Metabolites were identified in the faeces, urine, bile (bile duct cannulated rats) and plasma for both radiolabels. The main route of excretion was in the faeces via the bile; urine was a minor route of excretion. Generally, metabolite profiles were similar irrespective of radiolabel apart from a few radiolabel-specific metabolites (except for plasma), dose (except for faeces) or sex. In total, up to 84.8% of the AD was identified, with all individual metabolites accounting for greater than 4.8% of the identified dose.

Faeces

In intact rats at 5 mg/kg bw, unchanged pydiflumetofen was a minor component only present up to 3.9% of the AD. However, at the higher doses (100-300 mg/kg bw) unchanged pydiflumetofen was a major component in faeces present up to 48.2% of the AD. This was also similar in bile duct cannulated (BDC) rats. In intact rats, the most abundant metabolite in faeces was SYN547897 (up to 10.5% AD) found in the high dose (100 mg/kg bw) females administered the phenyl radiolabel, whereas metabolites in BDC rats were relatively minor. Up to 31 unidentified components were present, over 10 of which accounted for 1.1-3.6% of the AD.

Bile

Unchanged pydiflumetofen was a minor component in bile present up to 0.2% of the AD. Metabolites

detected in bile were numerous, with at least 52 metabolites observed and generally comprised of glucuronide and cysteine conjugated metabolites. No phenyl specific metabolites of pydiflumetofen were detected in bile. However, the pyrazole specific metabolites SYN548264, SYN548263, SYN548265 glucuronide and desmethyl SYN548265 glucuronide were observed, with SYN548265 glucuronide the largest of these, accounting for up to 3.6% of the AD. SYN548264 and SYN548263 were the only non-conjugated metabolites observed in bile. Major metabolites were mostly whole molecule products of the parent, the most abundant being SYN547891 glucuronide (up to 9.7% AD in the 5 mg/kg bw females administered the phenyl radiolabel). Up to 25 unidentified components were present, over 10 of which accounted for 1.0-2.9% of the AD.

Urine

Unchanged pydiflumetofen was a minor component in urine present at $\leq 0.1\%$ of the AD. Metabolites detected in urine were numerous and generally comprised a low percentage of the AD. The major urinary metabolites were qualitatively similar between males and females and irrespective of dose, with no significant quantitative differences observed in urine from male compared with female rats. At least 41 metabolites were detected with the majority of metabolites identified as cleavage products of the parent molecule. The metabolite profile was largely similar between intact and BDC rats. The most abundant metabolite was 2,4,6-TCP sulphate present up to 14.9% AD (5 mg/kg bw males administered the phenyl radiolabel). Other significant metabolites included 2,4,6-TCP (6.6% AD in 5 mg/kg bw females administered the phenyl radiolabel) and the pyrazole-specific metabolite SYN548263 (8.9% AD in 5 mg/kg bw males administered the pyrazole radiolabel). Up to 35 unidentified components were present, five of which accounted for 1.1-4.4% of the AD.

Plasma

In general the major metabolites present in plasma were qualitatively similar irrespective of dose and sex. The major metabolites detected in plasma were cleavage products and therefore the profiles for the ^{14}C -metabolites were different between the rats administered the phenyl and pyrazole labelled dose. In general, the intact metabolites detected were qualitatively and quantitatively similar between the two labelled dose forms. Unchanged pydiflumetofen was present at 0.5-5.2% of the Total Radioactivity AUC (TRA). Major metabolites were cleavage products and therefore were different between the two radiolabels. The most abundant phenyl-specific component was 2,4,6-TCP sulphate (44.1% TRA in 300 mg/kg bw males). No other component represented greater than 10% TRA. However, HTCP sulphate was close at up to 9.3% TRA in 5 mg/kg bw females (6.1% TRA in 5 mg/kg bw males). The most abundant pyrazole-specific component was SYN508272 (14.8% TRA in 100 mg/kg bw females), with its precursor SYN548263 accounting for up to 8.1% TRA in 5 mg/kg bw females. Up to 34 unidentified components were present, none of which individually accounted for $> 3.6\%$ TRA.

Cage wash

All components identified in the cage wash were also present in the urine and/or faeces. No component was present $> 2.2\%$ of the AD.

Overall in this GLP and OECD TG-compliant metabolism study, pydiflumetofen was extensively metabolized via multiple first pass metabolic processes including hydroxylation, glucuronation, demethylation, dechlorination and cleavage to create phenyl- or pyrazole-specific metabolites. Only two metabolites (2,4,6 TCP sulphate in urine and plasma and SYN548272 in plasma) individually accounted for $>10\%$ of the AD. Numerous other metabolites were detected as cleavage products and as molecules that retained both the phenyl and pyrazole ring moieties. The intact metabolites detected were qualitatively and quantitatively similar between the two labels. The cleavage of the parent molecule occurred following hydroxylation of pydiflumetofen on the carbon adjacent to the trichlorophenyl ring to give SYN547948. This then cleaved to yield the 2,4,6 trichlorophenol (2,4,6-TCP) and SYN548263. 2,4,6-TCP was then sulphated, which accounted for the largest % of dose excreted at up to 14.9% of the AD. Other metabolites that retained the phenyl ring were hydroxyl 2,4,6-TCP (HTCP) sulphate and 2,4,6-TCP methanol (TCPM) glucuronide. SYN548263 was further metabolised by demethoxylation to SYN548264 and by N-demethylation. Amide hydrolysis of the pyrazole half molecules gave the pyrazole amide, SYN508272. Reduction of SYN548263 yielded the alcohol SYN548265. SYN548265 was further metabolised via demethylation and glucuronidation.

Numerous metabolites were observed that retained both the phenyl and pyrazole moieties. The primary metabolic routes for these metabolites included demethoxylation to SYN545547, N-dealkylation to SYN547891, single and di-hydroxylation, O-demethylation to SYN547890, oxidative dechlorination to SYN547894 and reductive dechlorination to SYN547893. The majority of these metabolites were also mono and di-hydroxylated and in many cases conjugated with glucuronide or in the case of SYN547894, glutathione.

These observations are consistent with the results of the preliminary study ([REDACTED] and [REDACTED], 2015) and excretion study ([REDACTED], 2015).

([REDACTED] and [REDACTED], 2015)

Report:	K-CA 5.1.1/06 [REDACTED] and [REDACTED] (2014). SYN545974 - Pharmacokinetics of SYN545974 in the Rat Following Multiple Oral and Single Intravenous Administration. [REDACTED] [REDACTED]. [REDACTED] Report No. 33409. Issue date 16 June 2014. Unpublished. Syngenta File No. SYN545974_10104.K-CA 5.1.1/04 [REDACTED] and [REDACTED]
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Guidelines: Not Applicable

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rat blood has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

The pharmacokinetics of SYN545974 were investigated following multiple (7 daily doses) oral gavage administrations of SYN545974 in 0.5% Tween 80 with 0.5% (w/v) carboxymethylcellulose over a dose range of 3-1000 mg/kg and a single intravenous administration of SYN545974 in DMSO: 10% aqueous hydroxypropyl- β -cyclodextrin (5:95 v/v) at a target dose level of 1 mg/kg. Groups of 4 male and 4 female rats per dose group each received a single daily oral dose for 7 days of 3, 10, 30, 100, 300, 500 or 1000 (males only) mg/kg or a single intravenous administration at 1 mg/kg. Serial blood samples from each rat obtained over a 24 hour period, following dosing on Day 1 (oral and intravenous) and Day 7 (oral only) were diluted with an equal volume of water and analysed using a validated LC-MSMS method. Pharmacokinetic parameters were determined which allowed the evaluation of kinetic changes according to the dose (dose effect), duration of administration (time effect) and possible sex differences (gender effect).

In general, systemic exposure to SYN545974 increased with dose following oral administration on Day 1. Overall, total systemic exposure to SYN545974 (based on geometric mean AUC(0-t) estimates) increased in an approximately proportional manner in males, but in a slightly sub-proportional manner across the dose range in females, with respective exponents of the power model of 1.1554 and 0.7267. It should be noted that in males, there are difficulties associated with assessing linearity with a sparse data set, especially at those doses below 30 mg/kg. Estimates of C_{max} generally increased in a sub-proportional manner across the entire dose range in both sexes, although in males the non-linearity was predominantly observed at the 500 and 1000 mg/kg/day doses. Median t_{max} and, where determined, mean t_{1/2} estimates tended to increase with increasing dose in both males and females.

Absolute oral bioavailability of SYN545974 (F) ranged from 2.3 to 6.3% in males and 4.8 to 36.8% in females, across the respective dose ranges, with oral bioavailability greatest in females between 3 and 30

mg/kg/day, where F was 21.0 to 36.8%.

Following repeat dosing, systemic exposure to SYN545974 (based on geometric mean C_{max} and AUC(0-t) estimates) was generally comparable between Days 1 and 7 at the 3 and 10 mg/kg/day doses in both sexes. Indeed, mean accumulation ratios (R_{ac}) were 0.9 and 1.1 for 3 and 10 mg/kg/day, respectively (derived for females only). However, systemic exposure was appreciably reduced on Day 7 compared to Day 1 for all subsequent doses, (mean R_{ac} estimates were 0.1 and 0.4 for all doses greater than 10 mg/kg/day) with the decrease more marked in males. Overall, total systemic exposure (AUC(0-t)) to SYN545974 increased in a sub-proportional manner across the dose range in males and females. In males, a 33-fold increase in dose from 30 mg/kg-1000 mg/kg resulted in a 7.6-fold increase in exposure. In males, linearity could not be assessed at doses below 30 mg/kg due to the sparse data set. In females, a 167-fold increase in dose from 3 mg/kg-500 mg/kg resulted in a 12-fold increase in exposure.

There was no trend to indicate consistent differences in median t_{max} and mean t_{1/2} parameters between sampling days for both males and females.

Mean total body SYN545974 clearance estimates (CL = 3414 and 2644 mL/h/kg for males and females, respectively) showed clearance was not limited hepatic and renal blood flow. Mean estimates of volume of distribution (V_d = 6134 and 6632 mL/kg for males and females, respectively) indicated extensive distribution of SYN545974, beyond the vasculature (total body water *ca* 668 mL/kg in rats). Mean t_{1/2} estimates were shorter compared to following oral administration indicating that the terminal elimination phase may not have been appropriately characterised after intravenous dosing or the absorption of SYN545974 is rate limited after oral dosing.

Systemic exposure to SYN545974 was consistently greater in females compared to males across doses and sampling days, with male to female exposure ratios of 0.1 to 0.6 after oral dosing and 0.7 following intravenous dosing. This sex difference was also observed in C_{max}, AUC(0-t) and , where estimated, AUC(0-inf) estimates with the difference in exposure to SYN545974 between males and females generally more marked at 3, 10 and 30 mg/kg/day than at the higher doses. No consistent sex-related trends were observed for all other parameter estimates.

After single administration, systemic exposure to SYN545974 increased in an approximately proportional manner in males, but in a slightly sub-proportional manner across the dose range in females, although a supra-proportional increase was noted between 3 and 300 mg/kg/day and 3 and 30 mg/kg/day in males and females, respectively. In males, a 33-fold increase in dose from 30 mg/kg-1000 mg/kg resulted in a 33-fold increase in exposure, but linearity could not be assessed below 30 mg/kg. In females, a 167-fold increase in dose from 3 mg/kg-500 mg/kg resulted in a 37-fold increase in exposure. After repeat administration, exposure increased sub-proportionally to dose in males (30- 1000 mg/kg) and females (30-500 mg/kg). In males, a 33-fold increase in dose from 30 mg/kg-1000 mg/kg resulted in a 7.6-fold increase in exposure, but linearity could not be assessed below 30 mg/kg. In females, a 167-fold increase in dose from 3 mg/kg-500 mg/kg resulted in a 12-fold increase in exposure. There was negligible accumulation of SYN545974 observed between Days 1 and 7 at the 3 and 10 mg/kg/day doses in females, with systemic exposure to SYN545974 being appreciably reduced at doses greater than 10 mg/kg/day on repeat oral administration in both sexes. Systemic exposure to SYN545974 was consistently greater in females compared to males throughout the study. No other consistent sex- related trends were observed.

MATERIALS AND METHODS

Materials:

Test Materials:

Name :	SYN545974
Source :	Syngenta Crop Protection
Physical state :	White powder

Batch reference :	2637-BA/110
Purity (%w/w) :	99.5 % w/w
Recertification date:	31 July 2013
Oral dose vehicle:	0.5% Tween 80 with 0.5% (w/v) carboxymethylcellulose in distilled water
Intravenous dose vehicle:	DMSO: 10% aqueous hydroxypropyl β -cyclodextrin (5:95 v/v)

Test Animals:

Species:	Rat
Strain:	Han Wistar
Number/sex:	32 male, 28 female
Age at dosing:	8-10 weeks
Weight at dosing:	260-342 g for males; 183-221 g for females (3 mg/kg) 251-294 g for males; 179-215 g for females (10 mg/kg) 237-306 g for males; 186-209 g for females (30 mg/kg) 242-302 g for males; 176-200 g for females (100 mg/kg) 268-320 g for males; 177-198 g for females (300 mg/kg) 243-308 g for males; 183-221 g for females (500 mg/kg) 256-308 g for males; (1000 mg/kg) 273-283 g for males; 174-189 g for females (1 mg/kg, iv)
Source:	
Housing:	Pre-study: multiply housed in solid bottomed polycarbonate cages with bedding On study: housed in pairs in polycarbonate cages with wire mesh floors
Acclimatisation period:	At least 8 days
Diet:	Certified Rodent Chow No. 5CR4 (14% protein) available <i>ad libitum</i>
Water:	Mains tap water <i>ad libitum</i>
Identification:	Tail marking and individual cage cards
Environmental conditions:	U31 and U32
Temperature:	20-24°C
Humidity:	47-61%
Air changes:	10-11 changes/h
Photoperiod:	Alternating 12-hour light and dark cycles

STUDY DESIGN AND METHODS

Study dates: Start: 08 May 2012; End: 16 June 2014

Dosing Regime:

For both oral and intravenous administration, four animals per dose group per sex were accurately weighed prior to dosing. The oral preparations were administered daily by plastic, gastric gavage at a target dose volume of 5 mL/kg/day to achieve target doses of 3, 10, 30, 100, 300, 500 and 1000 mg/kg/day. The intravenous preparation was administered once only by Vygon in the tail vein at a target dose volume of 5 mL/kg to achieve a target dose of 1 mg/kg.

Dosing and blood sampling regime in the rat

Group	Route	Dose		Animals		Sampling times (post dose on D1 and 7)
		mg/kg/day	mL/kg/day	Sex	Number	
1	Oral	3	5	Male	1-4	0.08 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24 hours
1		3		Female	5-8	
2		10		Male	9-12	
2		10		Female	13-16	
3		30		Male	17-20	
3		30		Female	21-24B	
4		100		Male	25-28	
4		100		Female	29-32	
5		300		Male	33-36	
5		300		Female	37-40	

6		500		Male	41-44	
6		500		Female	45-48	
7		1000		Male	49-52	
8A	IV	1		Male	57-60	
8A		1		Female	61-64	

Sample Collection and Bioanalysis

Blood samples (*ca* 0.1 mL) were collected from a tail vein of each rat (into plastic tubes with K2EDTA as the anti-coagulant) at defined intervals over a 24 hour period following dosing on Day 1 (oral and intravenous) and Day 7 (oral only). Immediately after collection, 50 µL of blood from each sample was accurately measured into an appropriately labelled plain plastic tube already containing 50 µL of deionised water. Samples were then placed on a roller mixer for a short time and then stored in a freezer set to maintain -80 °C until taken for analysis.

Deionised water was added to control rat (Han Wistar) blood (K2 EDTA as anticoagulant) to produce a blood: water (50:50, v/v) solution. Control matrix was stored a freezer set to maintain -20 °C when not in use. The samples were stored immediately on receipt in a freezer set to maintain -80°C pending analysis. Once aliquoted during the experimental phase, the remainder of each sample was returned to a freezer set to maintain -80°C.

The test samples were extracted and analysed along with the freshly prepared blank samples, calibration standards and quality control samples. Extracts obtained from control blood were chromatographed near the start of each relevant run, as were the calibration standard extracts (in ascending concentration order). The test sample extracts were chromatographed after the first set of quality control extracts and further sets of quality control sample extracts were interspersed between the test sample extracts where appropriate. A final set of quality control sample extracts was chromatographed after the final test sample extract. Each set of quality control samples consisted of one sample at each concentration level.

Vials containing acetonitrile were included in each run after standard and quality control sample extracts containing high concentrations of SYN545974 in order to demonstrate the absence of significant assay carryover influencing the data.

Pharmacokinetic evaluation

Pharmacokinetic parameters were estimated using WinNonlin pharmacokinetic software version 5.2.1 (Pharsight). Parameters were derived using a non-compartmental approach consistent with both extravascular and intravenous bolus routes of administration. All parameters were generated from individual SYN545974 concentrations in blood. Parameters were estimated using sampling times relative to the start of each dose administration (and were within an allowable deviation range ($\pm 10\%$), otherwise actual times were used (on one occasion the actual time was used (animal 6, 0.25 h timepoint)).

The area under the SYN545974 versus time curve (AUC) was calculated using the linear/log trapezoidal method. When practical, the terminal elimination phase of each concentration versus time curve was identified using at least the final three observed concentration values. The slope of the terminal elimination phase was determined using log linear regression on the unweighted concentration data. Parameters relying on the determination of the terminal elimination phase were reported but excluded from descriptive statistics if the coefficient of determination was less than 0.800 and/or if the extrapolation of the AUC to infinity represented more than 20% of the total area.

The ratio of AUC(0-24 h) between males and females was used to evaluate sex differences in systemic exposure and the accumulation ratio (Rac) of AUC(0-24 h) between Day 1 and 7 was used to evaluate changes in systemic exposure to SYN545974 with repeat dosing.

A non-linear power model was constructed to assess linearity in systemic exposure to SYN545974 (based on AUC(0-t) estimates) with increasing dose. The relationship between exposure and dose would be considered proportional when the exponent of the power model was equal to 1.

RESULTS

Pharmacokinetic Analysis

Mean pharmacokinetic data is reported in Tables 6.1.1-49 and 6.1.1-50. The influence of gender data (m/f ratio) is presented in Table 6.1.1-51.

Following oral dosing, SYN545974 was not quantifiable in all samples, particularly in males at lower dose levels (*i.e.* at 3 and 10 mg/kg). Following oral dosing, SYN545974 concentration *versus* time profiles were consistent with the extravascular route of administration, whereby a post dose absorption phase was evident, after which concentrations declined in a generally mono-exponential manner. After intravenous dosing, SYN545974 concentrations declined in an approximate bi-exponential manner.

Effect of increasing oral dose on Day 1 and Day 7

The relationship between systemic exposure to SYN545974 and dose following oral administration is presented in Figures 6.1.1-2 and 3 and in Tables 6.1.1-49 and 50.

In general, systemic exposure to SYN545974 increased with dose following oral administration on Days 1 and 7. On Day 1, total systemic exposure to SYN545974 (based on geometric mean AUC(0-t) estimates) increased in an approximately proportional manner in males, but in a slightly sub-proportional manner across the dose range in females, with respective exponents of the power model of 1.1554 and 0.7267 (Figure 6.1.1-2 and Table 6.1.1-49). However, in males, there were difficulties associated with assessing linearity with a sparse data set at doses below 30 mg/kg. A supra-proportional increase was noted between 3 and 300 mg/kg/day and 3 and 30 mg/kg/day in males and females, respectively, after which a sub-proportional increase in AUC(0-t) estimates was evident across the subsequent increasing doses.

Table 6.1.1-49: Mean whole blood concentrations (expressed as ng/ml) and pharmacokinetics parameters following a single oral administration of SYN545974 at 3, 10, 30, 100, 300, 500 and 1000 mg/kg to male and females rats

Males

Nominal time after dosing (h)	Group 1 (3 mg/kg)	Group 2 (10 mg/kg)	Group 3 (30 mg/kg)	Group 4 (100 mg/kg)	Group 5 (300 mg/kg)	Group 6 (500 mg/kg)	Group 7 (1000 mg/kg)
0.25	<LLOQ	<5.65	10.4	15.6	36.2	15.7	27.7
0.5	<5.08	<6.23	13.6	18.0	31.5	27.6	53.5
1	<5.51	9.95	31.6	44.2	84.1	49.9	112
2	<5.72	12.7	42.7	122	220	215	322
4	<LLOQ	8.16	31.7	264	458	349	568
6	<LLOQ	<5.58	22.5	227	610	379	606
8	<LLOQ	<5.34	16.8	152	538	321	548
10	<LLOQ	<LLOQ	<10.2	98.5	408	262	507
12	<LLOQ	<LLOQ	<6.11	45.6	341	165	467
24	<LLOQ	<LLOQ	<LLOQ	<5.01	29.3	15.9	51.0
C _{max} (ng/mL)	7.86	12.4	38.9	242	602	380	612
t _{max} (hours)	2.00	2.00	2.00	4.00	6.00	6.00	7.00
t _{1/2} (hours)	NC	NC	2.76	3.17	3.53	3.76	4.08
AUC ₍₀₋₄₎ (ng.h/mL)	11.22	41.65	227.4	1613	6196	3644	7546
AUC _(0-inf) (ng.h/mL)	NC	NC	323.6	1796	6358	3737	7855
F (%)	NC	2.8*	3.0*	6.0	6.3	2.3	2.6

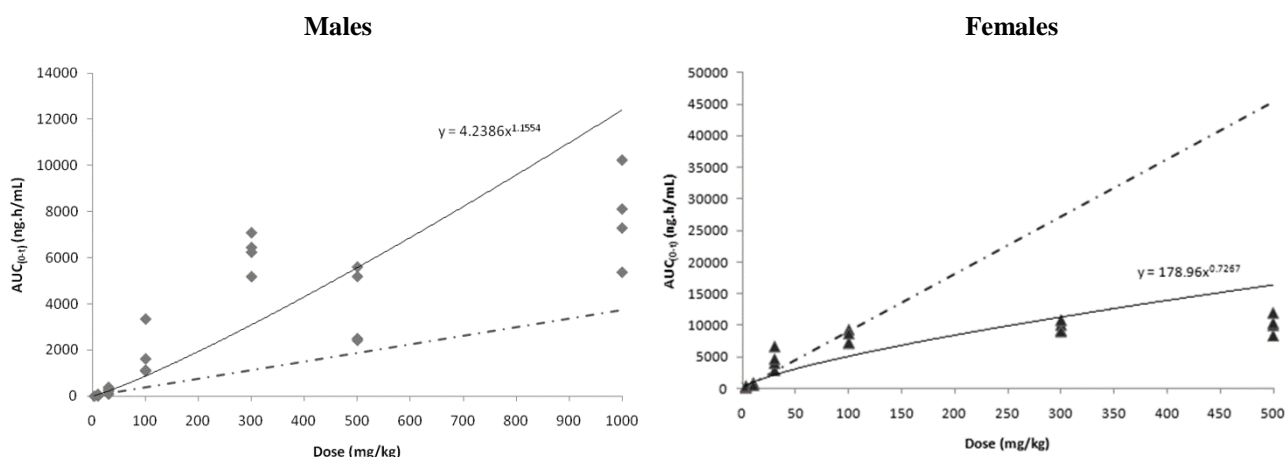
* Value considered unreliable (estimation of geometric mean AUC(0-24h) used in calculation of F included individual AUC(0-24h) derived by extrapolation where coefficient of determination was less than 0.800 and/or the extrapolation of the AUC to infinity represents more than 20% of the total area.

NC = Not calculable

Females

Nominal time after dosing (h)	Group 1 (3 mg/kg)	Group 2 (10 mg/kg)	Group 3 (30 mg/kg)	Group 4 (100 mg/kg)	Group 5 (300 mg/kg)	Group 6 (500 mg/kg)
0.25	27.3	30.1	87.1	117	215	195
0.5	47.6	68.9	206	195	413	264
1	82.6	143	406	353	444	369
2	63.5	176	490	418	572	534
4	31.7	97.8	471	648	567	619
6	14.9	50.0	452	630	585	583
8	10.2	27.7	303	500	626	620
10	<6.66	18.2	210	576	556	525
12	<5.46	10.9	125	500	543	545
24	<LLOQ	<LLOQ	<10.8	31.2	111	166
C _{max} (ng/mL)	76.0	178	527	674	639	640
t _{max} (hours)	1.00	2.00	3.00	5.00	7.00	8.00
t _{1/2} (hours)	2.74	2.96	3.00	3.15	5.69	7.02
AUC _(0-t) (ng.h/mL)	273	774	4410	8124	9827	10170
AUC _(0-inf) (ng.h/mL)	296	820	4489	8266	10740	11060
F (%)	23.0	21.0	36.8	20.8	7.6	4.8

Figure 6.1.1-2: Relationship between AUC(0-t) and Dose of SYN545974 in Male and Female Rats Following Oral Administration of SYN545974: Day 1



Dotted line = Dose-proportional relationship passing through the parameter at the 3 mg/kg/day dose level. However, it should be noted that in males, there are difficulties associated with assessing linearity with a sparse dataset, especially at those doses below 30 mg/kg. Solid line = Power function obtained from linear regression from log parameter against log dose.

On Day 7, total systemic exposure to SYN545974 increased in sub-proportional manner across the dose range in males and females, with respective exponents of the power model of 0.6737 and 0.4637 (Figure 6.1.1-3). Estimates of C_{max} generally increased in a sub-proportional manner across the entire dose range in both sexes on both sample days although in Day 1 males the non-linearity was predominantly observed at the 500 and 1000 mg/kg/day doses. Median t_{max} and, where determined, mean t_{1/2} estimates tended to increase with increasing dose in both males and females (Table 6.1.1-50).

Absolute oral bioavailability (F) of SYN545974 ranged from 2.3 to 6.3% in males and 4.8 to 36.8% in females, across the respective dose ranges, with oral bioavailability greatest in females between 3 and 30 mg/kg/day, where F was 21.0 to 36.8%.

Table 6.1.1-50: Mean whole blood concentrations (expressed as ng/ml) and pharmacokinetics parameters following daily oral administration for 7 days of SYN545974 at 3, 10, 30, 100, 300, 500 and 1000 mg/kg to male and females rats

Males

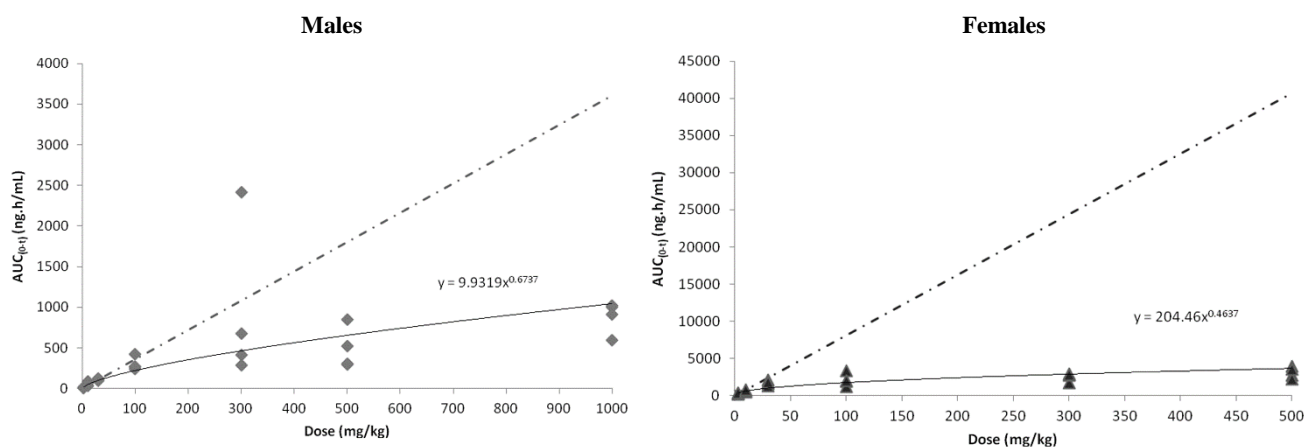
Nominal time after dosing (h)	Group 1 (3 mg/kg)	Group 2 (10 mg/kg)	Group 3 (30 mg/kg)	Group 4 (100 mg/kg)	Group 5 (300 mg/kg)	Group 6 (500 mg/kg)	Group 7 (1000 mg/kg)
0.25	<5.33	<6.51	<5.96	<6.72	28.5	9.78	20.9
0.5	<5.81	<7.75	7.00	8.28	35.2	12.4	21.6
1	<5.54	<9.74	11.2	15.5	36.5	14.7	25.3
2	<5.47	<12.8	17.4	27.4	57.0	21.8	29.9
4	<5.92	<8.56	13.5	31.5	53.1	27.0	35.6
6	<LLOQ	<6.76	10.1	33.6	50.8	34.7	47.5
8	<LLOQ	<5.19	7.92	25.4	78.6	30.3	40.8
10	<LLOQ	<LLOQ	<6.31	22.9	66.6	33.8	47.7
12	<LLOQ	<LLOQ	<5.16	17.6	54.0	22.5	46.9
24	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<12.8	<11.3	30.3
C _{max} (ng/mL)	8.5	14.9	17.2	34.3	63.3	41.9	64.5
t _{max} (hours)	2.3	2.00	2.00	6.00	10.00	7.00	9.00
t _{1/2} (hours)	NC	NC	NC	3.57	6.34	3.37	5.85
AUC ₍₀₋₄₎ (ng.h/mL)	13.5	57.8	109	291	666	448	832
AUC _(0-inf) (ng.h/mL)	NC	NC	NC	391	2725	429	1096
R _{ac}	NC	NC	NC	0.2	0.2	0.1	0.1

NC = Not calculable

Females

Nominal time after dosing (h)	Group 1 (3 mg/kg)	Group 2 (10 mg/kg)	Group 3 (30 mg/kg)	Group 4 (100 mg/kg)	Group 5 (300 mg/kg)	Group 6 (500 mg/kg)
0.25	18.6	29.8	56.0	27.5	30.3	26.8
0.5	44.0	58.5	87.6	53.1	32.0	33.1
1	77.7	121	168	83.6	41.6	43.5
2	65.2	147	259	199	80.8	60.1
4	24.3	71.2	233	278	140	161
6	11.9	35.5	119	199	167	195
8	<7.51	19.9	89.4	146	198	222
10	<5.37	17.0	72.2	109	246	250
12	<LLOQ	11.7	38.6	60.4	161	239
24	<LLOQ	<LLOQ	<8.53	<6.35	14.2	22.0
C _{max} (ng/mL)	76.5	146	272	259	252	286
t _{max} (hours)	1.00	2.00	3.00	4.00	9.00	10.00
t _{1/2} (hours)	2.19	2.99	4.43	2.89	3.46	3.24
AUC ₍₀₋₄₎ (ng.h/mL)	244	612	1674	1946	2471	3035
AUC _(0-inf) (ng.h/mL)	264	768	1868	2045	2544	3438
R _{ac}	0.9	1.1	0.4	0.3	0.3	0.3

Figure 6.1.1-3: Relationship between AUC(0-t) and Dose in Male and Female Rats Following Oral Administration of SYN545974: Day 7



Solid line = Power function obtained from linear regression from log parameter against log dose.

Effect of repeat oral dosing

Following repeat dosing, systemic exposure to SYN545974 (based on geometric mean C_{max} and AUC(0-t) estimates) was generally comparable between Days 1 and 7 at the 3 and 10 mg/kg/day doses in females. In males, there were difficulties associated with assessing C_{max} and AUC(0-t) at doses below 30 mg/kg. Mean accumulation ratios (R_{ac}) were 0.9 and 1.1 for 3 and 10 mg/kg/day, respectively (derived for females only). However, systemic exposure was appreciably reduced on Day 7 compared to Day 1 for all subsequent doses (mean R_{ac} estimates were 0.1 and 0.4 for all doses greater than 10 mg/kg/day) with the decrease more marked in males. There was no trend to indicate consistent differences in median t_{max} and mean t_{1/2} parameters between sampling days for both males and females (Table 6.1.1-50).

Effect of sex

Systemic exposure to SYN545974 was consistently greater in females compared to males across doses and sampling days, with male to female exposure ratios of 0.1 to 0.6 after oral dosing and 0.7 following intravenous dosing (Table 6.1.1-51). This sex difference was observed in C_{max}, AUC(0-t) and , where estimated, AUC(0-inf) estimates with the difference in exposure to SYN545974 between males and females generally more marked at 3, 10 and 30 mg/kg/day than at the higher doses. No consistent sex-related trends were observed for all other parameter estimates.

Table 6.1.1-51: Assessment of absolute bioavailability, gender differences and changes in systemic exposure to SYN545974 in rats following single or daily oral and single intravenous bolus administration of SYN545974

Gender	Phase	Dose (mg/kg/day)	F (%)	Mean R _{ac}	Dose Route	Phase	Dose (mg/kg/day)	Day	M/F Ratio
Male	1	3	NC	NC	Oral	1	3	1	NC
	2	10	2.8*	NC		7		7	0.3
	3	30	3.0*	NC		1		1	0.1
	4	100	6.0	0.2		7		7	0.2
	5	300	6.3	0.2		3	30	1	0.1
	6	500	2.3	0.1		7		7	0.1
	7	1000	2.6	0.1		4	100	1	0.2
Female	1	3	23.0	0.9		7		7	0.2
	2	10	21.0	1.1		5	300	1	0.6
	3	30	36.8	0.4		7		7	0.4
	4	100	20.8	0.3		6	500	1	0.4
	5	300	7.6	0.3		7		7	0.2
	6	500	4.8	0.3		IV Bolus	8	1	1
									0.7

NC = not calculated

* Value considered unreliable (estimation of geometric mean AUC(0-24h) used in calculation of F included individual AUC(0-24h) derived by extrapolation where 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.

Effect of single intravenous dosing

Mean total body SYN545974 clearance estimates (CL = 3414 and 2644 mL/h/kg for males and females, respectively) were less than the combined known hepatic and renal blood flow rates (*ca.* 3312 and 2208 mL/h/kg, respectively, in rats) indicating that clearance of SYN545974 was not limited by hepatic plasma and blood flow rates. Mean estimates of volume of distribution (V_d = 6134 and 6632 mL/kg for males and females, respectively) indicated extensive distribution of SYN545974 beyond the vasculature (total body water *ca.* 668 mL/kg in rats). Mean t_{1/2} estimates were shorter compared to those following oral administration indicating that the terminal elimination phase may not have been appropriately characterised after intravenous dosing or the absorption of SYN545974 is rate limited after oral dosing.

CONCLUSION:

The pharmacokinetics of non-labelled pydiflumetofen were investigated in a GLP study following multiple (7 daily doses) oral gavage administrations of pydiflumetofen over a dose range of 3-1000 mg/kg

bw and a single iv administration of pydiflumetofen at a target dose level of 1 mg/kg bw. Groups of 4 male and 4 female rats per dose group each received a single daily oral dose for 7 days of 3, 10, 30, 100, 300, 500 or 1000 (males only) mg/kg bw or a single iv administration at 1 mg/kg bw. Serial blood samples from each rat obtained over a 24 hour period, following dosing on day 1 (oral and iv) and day 7 (oral only) were analysed using a validated LC-MSMS method. Pharmacokinetic parameters were determined which allowed the evaluation of kinetic changes according to dose (dose effect), duration of administration (time effect) and possible sex differences (gender effect).

Oral administration

On day 1 (single exposure), after oral administration, total systemic exposure to pydiflumetofen (based on geometric mean AUC(0-t) estimates) increased rapidly with dose at lower doses, but increased sub-proportionally at higher doses in females. Systemic exposure increased in an approximately proportional manner in males. However, in males linearity could not be determined for doses below 30 mg/kg bw as pydiflumetofen levels were below the limit of quantification. Estimates of C_{max} generally increased in a sub-proportional manner across the entire dose range in both sexes, although in males the non-linearity was predominantly observed at the 500 and 1000 mg/kg bw doses.

On day 7 (repeat exposure), overall systemic exposure increased sub-proportionally with dose for both sexes. In males, a 33-fold increase in dose from 30 mg/kg-1000 mg/kg resulted in a 7.6-fold increase in exposure. In males, linearity could not be assessed at doses below 30 mg/kg due to the sparse data set. In females, a 167-fold increase in dose from 3 mg/kg-500 mg/kg resulted in a 12-fold increase in exposure.

Systemic exposure was comparable between days 1 and 7 in both sexes in the low dose groups (3-10 mg/kg bw/d). Indeed, mean accumulation ratios (R_{ac}) were 0.9 and 1.1 for 3 and 10 mg/kg/d, respectively (derived for females only). However, at higher doses, systemic exposure was reduced on day 7 compared to day 1, with the decrease more marked in males. Overall, there was negligible accumulation of pydiflumetofen between days 1 and 7 at the 3 and 10 mg/kg bw/d doses in females, with systemic exposure being appreciably reduced at doses greater than 10 mg/kg bw/d on repeat oral administration in both sexes.

Sex differences

Systemic exposure (C_{max}, AUC_(0-t) and AUC_(0-inf)) was consistently greater in females compared to males at all doses and sampling days, especially from 3-30 mg/kg bw/d with male to female exposure ratios of 0.1 to 0.6 after oral dosing and 0.7 following iv dosing. No other sex-related differences were observed.

Intravenous administration

After single iv administration, systemic exposure decreased steadily. Mean total body clearance was not limited by hepatic and renal blood flow rates. Pydiflumetofen was extensively distributed beyond the circulatory system. Shorter mean t_{1/2} estimates compared to those calculated following oral administration suggested the terminal elimination phase may not have been appropriately characterized after iv dosing, or pydiflumetofen absorption is rate limited after oral dosing.

(██████████ and ██████████, 2014)

Report:	K-CA 5.1.1/07 ██████████ and ██████████ (2014a). SYN545974 - Pharmacokinetics of SYN545974 in the Mouse Following Multiple Oral and Single Intravenous Administration. ██████████. ██████████ Report No. 33408. Issue date 19 August 2014. Unpublished. Syngenta File No. SYN545974_10124.
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Guidelines: Not Applicable

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in mouse blood has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

This study investigated the pharmacokinetics of SYN545974 in mice following seven daily single oral doses of SYN545974 and a single intravenous dose. SYN545974 was dosed by gavage in 0.5% (w/v) carboxymethylcellulose containing 0.5% (v/v) Tween 80 at 10, 30, 100, 200, 300, 500, 750 or 1000 mg/kg. The single intravenous bolus dose was administered in DMSO: 10% (w/v) aqueous hydroxypropyl- β -cyclodextrin (5:95, v/v) at a target dose of 1 mg/kg. Groups of male and female mice each received either a single oral dose or a single daily oral dose for 7 days. A further group of male and female mice each received a single intravenous bolus administration at 1 mg/kg. Serial and terminal blood samples from each mouse obtained over a 24 hour period following dosing on Day 1 (oral and intravenous) and Day 7 (oral only) were diluted with an equal volume of water and analysed using a validated LC-MSMS method. Pharmacokinetic parameters were determined which allowed the evaluation of potential kinetic changes according to the dose administered (dose effect), duration of administration (time effect) and sex differences (gender effect).

In general, systemic exposure to SYN545974 increased with dose following oral administration on Days 1 and 7. Overall, on Day 1, exponents of the power model for AUC(0-t) were 0.9 and 1.0, in males and females, respectively, indicating a trend to a proportional increase in total systemic exposure to SYN545974 across the 10 to 1000 mg/kg dose range. However, this increase in AUC(0-t) was characterised by supra-proportional increase between 10 and 300 mg/kg in males and female after which a sub-proportional increase in AUC(0-t) estimates was evident across the subsequent increasing doses. On Day 7, AUC(0-t) increased in an overall sub-proportional manner with exponents of the power model of 0.7 and 0.8 in males and females, respectively. However, the non-linearity of AUC(0-t) was characterised in males by a supra-proportional increase between 10 and 100 mg/kg after which a sub-proportional increase was evident from 100 to 1000 mg/kg and in females by a generally proportional increase from 10 to 300 mg/kg, after which AUC(0-t) increased sub-proportionally across the subsequent increasing doses. Estimates of C_{max} increased in a sub-proportional manner across the dose range on Days 1 and 7 in both sexes. The t_{max} and, where determined, t_{1/2} estimates were generally comparable across the dose range in both males and females.

Absolute oral bioavailability of SYN545974 (F) was low, ranging from 3.6 to 10% in males and 3.1 to 7.9 % in females, across the respective dose ranges.

Following repeat dosing, systemic exposure to SYN545974 (based on C_{max} and AUC(0-t)) was reduced on Day 7 compared to Day 1 in both sexes with Rac AUC(0-24 h) being between 0.1 and 0.4 for all doses.

The observed t_{max} was longer on Day 7 than on Day 1 for the higher dose groups; 200 (males only) 300, 500, 750 and 1000 mg/kg/day.

Mean total body clearance was 7100 and 6750 mL/h/kg for males and females, respectively. Mean estimates of volume of distribution (V_d = 6490 and 13500 mL/kg for males and females, respectively) indicated extensive distribution of SYN545974 beyond the vasculature (total body water *ca* 725 mL/kg in mice).

On Day 1, systemic exposure to SYN545974 was generally comparable between sexes with male to female exposure ratios between 1.0 and 1.4 across all doses, except for the 30 mg/kg dose group where the male to female systemic exposure ratio was 1.9.

Following repeat dosing, overall systemic exposure to SYN545974 exhibited little difference between sexes at the 10, 30 and 100 mg/kg dose groups. However, at higher doses (200 to 1000 mg/kg) systemic exposure (based on AUC(0-24)) was up to 2.8-fold greater in females than in males. No consistent sex-related trends were observed for all other parameter estimates.

Overall, total systemic exposure to SYN545974 increased in a generally proportional manner on Day 1 and in a sub-proportional manner on Day 7 in males and females. C_{max} increased sub-proportionally across the dose range on Days 1 and 7 in both sexes. Absolute oral bioavailability of SYN545974 was very low.

Systemic exposure to SYN545974 was appreciably reduced at doses greater than 10 mg/kg on repeat oral administration. Clearance of SYN545974 was less than the known combined hepatic and renal blood flow rates in mice, with SYN545974 indicating extensive distribution beyond the central circulation.

Systemic exposure to SYN545974 was generally comparable between sexes on Day 1. Where a trend was observed, systemic exposure to SYN545974 was greater in female than in males at doses 200 to 1000 mg/kg. No other consistent sex-related trends were observed

MATERIALS AND METHODS

Materials:

Test Materials:

Name :	SYN545974
Source :	Syngenta Crop Protection
Physical state :	White powder
Batch reference :	2637-BA/110
Purity (%w/w) :	99.5 % w/w
Recertification date:	31 July 2013
Oral dose vehicle:	0.5% (w/v) carboxymethylcellulose (CMC) containing 0.5% (v/v) Tween 80 in distilled water
Intravenous dose vehicle:	DMSO: 10% (w/v) aqueous hydroxypropyl β -cyclodextrin (5:95, v/v)

Test Animals:

Species:	Mouse
Strain:	CD-1
Number/sex:	144 male and 144 female
Age at dosing:	Groups 1, 4-9; 9-11 weeks Group 2; 10-12 weeks Group 3; 9-10 weeks
Weight at dosing:	Group 1; males 36-42 g, females 24-34 g Group 2; males 36-46 g, females 26-32 g Group 3; males 36-44 g, females 26-32 g Group 4; males 36-44 g, females 28-32 g Group 5; males 35-45 g, females 22-31 g Group 6; males 36-44 g, females 29-36 g Group 7; males 33-45 g, females 26-33 g Group 8; males 37-43 g, females 26-34 g Group 9; males 36-45 g, females 26-34 g
Source:	██████████
Housing:	Pre-study: singly housed (males) and group housed (females) in solid bottomed cages with wood shavings. On study: singly housed (males) and group housed (females) in cages with wire mesh floors.
Acclimatisation period:	9 days (10 mg/kg), 15 days (100 mg/kg), 9 days (1 mg/kg), 8 days (30 mg/kg), 8 days (300 mg/kg), 8 days (500 mg/kg), 8 days (1000 mg/g), 6 days (200 mg/kg), 6
Diet:	██████████ Certified Rodent Chow No. 5CR4 (14% protein) available <i>ad libitum</i> , except for Phase 5, where ██████████ RM1 was fed in error.
Water:	Mains tap water <i>ad libitum</i>
Identification:	Ear punch (Phase 1), tail marking and individual cage cards
Environmental conditions:	U30, U34, U37
Temperature:	19-24°C
Humidity:	33-87%
Air changes:	10-11 changes/h

Photoperiod:	Alternating 12-hour light and dark cycles
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STUDY DESIGN AND METHODS

Study dates: Start: 02 April 2012; End: 19 August 2014

Dosing Regime:

For both oral and intravenous administration, each animal was accurately weighed prior to dosing. Dosing syringes were charged with the appropriate volume of dose formulation. The dosing syringes were weighed prior to and following each dose. The actual dose received by each animal was determined with reference to the mean calculated concentration based on the pre-dose and post-dose concentration analysis and the weight of dose administered. The oral preparations were administered daily by gastric gavage at a target dose volume of 4.0-5.4 mL/kg/day to achieve target doses of 10, 30, 100, 200, 300, 500, 750 and 1000 mg/kg/day. The intravenous preparation was administered as a bolus, once only in the tail vein at a target dose volume of 5 mL/kg to achieve a target dose of 1 mg/kg. The study design and sampling regimen is shown below in the tables below.

Dosing and day 1 blood sampling regime in the mouse

Dosing and day 1 blood sampling regime in the mouse							
Group	Route	Dose		Animals		Sampling times (hours after dosing)	
		mg/kg	volume (mL/kg)	Sex	Number		
1	Oral	10	5	Male	1-4	1, 4, 12*	
1					5-8	2, 8, 24*	
1				Female	9-12	1, 4, 12*	
1					13-16	2, 8, 24*	
2		100		Male	33-36	1, 4, 12*	
2					37-40	2, 8, 24*	
2				Female	41-44	1, 4, 12*	
2					45-48	2, 8, 24*	
3	Intravenous	1		Male	65-68	5 min, 2, 8*	
3					69-72	0.5, 4, 12*	
3					73-76	1, 6, 24*	
3				Female	77-80	5 min, 2, 8*	
3					81-84	0.5, 4, 12*	
3					85-88	1, 6, 24*	
4	Oral	30		Male	89-92	1, 3, 4*	
4					93-96	0.5, 2, 6, 8*	
4				Female	97-100	1, 3, 4*	
4					101-104	0.5, 2, 6, 8*	
5		300		Male	121-124	1, 4, 12*	
5					125-128	0.5, 2, 8*	
5				Female	129-132	1, 4, 12*	
5					133-136	0.5, 2, 8*	
6		500		Male	153-156	1, 4, 12*	
6					157-160	0.5, 2, 8, 24*	
6				Female	161-164	1, 4, 12*	
6					165-168	0.5, 2, 8, 24*	
7		1000		Male	185-188	1, 4	
7					189-192	0.5, 2, 8, 24*	
7					217-220	12*	
7				Female	193-196	1, 4	
7					197-200	0.5, 2, 8, 24*	
7					221-224	12*	
8		200		Male	217R-220R	1, 4, 12*	
8					221R-224R	0.5, 2, 8, 24*	
8				Female	225-228	1, 4, 12*	
8					229-232	0.5, 2, 8, 24*	
9		750		Male	249-252	1, 4, 12*	
9					253-256	0.5, 2, 8, 24*	
9				Female	257-260	1, 4, 12*	
9					261-264	0.5, 2, 8, 24*	

* = Terminal blood sample

R = As these animal numbers had already been used for the replacement 12 h animals, subsequent animals were suffixed with an 'R'.

Dosing and day 7 blood sampling regime in the mouse

Group	Route	Dose		Animals		Sampling times (hours after dosing)
		mg/kg/day	volume (mL/kg)	Sex	Number	
1	Oral	10	5	Male	17-20	1, 4, 12*
1					21-24	2, 8, 24*
1				Female	25-28	1, 4, 12*
1					29-32	2, 8, 24*
2		100		Male	49-52	1, 4, 12*
2					53-56	2, 8, 24*
2				Female	57-60	1, 4, 12*
2					61-64	2, 8, 24*
4		30		Male	105-108	1, 3, 4*
4					109-112	0.5, 2, 6, 8*
4				Female	113-116	1, 3, 4*
4					117-120	0.5, 2, 6, 8*
5		300		Male	137-140	1, 4, 12*
5					141-144	0.5, 2, 8*
5				Female	145-148	1, 4, 12*
5					149-152	0.5, 2, 8*
6		500		Male	169-172	1, 4, 12*
6					173-176	0.5, 2, 8, 24*
6				Female	177-180	1, 4, 12*
6					181-184	0.5, 2, 8, 24*
7		1000		Male	201-204	1, 4, 12*
7					205-208	0.5, 2, 8, 24*
7				Female	209-212	1, 4, 12*
7					213-216	0.5, 2, 8, 24*
8		200		Male	233-236	1, 4, 12*
8					237-240	0.5, 2, 8, 24*
8				Female	241-244	1, 4, 12*
8					245-248	0.5, 2, 8, 24*
9		750		Male	265-268	1, 4, 12*
9					269-272	0.5, 2, 8, 24*
9				Female	273-276	1, 4, 12*
9					277-280	0.5, 2, 8, 24*

* = Terminal blood sample

Sample Collection and Bioanalysis

Serial blood samples (*ca* 0.05 mL) were collected via saphenous venepuncture of each mouse (into tubes containing K2 EDTA as the anticoagulant) at two to three defined intervals following dosing on Day 1 (oral and intravenous) and Day 7 (oral only). The serial bleeds were then followed by a terminal bleed and *ca* 0.5-1 mL collected via cardiac puncture (see Section 3.6 for time points) to generate a composite concentration-time profile for each dose group with n=4 samples/time point.

Immediately after collection, 50 µL of blood from each sample was accurately measured into an appropriately labelled plain tube already containing 50 µL of deionised water and stored in a freezer set to maintain -80°C until analysed. The lower limit of quantification (LLOQ) was 5 ng SYN545974/mL blood.

The test samples were extracted and analysed along with the freshly prepared blank samples, calibration standards and quality control samples. Extracts obtained from control blood were chromatographed near the start of each relevant run, as were the calibration standard extracts (in ascending concentration order). The test sample extracts were chromatographed after the first set of quality control extracts and further sets of quality control sample extracts were interspersed between the test sample extracts where appropriate. A final set of quality control sample extracts was chromatographed after the final test sample extract. Each set of quality control samples consisted of one sample at each concentration. Vials containing acetonitrile were included in each run after standard and quality control sample extracts.

containing high concentrations of SYN545974 in order to demonstrate the absence of significant assay carryover influencing the data.

Pharmacokinetic evaluation

Pharmacokinetic parameters were obtained using WinNonlin pharmacokinetic software version 5.2.1 (Pharsight) using a non-compartmental approach consistent with each route of administration. All parameters were generated from arithmetic mean SYN545974 concentrations in blood from Days 1 and 7, where applicable. Arithmetic mean blood concentrations were derived from 4 animals/sex/group/time point/sampling occasion, wherever practical. Parameters were estimated using sampling times relative to the start of each dose administration (within an allowable deviation range ($\pm 10\%$), otherwise actual times were used).

The area under the SYN545974 versus time curve (AUC) was calculated using the linear/log trapezoidal method. When practical, the apparent terminal phase of each concentration versus time curve was identified using at least the final three observed concentrations. The slope of the apparent terminal elimination was determined using log linear regression on the unweighted concentration data. Parameters relying on the determination of the terminal elimination phase were reported and highlighted if the coefficient of determination was less than 0.800 and/or if the extrapolation of the AUC to infinity represented more than 20% of the total area.

In addition, ratios to evaluate the influence of gender between sexes (calculated as the ratio of AUC(0-24 h) between males and females) and changes in systemic exposure to SYN545974 with repeat dosing (accumulation ratio Rac of AUC(0-24 h) between Day 1 and 7) were estimated.

A non-linear power model was constructed to assess dose proportionality in systemic exposure to SYN545974 (based on AUC(0-t)) with increasing dose. The relationship between exposure and dose would be considered in proportion when the exponent of the power model was equal to 1.

RESULTS

Following oral dosing, SYN545974 was quantifiable in at least one sample per timepoint up to between 2 to 8 h for the 10 and 30 mg/kg dose groups, between 8 to 12 h following 100 mg/kg, up to 12 h for 200, 300, 500 and 750 mg/kg dose groups and between 12 and 24 h for the 1000 mg/kg dose group in both sexes on Days 1 and 7. Following intravenous dosing, SYN545974 was quantifiable up to 4 h in at least 1 sample per time point in males and females.

Following oral dosing, SYN545974 concentration versus time profiles were consistent with the extravascular route of administration, whereby a post dose absorption phase was evident, after which concentrations declined in a generally mono-exponential manner where concentrations were quantifiable beyond 4 h following dosing. After intravenous dosing, SYN545974 concentrations also declined in an approximate mono-exponential manner.

In general, systemic exposure to SYN545974 increased with dose following oral administration on Days 1 and 7. Overall, on Day 1, exponents of the power model for AUC(0-t) were 0.9320 and 0.9766 (Figure 6.1.1-4), in males and females, respectively, indicating a trend to a proportional increase in total systemic exposure to SYN545974 across the 10 to 1000 mg/kg dose range. However, this increase in AUC(0-t) was characterised by supra-proportional increase between 10 and 300 mg/kg in males and female after which a sub-proportional increase in AUC(0-t) estimates was evident across the subsequent increasing doses. On Day 7, AUC(0-t) increased in an overall sub-proportional manner with exponents of the power model of 0.6894 and 0.8139 in males and females, respectively (Figure 6.1.1-4). However, the non-linearity of AUC(0-t) was characterised in males by a supra-proportional increase between 10 and 100 mg/kg after which a sub-proportional increase was evident from 100 to 1000 mg/kg and in females by a generally proportional increase from 10 to 300 mg/kg, after which AUC(0-t) increased sub-proportionally across the subsequent increasing doses. Estimates of C_{max} increased in a sub-proportional manner across the dose range on Days 1 and 7 in both sexes. t_{max} and, where determined, t_{1/2} estimates were generally

comparable across the dose range in both males and females.

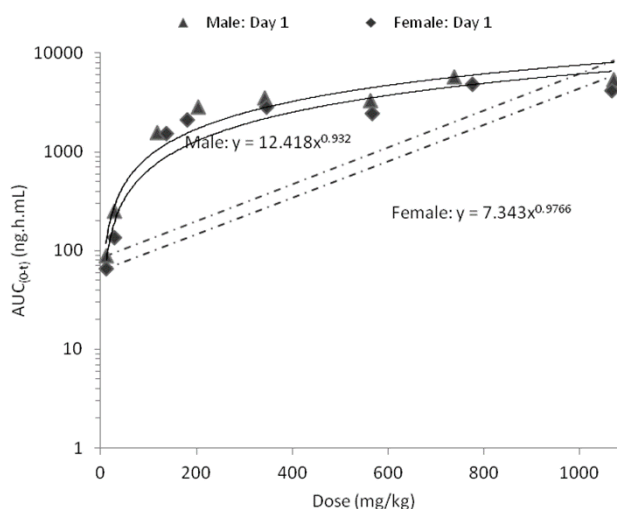
Absolute oral bioavailability of SYN545974 (F) was very low with reliable estimates ranging from 3.56 to 10.0 % in males and 3.07 to 7.87 % in females, across the respective dose ranges.

Mean total body clearance (Cl) was 7100 and 6750 mL/h/kg for males and females, respectively. Mean estimates of volume of distribution (Vd = 6490 and 13500 mL/kg for males and females, respectively) indicated extensive distribution of SYN545974 beyond the vasculature (total body water *ca* 725 mL/kg in mice).

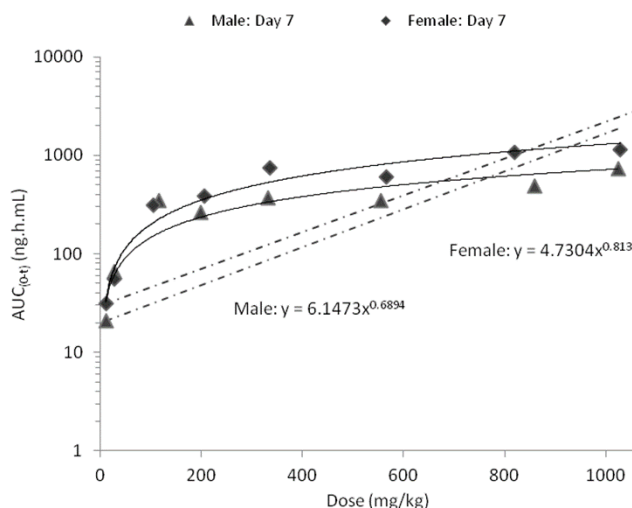
On Day 1, systemic exposure to SYN545974 was generally comparable between sexes with male to female exposure ratios between 1.0 and 1.4 across all doses, except for the 30 mg/kg dose group where the male to female systemic exposure ratio was 1.9.

Following repeat dosing, overall systemic exposure to SYN545974 exhibited little difference between sexes at the 10, 30 and 100 mg/kg dose groups. However, at higher doses (200 to 1000 mg/kg) systemic exposure (based on AUC(0-24)) was up to 2.8-fold greater in females than in males. No consistent sex-related trends were observed for all other parameter estimates.

Figure 6.1.1-4: Relationship between AUC(0-t) and Dose of SYN545974 in Male and Female Mouse Following Oral Administration of SYN545974 on Day 1 and Day 7



Dotted line = Dose-proportional relationship through the parameter estimates at the 10 and 1000 mg/kg doses. Solid line = Power function obtained from linear regression from log parameter against log dose.



CONCLUSION:

This GLP study investigated the pharmacokinetics of non-labelled pydiflumetofen in mice following seven daily single oral doses and a single iv dose. Pydiflumetofen was dosed by gavage at 10, 30, 100, 200, 300, 500, 750 or 1000 mg/kg bw. The single iv dose was administered at a target dose of 1 mg/kg bw. Groups of male and female mice each received either a single oral dose or a single daily oral dose for 7 days. A further group of male and female mice each received a single iv administration. Serial and terminal blood samples from each mouse obtained over a 24 hour period following dosing on day 1 (oral and iv) and day 7 (oral only) were analysed using a validated LC-MSMS method. Pharmacokinetic parameters were determined which allowed the evaluation of potential kinetic changes according to dose administered (dose effect), duration of administration (time effect) and sex differences (gender effect).

Oral administration

After oral administration, on day 1 (single exposure), systemic exposure to pydiflumetofen (based on AUC(0-t) estimates) increased rapidly with dose at lower doses, but increased sub-proportionally at higher doses (from 100 mg/kg bw in both sexes). On day 7 (repeat exposure), overall systemic exposure increased sub-proportionally with dose for both sexes. However, the non-linearity of the AUC(0-t) was characterised in males by a supra-proportional increase between 10 and 100 mg/kg bw/d after which a sub-proportional increase was evident from 100 to 1000 mg/kg bw/d and in females by a generally proportional increase from 10 to 300 mg/kg bw/d, after which AUC(0-t) increased sub-proportionally across the subsequent increasing doses. Generally, systemic exposure was lower on day 7 compared to day 1 in both sexes with $\text{Rac AUC}(0-24 \text{ h})$ being between 0.1 and 0.4 for all doses. However, there were no consistent differences in t_{max} and $t_{1/2}$ for either sex caused by repeat dosing.

Sex differences

Systemic exposure was generally comparable between the sexes on day 1 except at 30 mg/kg bw where it was higher in males. On day 7, systemic exposure was comparable between males and females at low doses up to 100 mg/kg bw/d, above which systemic exposure ($\text{AUC}(0-24 \text{ h})$) was greater in females. No consistent sex-related differences were observed for all other pharmacokinetic parameters.

Intravenous administration

After iv administration, systemic exposure decreased steadily in an approximate mono-exponential manner. Mean total body clearance was not limited by hepatic and renal blood flow rates, indicating that pydiflumetofen was extensively distributed beyond the circulatory system.

(██████████ and ██████████, 2014a)

Report:	K-CA 5.1.1/08 ██████████ (2015). SYN545974 – The Excretion and Biotransformation of [Phenyl- ^{14}C] and [Pyrazole-5- ^{14}C]-SYN545974 Following Single Oral Administration in the Mouse. ██████████ ██████████ Report No. 35415. Issue date 03 August 2015. Unpublished. Syngenta File No. SYN545974_10257.
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Guidelines: Metabolism – Mouse; OECD 417 (2010); EPA OPPTS 870.7485 (1998); EC 1107/2009 (2009); EU 283/2013 (2013); JMAFF 12 Nohsan No 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

The excretion of [phenyl-U-14C] and [pyrazole-5-14C]-SYN545974 was investigated following oral doses of 10 or 300 mg/kg, to groups of 4 male and 4 female 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.

Following a single oral administration of phenyl or pyrazole labelled [14C]-SYN545974 at 10 mg/kg to mice, a mean of 97-103% of the administered dose was eliminated in urine and feces (including cage wash) over seven days. The majority of administered radioactivity (87-97%) was excreted in the first 24 hours. The routes and rates were similar for both radiolabels and for both males and females, with the majority of the dose excreted in the feces (63-79%). Urinary excretion accounted for 15-30% of the dose.

Following a single oral administration of phenyl or pyrazole labelled [14C]-SYN545974 at 300 mg/kg to mice, a mean of 95-110% of the administered dose was eliminated in urine and feces (including cage wash) over seven days. The majority of administered radioactivity (88-103%) was excreted in the first 24 hours. The routes and rates were similar for both radiolabels and for both males and females, with 76-94% of the dose excreted in the feces and 7.2-15% in the urine.

Following a single oral administration of phenyl or pyrazole labelled [14C]-SYN545974 excretion was essentially complete in all animals by 168 h post dose with 0.3% or less remaining in the carcass and gastrointestinal tract.

[14C]-SYN545974 was extensively metabolised giving rise to numerous metabolites. In total up to 86% of the administered dose was identified. Across all individual samples up to 24 unknown metabolites accounting in total for 0.0-15.9% dose were observed with no unidentified component accounting for greater than 3.6% dose.

The major metabolites present in urine and feces were qualitatively and quantitatively similar between males and females and across doses. Some quantitative differences were observed in feces from male compared with female mice, but these were not considered significant. Unchanged SYN545974 was not detected in urine. In the feces from the 10 mg/kg oral dose group, unchanged parent accounted for less than 4.4% of the dose. However, in the 300 mg/kg dose group, unchanged SYN545974 was the major component recovered at up to 48.8% of the administered dose.

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 parent and included the phenyl derived metabolites 2,4,6-trichlorophenol (TCP) sulphate, 3-hydroxy-2,4,6-TCP sulphate and 2,4,6-trichlorophenol glucuronide. The major pyrazole derived metabolite was identified as SYN548263. Other components identified included demethylated and/or hydroxylated SYN545974 and their corresponding glucuronide and sulphate conjugates.

Metabolites detected in feces from mice were mostly identified as demethylated and/or hydroxylated and/or dechlorinated SYN545974. Only one label specific metabolite was observed and this was the pyrazole containing metabolite SYN548263 at up to 4.7% dose. SYN545974 was the major component recovered at up to 48.8% of the administered dose. Desmethyl-hydroxy SYN545974 isomers and didesmethyl hydroxy SYN545974 isomers accounted for a large % of the administered dose in feces at up to 14.0% and 13.5% respectively. SYN547897 was detected in nearly all samples and accounted for 2.9- 11.4% dose. Only two other metabolites accounted for >5% of the dose and these were SYN547890 (6.0%) and dihydroxy SYN545974 (5.8%).

Irrespective of radiolabel, dose or sex, following a single oral administration of [¹⁴C]-SYN545974, the majority of dose related radioactivity was eliminated by 24 hours post dose and excretion was essentially complete by 168 h. The major route of elimination was *via* the feces, with urinary

elimination playing a minor role. Based on the % of parent in the feces at 300 mg/kg compared to the 10 mg/kg dose, suggests that nearly up to 50% of the 300 mg/kg dose is unabsorbed.

Up to 86% of the administered radioactivity was identified following a single oral dose of SYN545974 to mice. In general the major metabolites present were qualitatively and quantitatively similar between males and females and across dose rates with no significant quantitative differences observed from male compared with female mice.

SYN545974 was extensively metabolised in mouse via demethylation, hydroxylation, and dechlorination together with glucuronide and sulphate conjugates with the potential for multiple isomers within most types. The molecule also cleaves at the benzylic carbon to yield the phenyl metabolite TCP and pyrazole metabolite SYN548263. These cleavage products were further metabolised via direct glucuronidation and sulphation and also following hydroxylation and sulphation to 3-hydroxy-TCP sulphate.

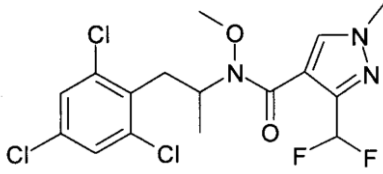
The biotransformation proceeded by:

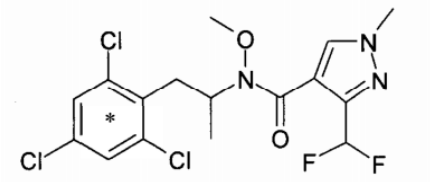
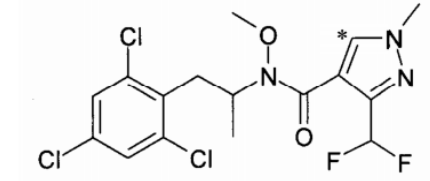
- Hydroxylation to give hydroxylated and dihydroxylated metabolite isomers.
- Demethylation to SYN547890.
- Demethoxylation to SYN545547, accompanied by hydroxylation.
- Hydroxylation and demethylation to give desmethyl hydroxy metabolites and didesmethyl hydroxy metabolites.
- Cleavage of SYN545974 to give the pyrazole metabolite SYN548263 and the phenyl metabolite 2,4,6 TCP.
- Dechlorination and hydroxylation to SYN547894 and other dechlorinated hydroxy and dechlorinated dihydroxy metabolites.
- Glucuronic acid conjugation and sulphate conjugation.

MATERIALS AND METHODS

Materials:

Unlabelled Test Material:

Name :	SYN545974
Structure :	
Source :	Syngenta Crop Protection Inc Münchwilen AG
Physical state :	Off white powder
Batch reference :	SMU2EP12007
Purity (%w/w) :	98.5
Radiolabelled Test Material:	[Phenyl-U- ¹⁴ C]-SYN545974
Radiochemical purity:	98.9%

Source:	Syngenta Crop Protection LLC
Lot/Batch number:	RDR-XIX-18
Structure:	 <p>* position of [¹⁴C]-label</p>
Radiolabelled Test Material:	[Pyrazole-5- ¹⁴ C]-SYN545974
Radiochemical purity:	98.5%
Source:	Selcia
Lot/Batch number:	5318JYC001-1
Structure:	 <p>* position of [¹⁴C]-label</p>

Vehicle: 0.5% (w/v) aqueous carboxymethylcellulose (CMC) containing 0.5% Tween 80.

Preparation of dosing solutions: Each dose preparation was formulated as a homogenous suspension in 0.5% aqueous CMC containing 0.5% Tween 80. Each dose preparation was prepared using [14C]-SYN545974 and non-radiolabelled SYN545974 to achieve the correct dose concentration and specific activity.

Test Animals:	
Species:	Mouse
Strain:	CD-1
Age/weight at dosing:	8- 10 weeks Group 1: 26-33 g (males), 22-30 g (females) Group 2: 29-31 g (males), 21-25 g (females) Group 3: 29-36 g (males), 23-28 g (females) Group 4: 28-32 g (males), 24-29 g (females)
Source:	██████████
Housing:	During the pre-study holding period, mice were singly (males) or multiply (female) housed by sex in solid bottomed polypropylene and stainless steel cages with bedding. On study, animals were housed singly in all-glass metabolism cages designed for the separate quantitative collection of urine and feces.
Acclimatisation period:	At least 5 days prior to dosing.
Diet:	Rat and Mouse No.1 maintenance diet, ██████████ ██████████ <i>Ad libitum</i>
Water:	Tap water <i>ad libitum</i>
Environmental conditions:	Temperature: 20 – 21°C Humidity: 24- 59% ^A Air changes: 10-11 changes/hour Photoperiod: 12 hours light / 12 hours dark
	A = On several occasions (11-12 and 14 March 2013) the humidity dropped to a range of 24-39%. In the opinion of the Study Director this had no impact on the study outcome.

Study Design and Methods:

Experimental Dates: Start: 10 February 2014 End: 18 December 2014

Group Arrangements: Animals were assigned to 4 groups as shown in the table below.

Dosing groups for excretion studies for [¹⁴C]-SYN545974

Test Group	Dose (mg/kg)	Number/sex	Remarks
Group 1 Excretion and metabolite identification	10 (Phenyl)	4 male, 4 female	Excreta collection over 168 hours.
Group 2 Excretion and metabolite identification	10 (Pyrazole)	4 male, 4 female	Excreta collection over 168 hours.
Group 3 Excretion and metabolite identification	300 (Phenyl)	4 male, 4 female	Excreta collection over 168 hours.
Group 4 Excretion and metabolite identification	300 (Pyrazole)	4 male, 4 female	Excreta collection over 168 hours.

Dosing and sample collection:

A single oral dose of [¹⁴C]-SYN545974 suspended in CMC was administered to each mouse by gavage at a target dose rate of 5 mL/kg. Animals in group 1 and group 2 received a low dose corresponding to 10 mg/kg. Animals in group 3 and 4 received a high dose corresponding to 300 mg/kg. All animals received target radioactive doses of 5 MBq/kg.

Urine and feces were collected individually and separately. Urine and feces were frozen immediately upon collection. At the end of each feces collection period, cage wash samples were collected (water).

Animals were humanely killed by CO₂ narcosis. The gastrointestinal tract (and contents) and carcass were retained separately.

All samples were analysed for radioactivity by liquid scintillation counting either directly or following sample oxidation.

Excretion studies:

Urine was collected at intervals of 8, 24, 48, 72, 96, 120, 144 and 168 hours after dosing. Feces and cage wash were collected at intervals of 24, 48, 72, 96, 120, 144 and 168 hours after dosing.

Metabolite characterization studies: Representative samples of urine and feces samples from each group were pooled for metabolic identification and quantification. Additional cage wash samples were also selected for metabolic identification and quantification.

Urine and feces sample pools were prepared by combining a fixed percentage (25%) by weight of each sample at selected time points from each animal. Time points were selected to represent ≥95% of the radioactivity in each sample type. Cage wash sample pools were prepared by combining a fixed percentage (100%) by weight of each sample at selected time points from selected animals (individually containing ≥5% of the recovered radioactivity).

A subsample of each urine pool (1.5 mL) was centrifuged (*ca* 14000 rpm, *ca* 10 min) to remove particulates. The radioactive content of the supernatant was determined by liquid scintillation counting (LSC). The pellet was re-suspended in water and analysed by LSC.

A subsample of each feces pool (*ca* 10 g) was mixed with acetonitrile (3 volumes solvent per gram sample) and shaken for *ca* 5 min using a mechanical shaker. The supernatant was decanted following centrifugation (*ca* 4000 rpm, *ca* 10 min) and the pellet extracted twice more with acetonitrile. The resulting supernatants were combined to produce a single acetonitrile extract for each sample. The remaining pellet was then extracted once with acetonitrile: water (9:1, v/v) then once with acetonitrile: water (1:1, v/v). The radioactive content of each extract and PES was determined by combustion and/or

LSC.

Each acetonitrile and acetonitrile/water extract was combined to form a single extract for each sample. Each combined extract was partitioned three times with an equal volume of hexane. Radioactivity recovered in the hexane fraction was not deemed significant and no further work was done on the hexane fractions. The aqueous fractions were reduced to ≤ 3 mL under a stream of nitrogen gas then reconstituted by addition of acetonitrile (1 mL) and water such that the final sample was 5 mL in acetonitrile: water (1:4, v/v).

A subsample of each cage wash pool (150 mL) was concentrated to dryness under nitrogen. Each sample was reconstituted with the aid of sonication for *ca* 30 min to a final volume of 5 mL in acetonitrile: water (1:9, v/v). The reconstituted cage wash samples were filtered (Ultracel-PL centrifuge filters) with the aid of centrifugation (*ca* 4000 rpm, *ca* 20 min). The formation of a pellet of particulate material blocked the filter. Material that had passed through the filter (filtrate) and the supernatant of material that had not passed through the filter were removed, combined and filter again with the aid of centrifugation. Again, filtration was incomplete due to formation of a pellet. The filtrate and supernatant were removed and combined. For the majority of cage wash samples the recovery of radioactivity in the combined filtrate and supernatant was sufficient and radioactivity associated with the filter and/or pellet did not require to be quantified. For two cage wash samples the recovery of radioactivity in the filtrate/supernatant was low. The remaining radioactivity was assumed to be associated with the filter and/or the pellet but this was not quantified.

Metabolites were identified by LC-MS using a combination of comparative chromatography with authentic reference standards, accurate mass measurement and MSⁿ fragmentation. Co-chromatography with reference chemicals was used to confirm the identification of metabolites. Metabolites were quantified by radiochemical detection.

Statistics: Not applicable.

RESULTS

Excretion:

The recovery of radioactivity in excreta, following administration of a single oral dose of [14C]-SYN545974 at doses of 10 or 300 mg/kg are presented in Tables 6.1.1-49 and 6.1.1-50.

Table 6.1.1-49: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [Phenyl-14C]-SYN545974 to mice

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 1 10 mg/kg		Group 3 300 mg/kg	
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-8 h	°3.7 9.5	11	3.3	°6.1 5.6
	8-24 h		10	3.1	
	24-48 h	1.5	1.1	0.7	2.1
	48-72 h	0.3	0.3	0.1	0.3
	72-96 h	<0.1	0.2	<0.1	0.1
	96-120 h	<0.1	0.1	°<0.1	°0.3
	120-144 h	<0.1	<0.1	°<0.1	0.1
	144-168 h	<0.1	<0.1	°<0.1	°0.2
	<i>Subtotal</i>	<i>15</i>	<i>23</i>	<i>7.2</i>	<i>15</i>
Feces	0-24 h	68	59	82	71
	24-48 h	4.6	2.6	1.9	3.3
	48-72 h	0.8	0.4	0.6	0.7
	72-96 h	0.3	1.0	0.1	0.3

	96-120 h	0.1	0.1	<0.1	0.3
	120-144 h	<0.1	0.1	<0.1	0.1
	144-168 h	<0.1	<0.1	°<0.1	0.3
	<i>Subtotal</i>	73	63	84	76
Cage wash		8.4	11	4.0	7.1
GI tract		°<0.1	<0.1	°<0.1	0.1
GI tract contents		°<0.1	<0.1	°<0.1	<0.1
Carcass		°0.1	°<0.1	°0.1	°0.1
Total Recovery		97	98	96	98

° = Below limit of detection

Table 6.1.1-50: Recovery of radioactivity in excreta and tissues after administration of a single oral dose of [Pyrazole-¹⁴C]-SYN545974 to mice

		Group mean excretion data (percentage of radioactive dose recovered)			
		Group 2 10 mg/kg		Group 4 300 mg/kg	
		Male (n=4)	Female (n=4)	Male (n=4)	Female (n=4)
Urine	0-8 h	7.3	23	2.8	5.5
	8-24 h	6.5	6.3	4.3	3.3
	24-48 h	1.3	0.7	0.9	1.1
	48-72 h	0.2	0.1	0.2	0.3
	72-96 h	0.1	<0.1	<0.1	0.1
	96-120 h	<0.1	°<0.1	<0.1	°0.1
	120-144 h	°<0.1	°<0.1	°<0.1	°<0.1
	144-168 h	°<0.1	°<0.1	°<0.1	°<0.1
	<i>Subtotal</i>	15	30	8.3	10
Feces	0-24 h	68	59	90	78
	24-48 h	9.3	3.4	4.1	2.0
	48-72 h	0.8	0.3	0.5	0.3
	72-96 h	0.2	0.1	0.1	0.1
	96-120 h	0.1	0.1	<0.1	°0.1
	120-144 h	<0.1	°<0.1	°<0.1	°0.1
	144-168 h	<0.1	°<0.1	°<0.1	°<0.1
	<i>Subtotal</i>	79	63	94	80
Cage wash		6.5	10	7.2	6.8
GI tract		<0.1	°<0.1	°<0.1	°<0.1
GI tract contents		<0.1	°<0.1	°<0.1	°<0.1
Carcass		°0.1	°0.1	°0.1	°0.3
Total Recovery		101	103	110	98

* = Below limit of detection

Single low dose: As summarized in Tables 6.1.1-49 and 6.1.1-50, following a single oral dose of phenyl or pyrazole labelled [14C]-SYN545974 at 10 mg/kg to male and female mice, the major route of elimination was *via* the feces, accounting for around 63-79% of the dose. Urinary excretion accounted for 15-30% of the dose.

Single high dose: As summarized in Tables 6.1.1-49 and 6.1.1-50, following a single oral dose of phenyl or pyrazole labelled [14C]-SYN545974 at 300 mg/kg to male and female mice, the major route of elimination was *via* the feces, accounting for around 76-94% of the dose. Urinary excretion accounted for 7.2-15% of the dose.

Metabolite characterization studies: Representative pooled samples of urine, feces and cage wash were analysed to determine the metabolic profile. The following metabolites of SYN545974 shown in Table 6.1.1-51 were identified in the mice.

Table 6.1.1–51: Metabolites of [^{14}C]-SYN545974 found in mice

Metabolite Name	Structure
SYN545974	
SYN547890	
SYN547897	
Hydroxylated Metabolites	Hydroxy SYN545974
Dihydroxylated Metabolites	Dihydroxy SYN545974
Hydroxylated Desmethyl Metabolites	Desmethyl hydroxy SYN545974

Table 6.1.1–51: Metabolites of [14C]-SYN545974 found in mice (continued)

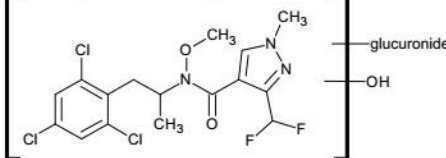
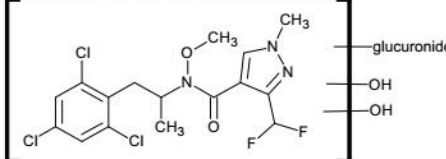
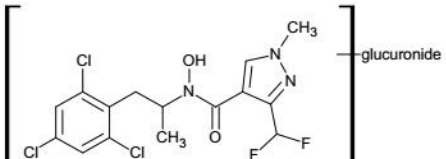
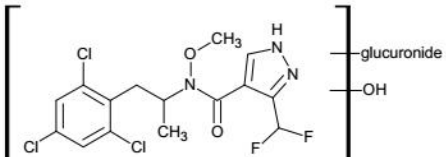
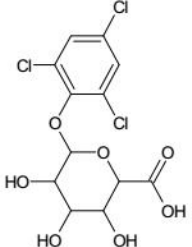
Metabolite Name	Structure
Glucuronide Conjugates	<p>Hydroxy SYN545974 glucuronide</p>  <p>Dihydroxy SYN545974 glucuronide</p>  <p>Desmethyl SYN545974 glucuronide</p>  <p>Desmethyl hydroxy SYN545974 glucuronide</p> 
Glucuronide Conjugates (continued)	<p>(2,4,6 TCP) Glucuronide</p> 

Table 6.1.1–51: Metabolites of [14C]-SYN545974 found in mice (continued)

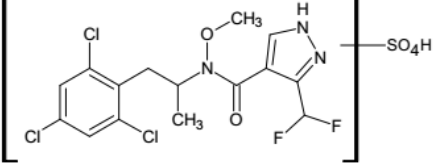
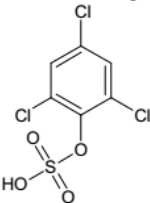
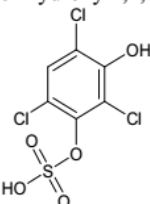
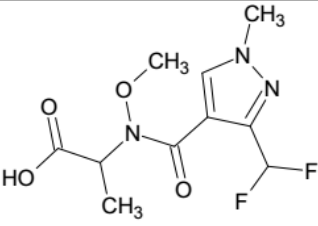
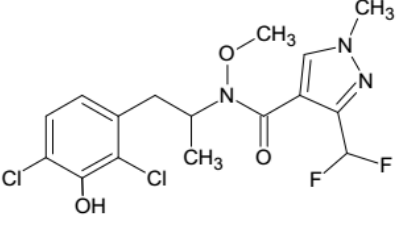
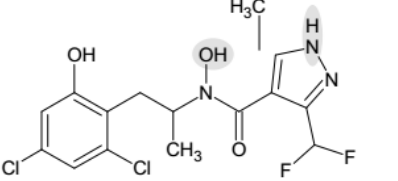
Metabolite Name	Structure
Sulphate Conjugates	<p>Desmethyl hydroxy SYN545974 sulphate</p>  <p>2,4,6 TCP sulphate</p>  <p>3-Hydroxy 2,4,6 TCP sulphate</p> 
Pyrazole Half Molecule Metabolites	 <p>SYN548263</p>
Dechlorinated and Hydroxylated Metabolites	<p>Dechlorinated hydroxy SYN545974</p>  <p>Dechlorinated desmethylhydroxy SYN545974</p> 

Table 6.1.1–52: Metabolite profile in urine of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg

Sample code			U1	U2	U5	U6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Urine			
			0-48 h		0-72 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	12.4	ND	ND	0.16	ND
Unassigned	NA	23.4	ND	0.26	ND	ND
Unassigned	NA	23.9	ND	0.36	ND	ND
Unassigned	NA	25.5	0.34	0.21	ND	ND
Unassigned	NA	26.4	ND	0.2	ND	ND
Unassigned	NA	27.7	0.35	0.47	ND	ND
Unassigned	NA	28	ND	0.43	0.24	ND
Unassigned	NA	29.1	0.51	0.6	ND	0.29
Desmethyl hydroxy SYN545974 glucuronide	604.0462	30.1	1.53	1.72	0.46	0.91
Dihydroxy SYN545974 glucuronide	634.0568					
Unassigned	NA	31.1	0.44	0.93	0.16	0.31
3-Hydroxy 2,4,6 TCP sulphate	290.8684	31.5	1.3	4.06	0.23	ND
2,4,6 TCP glucuronide	370.9498	32.2	ND	ND	1.06	3.24
Desmethyl hydroxy SYN545974 sulphate	505.9564	32.2	ND	ND	0.15	ND
Hydroxy SYN545974 glucuronide	618.0619	33.7	2.05	2.67	0.09	1.45
Desmethyl hydroxy SYN545974 glucuronide	604.0462	33.9	ND		ND	
Unassigned	NA	34	ND	ND	0.76	ND
Desmethyl hydroxy SYN545974 glucuronide	604.0462	34.8	0.18	0.77	0.25	0.92
Unassigned	NA	36	ND	ND	ND	0.31
Didesmethyl hydroxy SYN545974	NA	36.8	0.3	0.5	ND	ND
Dihydroxy SYN545974	NA	37.8	0.22	0.21	ND	ND
Desmethyl hydroxy SYN545974 glucuronide	604.0462	39.3	0.19	0.23	0.17	0.8
Unassigned	NA	40.5	0.25	0.69	ND	0.61
Desmethyl hydroxy SYN545974	428.0142	41.6	0.29	ND	0.21	ND
Desmethyl hydroxy SYN545974 sulphate	505.9564	42.0	0.14	0.21	0.19	0.35
Unassigned	NA	43.3	0.15	ND	ND	ND
2,4,6 TCP sulphate	274.8745	43.7	5.32	6.38	2.28	3.59
Unassigned	NA	46.1	ND	0.5	ND	ND

Table 6.1.1–52: Metabolite profile in urine of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (continued)

Sample code			U1	U2	U5	U6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Urine			
			0-48 h		0-72 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Desmethyl hydroxy sulphate	505.9564	47.6	0.2	0.64	0.08	0.29
Desmethoxy-hydroxy SYN545974	412.0192	49.9	ND	0.22	ND	0.38
Dechlorinated desmethyl hydroxyl SYN545974	394.0531	50.4	0.77	0.16	0.61	0.4
Total Assigned			12.49	17.77	5.78	12.33
Total Unassigned			2.04	4.65	1.32	1.52
PES			0.19	0.32	0.1	0.23
Total			14.7	22.7	7.2	14.1
Losses on processing			-0.02	-0.04	0.00	0.02

Table 6.1.1–53: Metabolite profile in urine of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg

Sample code			U3	U4	U7	U8
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Urine			
			0-48 h		0-72 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	3.9	ND	0.19	ND	ND
Unassigned	NA	7.9	ND	1.39	0.31	0.25
Unassigned	NA	9	0.63	0.5	ND	ND
Unassigned	NA	14.9	0.27	0.49	ND	ND
Unassigned	NA	17.5	1.38	2.47	0.43	0.48
Unassigned	NA	18.8	ND	ND	ND	0.22
Unassigned	NA	20.6	ND	0.4	0.39	ND
Unassigned	NA	21.1	0.59	1.05	0.22	0.19
Unassigned	NA	22.2	ND	0.44	ND	ND
Unassigned	NA	23.4	2.39	3.59	0.9	0.92
Unassigned	NA	24	ND	0.89	0.45	ND
SYN548263	278.0947	26.1	6.19	9.35	2.17	3.91
Desmethyl hydroxy SYN545974 glucuronide	604.0462	30.1	1.75	2.27	0.36	0.56
Dihydroxy SYN545974 glucuronide	634.0568		ND	ND		
Unassigned	NA	31.1	ND	0.87	ND	0.35
Unassigned	NA	31.9	ND	0.48	0.2	ND
Unassigned	NA	32.8	ND	0.83	0.26	ND
Hydroxy SYN545974 glucuronide	618.0619	33.7	1.09	1.94	0.39	1.26
Desmethyl hydroxy SYN545974 glucuronide	604.0462	33.7	ND	ND		
Unassigned	NA	35.1	ND	ND	0.58	0.45
Unassigned	NA	37.7	ND	ND	0.25	ND
Unassigned	NA	38.4	ND	ND	0.15	ND
Unassigned	NA	39.4	ND	ND	0.15	0.23
Hydroxy SYN545974 glucuronide	618.0619	40.4	ND	0.83	0.12	0.14
Hydroxy SYN545974 glucuronide	618.0619	41.8	ND	0.34	ND	0.24
Unassigned	NA	44.4	ND	ND	ND	0.18

Table 6.1.1–53: Metabolite profile in urine of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (continued)

Sample code			U3	U4	U7	U8
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Urine			
			0-48 h		0-72 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	47.1	ND	0.4	ND	ND
Desmethyl hydroxy SYN545974 sulphate	505.9664	47.6	0.32	0.7	ND	0.38
Unassigned	NA	49	0.41	ND	0.1	ND
Unassigned	NA	63.7	ND	ND	0.34	ND
Unassigned	NA	83.1	ND	ND	0.39	0.28
Total Assigned			9.35	15.43	3.04	6.49
Total Unassigned			5.67	13.99	5.12	3.55
PES			0.18	0.48	0.05	0.16
Total			15.20	29.90	8.20	10.20
Losses on processing			0.00	0.00	-0.01	0.00

Table 6.1.1–54: Metabolite profile in feces of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg

Sample code			F1	F2	F5	F6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Feces			
			0-48 h		0-48 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Femal
Unassigned	NA	13.8	0.56	ND	ND	ND
Unassigned	NA	24.5	0.87	0.53	ND	ND
Unassigned	NA	26.1	1.15	ND	ND	ND
Unassigned	NA	29.3	0.65	0.72	ND	ND
Unassigned	NA	30.3	0.6	ND	ND	ND
Unassigned	NA	32.2	1.22	1.24	ND	ND
Unassigned	NA	33	0.47	0.78	ND	ND
Unassigned	NA	33.6	1.37	ND	ND	ND
Didesmethyl dihydroxy SYN545974	413.9985	36.8	2.62	7.32	ND	0.5
Dihydroxy SYN545974	458.0247	37.8	4.64	4.41	0.99	0.84
Unassigned	NA	38.9	1.13	ND	ND	ND
Desmethyl hydroxy SYN545974	428.0142	40.6	1.04	1.57	2.05	1.13
Desmethyl hydroxy SYN545974	428.0142	41.6	4.12	2.31	0.66	0.51
Desmethyl hydroxy SYN545974	428.0142	42.1	ND	ND	0.4	0.41
Didesmethyl hydroxy SYN545974	413.9985	45.5	4.09	6.22	2.99	1.73
Didesmethyl SYN545974	398.0036	46.3	1.22	ND	ND	ND
Unassigned	NA	46.7	0.85	ND	ND	ND
Desmethyl hydroxy SYN545974	428.0142	48.6	6	6.84	4.97	2.83
Desmethoxy hydroxy SYN545974	412.0192	49.9	1.32	ND	ND	ND
Unassigned	NA	50.1	1.22	ND	ND	ND
Unassigned	NA	51.3	1.27	ND	ND	ND

Unassigned	NA	52.3	2.15	1.05	ND	ND
Unassigned	NA	54.3	0.75	ND	ND	ND
Dechlorinated hydroxy SYN545794	408.0688	55.6	4.18	2.55	0.75	0.76
Didesmethyl SYN545974	398.0036					
SYN547897	442.0298	56.5	7.2	5.77	4.19	2.9
Unassigned	NA	58.8	ND	1.41	ND	ND
SYN547890	412.0192	59.4	2.48	2.01	1.91	1.92

Table 6.1.1–54: Metabolite profile in feces of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (continued)

Sample code			F1	F2	F5	F6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Feces			
			0-48 h		0-48 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	62.6	1.26	ND	ND	ND
SYN545974	426.0349	66.8	4.42	1.08	48.79	47
Unassigned	NA	70.6	0.42	ND	ND	ND
Total Assigned			43.33	40.08	67.7	60.53
Total Unassigned			15.94	5.73	0	0
Not profiled			0.72	0.43	3.67	2.9
PES			11.34	12.48	9.67	5.33
Total			72.2	61.5	83.4	74
Losses on processing			0.87	2.78	2.36	5.24

Table 6.1.1–55: Metabolite profile in feces of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg

Sample code			F3	F4	F7	F8
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Feces			
			0-48 h		0-48 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	9.5	ND	0.51	ND	ND
Unassigned	NA	11.5	0.84	ND	ND	ND
Unassigned	NA	16.6	ND	0.57	ND	ND
Unassigned	NA	18.6	ND	0.86	ND	1.81
Unassigned	NA	24.3	ND	0.75	ND	3.12
SYN548263	278.0947	26.1	2.41	1.48	4.74	3.72
Unassigned	NA	32.2	1.13	2.73	ND	2.01
Unassigned	NA	34.1	0.95	ND	ND	ND
Didesmethyl hydroxy SYN545974	413.9985	36.8	1.58	5.78	1.69	3.27
Dihydroxy SYN545974	458.0247	37.8	5.76	3.75	4.65	2.32
Desmethyl hydroxy SYN545974	428.0142	40.6	1.45	2.65	2.2	2.13
Desmethyl hydroxy SYN545974	428.0142	41.6	3.53	3.49	2.27	ND
Desmethyl hydroxy SYN545974	428.0142	42.1	ND	ND	1.8	ND

Unassigned	NA	44.2	ND	1.07	ND	ND
Unassigned	NA	44.9	ND	1.13	ND	ND
Didesmethyl hydroxy SYN545974	413.9985	45.5	5.08	3.48	2.15	2.57
Unassigned	NA	46.4	1.16	ND	ND	ND
Unassigned	NA	47	1.23	ND	ND	ND
Desmethyl hydroxy SYN545974	428.0142	48.6	6.72	7.36	9.31	7.63
Desmethoxy hydroxy SYN545974	412.0192	49.9	1.7	0.97	ND	ND
Unassigned	NA	50	ND	0.79	ND	ND
Unassigned	NA	51.2	ND	0.58	ND	ND
Desmethyl hydroxy SYN545974	428.0142	52.3	1.39	0.46	ND	ND
Dechlorinated hydroxy SYN545974	408.0688	55.6	2.48	2.25	0.9	ND
Didesmethyl SYN545974	398.0036					
SYN547897	442.0298	56.5	11.35	5.35	6.73	4.15
SYN547890	412.0192	59.4	5.96	1.99	5.01	2.55

Table 6.1.1–55: Metabolite profile in feces of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (continued)

Sample code			F3	F4	F7	F8
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Feces			
			0-48 h		0-48 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	62.3	0.88	ND	ND	ND
SYN545974	426.0349	65.4	1.14	0.58	44.28	36.93
Unassigned	NA	67.2	1.39	0.45	ND	ND
Unassigned	NA	80.3	ND	0.51	ND	ND
Unassigned	NA	87.6	1.15	ND	ND	ND
Total Assigned			50.55	39.59	85.73	65.27
Total Unassigned			8.73	9.95	0	6.94
Not profiled			0.23	0.31	3.56	3.1
PES			11.9	10.17	4.6	4.3
Total			77.8	62.8	93.8	79.6
Losses on processing			6.39	2.78	-0.09	-0.01

Table 6.1.1–56: Metabolite profile in cage wash of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg

Sample code			CW1	CW2	CW5	CW6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Cage wash			
			0-24 h	0-48 h	0-24 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Unassigned	NA	3.2	ND	0.15	0.09	0.08
Unassigned	NA	24.6	0.15	ND	ND	ND
Unassigned	NA	26.1	ND	ND	0.11	0.11
Unassigned	NA	26.8	0.12	ND	ND	0.07

Unassigned	NA	27.6	0.11	ND	0.13	ND
Unassigned	NA	28.4	0.12	ND	ND	0.08
Unassigned	NA	28.8	0.32	0.35	0.08	0.07
Unassigned	NA	29.6	0.82	ND	0.07	0.06
Desmethyl hydroxy SYN545974 glucuronide	604.0462	30.1	ND	1.14	0.22	0.22
Unassigned	NA	31.8	0.22	0.47	0.09	ND
Unassigned	NA	32.3	0.14	0.18	0.25	0.21
Unassigned	NA	33.2	0.54	ND	ND	ND
Unassigned	NA	34.1	ND	ND	0.14	0.25
Hydroxy SYN545974 glucuronide	618.0619	34.7	ND	0.48	0.35	0.35
Unassigned	NA	35.2	ND	0.35	ND	ND
Unassigned	NA	35.9	0.08	0.36	ND	ND
Unassigned	NA	37.7	0.22	0.28	0.12	0.16
Unassigned	NA	38.3	ND	ND	ND	0.09
Dihydroxy SYN545974	458.0247	38.8	ND	0.3	0.27	0.26
Unassigned	NA	39.4	ND	ND	ND	0.06
Unassigned	NA	40.1	ND	ND	ND	0.09
Unassigned	NA	40.6	0.15	0.19	ND	0.07
Unassigned	NA	40.9	ND	ND	ND	0.07
Desmethyl hydroxy SYN545974	428.0142	41.6	ND	0.51	0.13	0.29
Desmethyl hydroxy SYN545974	428.0142	42.8	ND	ND	0.27	0.1
Desmethyl hydroxy SYN545974	428.0142	42.1	0.13	0.33	ND	0.1
Unassigned	NA	43.3	ND	ND	ND	0.11
Unassigned	NA	44.5	ND	0.2	0.06	0.09
Unassigned	NA	45.4	ND	ND	ND	0.08

Table 6.1.1–56: Metabolite profile in cage wash of mice following a single oral dose of [Phenyl-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (Continued)

Sample code			CW1	CW2	CW5	CW6
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Cage wash			
			0-24 h	0-48 h	0-24 h	
			10 mg/kg		300 mg/kg	
			Male	Female	Male	Female
Didesmethyl hydroxy SYN545974	413.9985	45.5	ND	ND	0.15	0.25
Unassigned	NA	47.5	0.26	ND	ND	0.13
Unassigned	NA	48.1	ND	ND	0.09	ND
Unassigned	NA	49	ND	ND	0.29	0.08
Desmethyl-hydroxy SYN545974	428.0142	49.6	ND	0.41	0.98	0.78
Desmethoxy-hydroxy SYN545974	412.0192	49.9	ND	ND	0.19	0.06
Unassigned	NA	51.8	ND	ND	1.47	1.05
Unassigned	NA	54.7	ND	ND	0.08	0.06
Unassigned	NA	55.1	ND	ND	ND	0.08
SYN547897	442.0298	56.5	0.08	ND	0.58	0.18
Dechlorinated hydroxy SYN545974	408.0688	58.9	ND	ND	0.07	0.07
Unassigned	NA	60.8	ND	ND	0.09	ND

SYN545974	426.0349	66.8	ND	0.14	0.08	0.09
Unassigned	NA	79.9	0.13	ND	ND	ND
Unassigned	NA	80.7	0.07	ND	ND	ND
Total Assigned			0.21	3.31	3.29	2.75
Total Unassigned			3.45	2.53	3.16	3.15
PES			2.75	3.26	NA	0.72
Total			8.1	12.5	7.9	7.7
Losses on processing			1.69	6.13	1.45	1.08

Table 6.1.1–56: Metabolite profile in cage wash of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (Continued)

Sample code			CW3	CW4	CW7
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Cage wash		
			0-24 h		0-24 h
			10 mg/kg		300 mg/kg
			Male	Female	Male
Unassigned	NA	2.8	ND	0.91	ND
Unassigned	NA	7.9	0.07	ND	ND
Unassigned	NA	8.3	0.06	0.08	ND
Unassigned	NA	8.7	ND	0.13	ND
Unassigned	NA	9.2	0.07	ND	ND
Unassigned	NA	9.8	0.06	ND	ND
Unassigned	NA	10.8	0.07	ND	ND
Unassigned	NA	14.4	ND	ND	0.11
Unassigned	NA	15	ND	ND	0.09
Unassigned	NA	15.8	0.13	0.19	0.27
Unassigned	NA	17.3	0.14	0.14	ND
Unassigned	NA	18	0.44	0.1	0.09
Unassigned	NA	19.8	0.1	0.07	ND
Unassigned	NA	21.1	0.07	0.11	ND
Unassigned	NA	23.4	0.87	0.7	0.72
Unassigned	NA	26.4	ND	0.12	ND
SYN548263	278.0947	26.1	1.33	1.56	1.69
Unassigned	NA	28.3	ND	0.1	ND
Unassigned	NA	29.2	0.06	ND	ND
Unassigned	NA	29.9	0.16	ND	0.09
Desmethyl hydroxy SYN545974 glucuronide	604.0462	30.1	0.41	0.26	0.2
Unassigned	NA	32	ND	0.15	ND
Unassigned	NA	33.1	ND	0.15	0.07
Hydroxy SYN545974 glucuronide	618.0619	33.7	0.18	0.12	0.2
Unassigned	NA	37.6	ND	0.23	ND
Unassigned	NA	38.4	ND	ND	0.11
Unassigned	NA	38.9	0.1	0.4	0.14
Unassigned	NA	40.1	ND	0.07	ND

Table 6.1.1–56: Metabolite profile in cage wash of mice following a single oral dose of [Pyrazole-¹⁴C]-SYN545974 at 10 mg/kg and 300 mg/kg (Continued)

Sample code			CW3	CW4	CW7
Assignment	<i>m/z</i> (+/-)	Approx RT (min)	% Dose in Cage wash		
			0-24 h		0-24 h
			10 mg/kg		300 mg/kg
			Male	Female	Male
Desmethyl hydroxy SYN545974 (a)	428.0142	41.6	ND	0.14	ND
Desmethyl hydroxy SYN545974 (a)	428.0142	42.1	ND	0.12	ND
Unassigned	NA	43.1	0.1	0.41	0.13
Unassigned	NA	46.4	0.18	0.2	0.2
Desmethyl-hydroxy SYN545974	428.0142	48.6	0.18	0.26	0.53
Desmethoxy-hydroxy SYN545974	412.0192	49.9	0.29	ND	ND
Unassigned	NA	55.1	0.09	ND	ND
Unassigned	NA	56.7	ND	0.04	ND
SYN547897	442.0298	56.5	0.38	0.2	0.24
Unassigned	NA	60.9	ND	0.08	0.07
SYN545974	426.0349	66.8	ND	ND	1.26
Unassigned	NA	79.4	ND	0.05	ND
Unassigned	NA	82.7	ND	ND	0.07
Total Assigned			2.77	2.66	4.12
Total Unassigned			2.77	4.43	2.16
PES			NA	0.55	0.22
Total			5.8	8.6	8.3
Losses on processing			0.26	0.96	1.80

SYN545974 metabolites in urine: Urine was the minor excretion route for SYN545974 and its related products with 7.2-30% dose recovered. Metabolites detected in urine were numerous and generally comprised a low percentage of the administered dose. Unchanged SYN545974 was a minor component in urine at $\leq 0.1\%$ of the dose. The major urinary metabolites were qualitatively similar between males and females and irrespective of dose, with no significant quantitative differences observed in urine between sexes.

At least 22 metabolites were detected, but only two metabolites accounted for $>5\%$ of the dose in urine. These were the phenyl specific metabolite 2,4,6-TCP (TCP) sulphate at up to 6.4% of the administered dose and the corresponding pyrazole specific metabolite, SYN548263, at up to 9.4% of the administered dose. Other phenyl specific metabolites identified were 3-hydroxy TCP and TCP glucuronide at $\leq 4.1\%$ and $\leq 3.2\%$ of the administered dose, respectively. However, no further pyrazole specific metabolites were identified in urine.

Metabolites containing both the phenyl and pyrazole rings were identified as various products of hydroxylation alone or in combination with demethylation or with demethylation and dechlorination. In most cases, these were excreted as their corresponding glucuronide or sulphate conjugate. No single metabolite containing both rings accounting for $>2.7\%$ of the dose.

Up to 9 unidentified components were observed in urine from the phenyl label dosed mice, but in total accounted for $\leq 4.7\%$ of the dose. In the urine from the pyrazole label dosed animals, up to 15 unidentified components were observed. In total, these accounted for $\leq 14\%$ dose with no individual component exceeding 3.6% dose.

SYN545974 metabolites in feces: After a 10 mg/kg dose, unchanged SYN545974 accounted for 0.6-4.4% dose in feces. However, in feces from animals that were dosed with 300 mg/kg, unchanged SYN545974 accounted for 37-49% dose.

At least 15 components were observed in feces. The most abundant metabolites, which accounted for >5% of the administered dose, were SYN547897 ($\leq 11\%$ dose), SYN547890 ($\leq 6.0\%$), a desmethyl hydroxy SYN545974 isomer ($\leq 9.3\%$), a dihydroxy isomer ($\leq 5.8\%$ dose) and a didesmethyl hydroxy SYN545974 isomer ($\leq 7.3\%$). The only cleavage product observed was the pyrazole metabolite SYN548263, which accounted for 1.5-4.7% of the dose. Metabolites containing both the phenyl and pyrazole rings were identified as various products of hydroxylation alone or in combination with demethylation and/or dechlorination. The most abundant metabolites were identified as desmethyl hydroxy SYN545974 ($\leq 9.3\%$ dose).

At least 17 components remained unidentified with no single component individually accounting for >3.1% dose.

SYN545974 metabolites in cage wash: At least 34 components were present in the cage wash from mice given either the phenyl or pyrazole ^{14}C labelled SYN545974 dose at 10 or 300 mg/kg. The radio profiles and the significant metabolites identified were consistent with those observed in the mouse excreta samples.

CONCLUSION:

The metabolism and excretion of [phenyl- ^{14}C] and [pyrazole-5- ^{14}C]-pydiflumetofen was investigated following oral doses of 10 or 300 mg/kg bw, to groups of 4 male and 4 female 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.

Excretion

Mean recovery of radioactivity was > 90% for all groups. Excretion was generally comparable irrespective of radiolabel and sex. After single oral administration of 10 mg/kg bw, the main excretion route was via the faeces for both radiolabels (up to 79 and 63% AD in males and females respectively) and urine acted as the minor route (up to 30% AD). In the high dose groups, faeces excretion increased up to 94% AD in males and 80% AD in females whereas urine excretion decreased for both sexes (7.2-15% AD). The majority of the administered radioactivity (87-97%) was excreted in the first 24 hours. Radioactivity was low in the carcass ($\leq 0.1\%$) for all groups indicating excretion was essentially complete by 168 h post-dose, with 0.3% or less remaining in the carcass and gastrointestinal tract. HSE notes that the expired air was not investigated as an excretion route in this study. However, considering expired air was not found to be a significant route of excretion in the rat and recovery of radioactivity was > 90% in all groups, excretion can be considered complete.

Biotransformation

Pydiflumetofen was extensively metabolized after a single oral dose in the mouse. Metabolites were identified in the faeces, urine and cage wash. Generally, metabolite profiles were similar irrespective of sex, dose and radiolabel (except for a limited number of radiolabel-specific metabolites). In total up to 86% of the AD was identified. Across all individual samples, up to 24 unknown metabolites accounting in total for 0.0-15.9% of the AD were observed, with no unidentified component accounting for greater than 3.6% of the AD.

Urine

Unchanged pydiflumetofen was not detected in urine above 0.1% of the AD in any group. The most abundant urinary metabolites were qualitatively similar between males and females and irrespective of dose, with no significant quantitative differences observed between sexes. Only two metabolites were present > 5% of the AD, the phenyl-specific metabolite 2,4,6-TCP sulphate (6.4% AD in 10 mg/kg bw females) and the pyrazole-specific metabolite SYN548263 (9.4% AD in 10 mg/kg bw females). No metabolites were present > 10% of the AD in the urine.

Faeces

At 10 mg/kg bw, unchanged pydiflumetofen was a minor component, only present up to 4.4% of the AD. However at 300 mg/kg bw, unchanged pydiflumetofen was a major component present up to 49% of the

AD. At least 15 components were observed in faeces. Seventeen components remained unidentified with no single component individually accounting for >3.1% of the AD. SYN547897 was present up to 11.35% of the AD in 10 mg/kg bw males (pyrazole radiolabel), however was < 10% (5.35%) of the AD in 10 mg/kg bw females (pyrazole radiolabel). Multiple metabolites were present > 5% of the AD in the faeces including SYN547890 (6.0% AD in 10 mg/kg bw males administered the pyrazole radiolabel), a desmethyl hydroxy SYN545974 isomer (up to 9.3% AD in 300 mg/kg bw males administered the pyrazole radiolabel) and a didesmethyl hydroxy SYN545974 isomer (7.3% AD in 10 mg/kg bw females administered the phenyl radiolabel). The only cleavage product observed was the pyrazole metabolite SYN548263, which accounted for 1.5-4.7% of the AD.

Cage wash

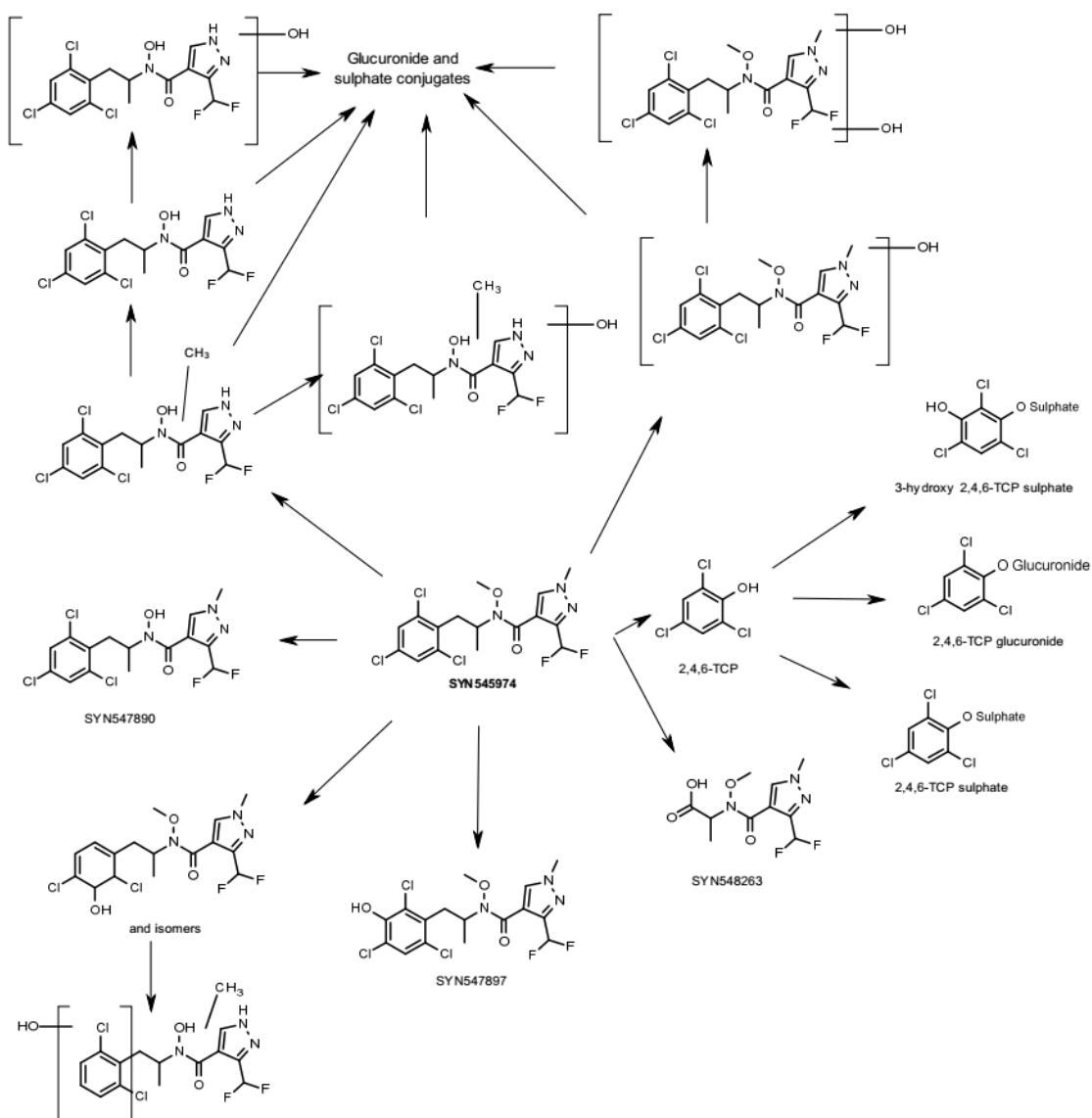
The metabolite profile for the cage wash was consistent with those of the urine and faeces irrespective of sex, dose or radiolabel.

Overall, under the conditions of this GLP and OECD TG-compliant metabolism and excretion study in the mouse, the main excretion route was via the faeces. The presence of unchanged pydiflumetofen in faeces indicated that up to 50% was unabsorbed at the high dose (300 mg/kg bw). Pydiflumetofen was extensively metabolized via metabolic processes including demethylation, hydroxylation, dechlorination, glucuronation and cleavage to create phenyl- or pyrazole- specific metabolites. These observations suggest that metabolism in the mouse is largely similar to that in the rat (██████████ and ██████████, 2015). No major metabolites (> 10% AD) were observed in the mouse (based on urine).

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 SYN545974 to give the pyrazole metabolite SYN548263 and the phenyl metabolite 2,4,6 TCP.
- Dechlorination and hydroxylation to SYN547894 and other dechlorinated hydroxy and dechlorinated dihydroxy metabolites.
- Glucuronic acid conjugation and sulphate conjugation.

Figure 6.1.1–5: Biotransformation Pathways Based on Identified Metabolites of SYN545974 in Mouse Urine, Feces and Cage Wash



(████████, ████████, ████████, and ████████, 2015)

Report: K-CA 5.1.1/09 ████████ (2015). SYN545974 - Oral (Gavage) Toxicokinetic Study in the Pregnant Rabbit. Final Report Amendment 3. ████████ Report No. ████████ Issue date 15 September 2015. Unpublished. Syngenta File No. SYN545974 10125.

Guidelines: Not Applicable

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rabbit blood has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

The objective of this study was to investigate the toxicokinetics of the test item, SYN545974, when administered daily, by oral gavage, to the pregnant rabbit from Days 6 to 27 of gestation.

Four groups of four sexually mature, timed-mated, female New Zealand White rabbits were dosed, once daily by oral gavage, with SYN545974 at 100, 300, 750 or 1000 mg/kg/day from Days 6 to 27 of gestation, inclusive.

Formulations used on Days 6, 13 and 27 of gestation were analysed to confirm homogeneity and determine achieved concentration. Animals were observed daily from arrival and weighed daily, from Days 5 to 28 of gestation inclusive. Food intake was recorded at intervals over Days 5 to 28 of gestation. Blood samples were taken at seven timepoints on each of Days 6, 13 and 27 of gestation for toxicokinetics. The animals were killed on Day 28 of gestation and a necropsy was performed including determination of pregnancy status and examination of the internal organs. SYN545974 was well tolerated when administered to the pregnant rabbit over Days 6 to 27 of gestation at doses of 100 mg/kg/day to 1000 mg/kg/day.

The toxicokinetics of SYN545974 in the pregnant rabbit were characterised by a sub-proportional increase in systemic exposure with dose, as measured by AUC₀₋₂₄, on all three days assessed. There were no deaths and clinical signs were restricted to one animal given 1000 mg/kg/day, with laboured breathing and inappetence of several days duration. With the exception of one female in the 100 mg/kg/day group, all animals were pregnant. There was no evidence of a treatment-related adverse effect on body weight or food intake. There were no treatment-related macroscopic post mortem findings.

The toxicokinetics of SYN545974 were characterised by a sub-proportional increase in systemic exposure with dose; with no apparent increase in systemic exposure between 750 and 1000 mg/kg/day.

MATERIALS AND METHODS

Materials:

Test Materials:

Name :	SYN545974
Source :	Syngenta Crop Protection
Physical state :	Off-white powder
Batch reference :	SMU2EP12007
Purity (%w/w) :	98.5 % w/w
Expiry date :	30 June 2016
Vehicle:	Aqueous carboxymethylcellulose (CMC), 1% (w/v)

Test Animals:

Strain and species	New Zealand White rabbit
Supplier	[REDACTED]
Number and sex	16 females
Mating	The females were obtained from the supplier time-mated. The day of mating was designated Day 0 of gestation. For mating, each female was mated with a stud male rabbit of the same strain and given an intravenous injection of 25IU Luteinising Hormone (LH) to stimulate ovulation.
Weight	On the first day of dosing, the females weighed 3.04 kg to 3.96 kg.
Age on arrival	3 to 4 months

Acclimatisation period	The animals were acclimatised within the study room for at least three days after arrival, towards the end of which they were re-examined to confirm their suitability for use.
Randomisation	Animals were allocated using a stratified body weight randomisation procedure based on individual body weights recorded on arrival (making sure that females mated with the same male were spread across the groups).
Identification	Each animal was individually identified by numbered ear tag.
Housing and environmental conditions	Females were housed individually in perforated-floor cages over paper lined trays.
	The study room was illuminated by fluorescent light set to give a cycle of 12 hours light and 12 hours dark and was air-conditioned. The target range for temperature was 16 °C to 20 °C. Recorded temperatures were within or marginally outside, these limits and ranged from 18 °C to 22 °C. Humidity was not controlled but was recorded
Diet and water	A pelleted diet, [REDACTED] Rabbit Diet (manufactured by [REDACTED]) supplied by [REDACTED] and mains tap water were freely available.
	From the day of arrival, approximately 30 g of hay was provided each day to animals as enrichment. Hay was supplied by [REDACTED].
Analysis	Certificates of analysis for pelleted diet, water, bedding and environmental enrichment devices, where supplied, are held centrally and retained within the [REDACTED] archives. It was considered that none of the contaminants that were monitored was present at a level that might have prevented the study objective from being achieved.

STUDY DESIGN AND METHODS

Study dates: Start: 25 July 2013; End: 7 November 2014

Dosing Regime:

Animals were assigned to 4 groups and administered SYN545974 suspended in 1% w/v aqueous CMC by gavage once daily as shown in the table below. Animals were dosed from Days 6 to 27 of gestation inclusive, at a dose volume of 5 mL/kg body weight, adjusted according to the most recent body weight.

Group	Number of females	Animal identification numbers	Dose (mg/kg/day) SYN545974	Target Formulation concentration (mg/mL)
1	4	35 - 38	100	20
2	4	39 - 42	300	60
3	4	43 - 46	750	150
4	4	47 - 50	1000	200

Sample Collection and Bioanalysis

Blood samples (0.1mL), were taken from the marginal ear vein of all animals on Days 6, 13 and 27 of gestation just before dosing and at 2, 4, 6, 8, 12 and 24 hours after dosing, into tubes containing K2EDTA anticoagulant. Immediately on sample collection, 0.05 mL of whole blood was accurately measured into a polypropylene tube containing exactly 0.05 mL of deionised water. Samples were mixed gently, then held on ice before being stored frozen (≤ -70 °C) until analysed. Residual whole blood was discarded. The blood:water samples were analysed for SYN545974 by LC-MS/MS.

Each analytical run consisted of eight calibration standards, processed in duplicate, Quality Control samples at three concentrations, processed at least in duplicate, and samples of blank rabbit plasma processed with and without internal standard. Study samples were bracketed by calibration standards and Quality Control samples in each analytical run. Only data from analytical runs which met SOP documented acceptance criteria were used.

Where study sample concentrations fell outside the analytical range of the method, or where analytical errors occurred, such samples were re-analysed singly, diluted appropriately with blank rabbit plasma if necessary. Where study samples were repeated, for example where unexpected concentrations within a pharmacokinetic profile were determined, the samples were repeated in duplicate and the final result was determined as per SOP requirements namely that 75 % of the calibration standards were required to be within ± 15 % of the nominal concentration (± 20 % at the LLQ), and 67 % of the QC samples were required to be within ± 15 % of the nominal concentration, including one at each concentration level.

Toxicokinetic evaluation

Toxicokinetic parameters were derived from the individual blood concentration-time profiles by non-compartmental analysis of the individual data using the validated system, Phoenix WinNonLin Professional 6.1. The reported toxicokinetic parameters consisted of the following:

C _{max}	the maximum observed blood concentration
T _{max}	the time C _{max} is observed
AUC ₀₋₂₄	the area under the blood concentration versus time curve from time zero to 24 h
R ₀	accumulation ratio calculated from the individual AUC ₀₋₂₄ (repeat dose) / AUC ₀₋₂₄ (single dose)

Nominal sampling times were used for all calculations of toxicokinetic parameters. AUC calculations were performed by the linear trapezoidal method, using raw data. Toxicokinetic parameters are displayed to three significant digits, except T_{max} which was displayed as nominal time. Group means were calculated for each day/group separately, as appropriate. Dose proportionality and degree of accumulation of the test item were evaluated by calculation of the appropriate ratios.

RESULTS

Mean formulation concentrations were within ± 6 % of the nominal concentration and all individual replicates within 9 % of nominal concentration on all three sampling occasions. All formulations were homogeneous with coefficients of variation of between 0.5 and 2.4 % (vs. acceptable limit of ≤ 5 %). All doses were based upon actual doses administered using the formulation concentrations.

The toxicokinetics of SYN545974 were consistent over Days 6, 13 and 27 of gestation as characterised by sub-proportional increases in peak concentration (C_{max}) and total systemic exposure (AUC₀₋₂₄), with no apparent increase in exposure between 750 and 1000 mg/kg/day (Table 6.1.1-57 - Table 6.1.1-59). Peak and total systemic exposure varied greatly between individual animals on each sampling occasion; however, there was a trend effect of sub-proportional increases in exposure despite the animal variability.

At dose levels of 100, 300, 750 or 1000 mg/kg/day animals were continuously exposed to SYN545974 over the 24 hours following dosing. T_{max} varied from 2 to 24 hours and, where reliably calculable, elimination half-lives (t_{1/2}) ranged from 5.3 to 7.5 hours.

On Day 13 of gestation there was no accumulation ranging from 0.6 to 0.9. By Day 27 of gestation, accumulation ratios were greater than 1.8 indicating possible accumulation. However, it should be noted that there was a high degree of individual variability.

With the exception of Female 38 (100 mg/kg/day) all animals were confirmed to be pregnant at necropsy.

Table 6.1.1-57: Mean Blood Concentrations (ng/mL) and Mean Toxicokinetic Parameters of SYN545974 on Day 6 of Gestation in Female Rabbits Following Oral (Gavage) Administration of SYN545974

Day 6	Dose (mg/kg/day)			
Timepoint (h)	100	300	750	1000
0	<5.0	<5.0	<5.0	<5.0
2	20.4	17.3	37.2	28.9

4	25.5	34.4	65.5	67.1
6	17.5	36.1	53.0	65.6
8	18.3	34.1	64.3	72.9
12	16.4	37.3	37.6	52.5
24	5.5	24.2	34.9	30.4
C _{max} (ng/mL)	26.4	44.1	71.2	79.3
t _{max} (hours)	2-8	2-24	4-8	4-12
t _{1/2} (hours)	NC	NC	NC	NC
AUC _{0-t last} (ng.h/mL)	254	722	1010	1140
AUC ₀₋₂₄ (ng.h/mL)	344	722	1010	1140
Dose-proportionality Ratio versus lowest dose		3.0	7.5	10.0
Dose-proportionality Ratio (C _{max})		1.7	2.7	3.0
Dose-proportionality Ratio (AUC ₀₋₂₄)		2.1	2.9	3.3

NC- not calculable

Table 6.1.1-58: Mean Blood Concentrations (ng/mL) and Mean Toxicokinetic Parameters of SYN545974 on Day 13 of Gestation in Female Rabbits Following Oral (Gavage) Administration of SYN545974

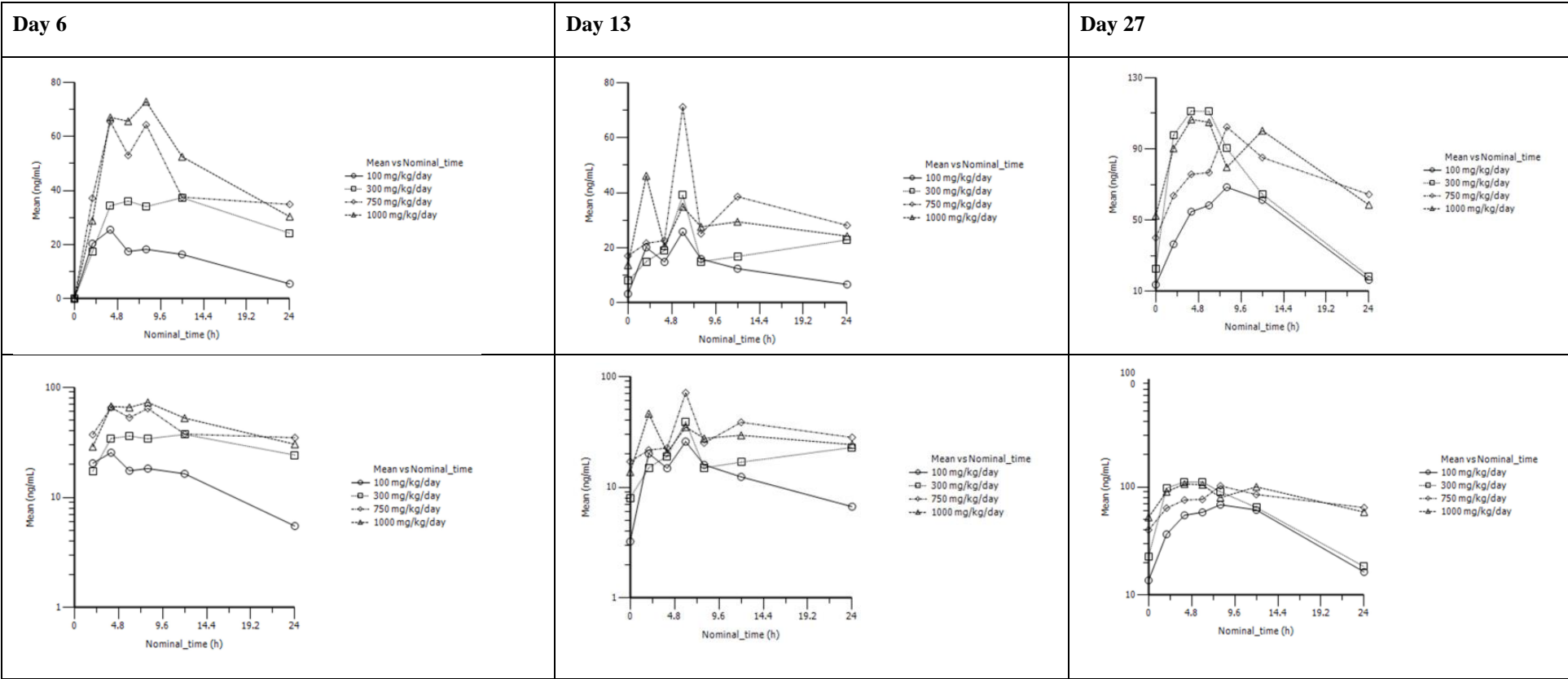
Day 13	Dose (mg/kg/day)			
Timepoint (h)	100	300	750	1000
0	3.23	7.98	17.0	13.7
2	20.1	15.0	21.6	46.1
4	14.8	19.0	22.6	20.7
6	25.8	39.1	71.1	34.9
8	15.9	15.0	25.1	27.6
12	12.4	16.8	38.6	29.4
24	6.67	22.9	28.1	24.2
C _{max} (ng/mL)	32.2	45.5	73.5	50.2
t _{max} (hours)	2-6	6-24	6-24	2-6
t _{1/2} (hours)	NC	NC	NC	NC
AUC _{0-t last} (ng.h/mL)	290	471	800	681
AUC ₀₋₂₄ (ng.h/mL)	314	471	800	681
Dose-proportionality Ratio versus lowest dose		3.0	7.5	10.0
Dose-proportionality Ratio (C _{max})		1.4	2.3	1.6
Dose-proportionality Ratio (AUC ₀₋₂₄)		1.5	2.5	2.2
R ₀ (AUC ₀₋₂₄ Day 13/Day 6)	0.9	0.7	0.8	0.6

Table 6.1.1-59: Mean Blood Concentrations (ng/mL) and Mean Toxicokinetic Parameters of SYN545974 on Day 27 of Gestation in Female Rabbits Following Oral (Gavage) Administration of SYN545974

Day 27	Dose (mg/kg/day)
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Timepoint (h)	100	300	750	1000
0	13.7	22.7	40.1	52.4
2	36.5	97.7	63.7	90.3
4	54.7	111	75.7	107
6	58.3	111	76.7	105
8	68.5	90.2	102	79.6
12	61.3	64.5	85.2	100
24	16.4	18.4	64.4	58.6
C _{max} (ng/mL)	87.5	118	102	116
t _{max} (hours)	4-12	4-8	8-8	4-12
t _{1/2} (hours)	NC	NC	NC	NC
AUC _{0-t last} (ng.h/mL)	1110	1560	1850	2050
AUC ₀₋₂₄ (ng.h/mL)	1110	1560	1850	2050
AUC _{INF} (ng.h/mL)	758	2300	NC	NC
Dose-proportionality Ratio versus lowest dose		3.0	7.5	10.0
Dose-proportionality Ratio (C _{max})		1.3	1.2	1.3
Dose-proportionality Ratio (AUC ₀₋₂₄)		1.4	1.7	1.8
R0 (AUC ₀₋₂₄ Day 27/Day 6)	3.2	2.2	1.8	1.8

Figure 6.1.1-6: Mean blood profiles of SYN545974 in female rabbits at 100, 300, 750 and 1000 mg/kg/day on Days 6, 13 and 27 of gestation



(upper: linear plot; lower: semi-log plot)

CONCLUSION:

The objective of this study was to investigate the pharmacokinetics of pydiflumetofen, when administered daily, by oral gavage, to pregnant rabbits. Four groups of 4 pregnant New Zealand White rabbits were dosed, once daily by oral gavage, with non-radiolabelled pydiflumetofen at 100, 300, 750 or 1000 mg/kg bw/d from days 6 to 27 of gestation, inclusive. Animals were observed daily from arrival and weighed daily. Food intake was recorded at intervals. Blood samples were taken at seven timepoints each on days 6, 13 and 27 of gestation for pharmacokinetic investigations. The animals were killed on day 28 of gestation and a necropsy was performed including determination of pregnancy status and examination of the internal organs.

Pydiflumetofen was well tolerated when administered to the pregnant rabbit over days 6 to 27 of gestation up to the limit dose of 1000 mg/kg bw/d. Clinical signs of toxicity (laboured breathing and inappetence over several days) were present only in one top-dose animal.

After single and multiple oral doses, systemic exposure (AUC) and maximum concentration in blood (C_{\max}) of pydiflumetofen increased sub-proportionally with dose. Systemic exposure and mean blood concentration seemed to plateau after 750 mg/kg bw. Bioaccumulation (R_0) was not apparent after 7 days dosing (gestation day 13); however it was up to 3.2 on GD 27. It was noted that variability between individual rabbits in all dose groups was very high which may affect the reliability of this study.

Mean $t_{1/2}$ could not be reliably calculated at any dose and individual values for t_{\max} varied from 2-24 hours in some groups. In addition, necropsy at the end of the study indicated one rabbit in the 100 mg/kg bw group was not pregnant and therefore was excluded from the group means.

Overall, under the conditions of this GLP-compliant but non-guideline pharmacokinetic study in pregnant rabbits, pydiflumetofen blood concentration and systemic exposure increased in a sub-proportional manner in relation to dose after single and multiple doses which is consistent with the rat and mouse pharmacokinetic findings. However, due to the unexplained high variability between individuals in all dose groups, the reliability of these findings is questioned.

(██████, 2015)

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

SYN545974 has been investigated for absorption by oral and intravenous routes and by oral route for metabolism and excretion in rats and mice (see section 6.1.1). In the absence of toxicity following administration of SYN545974 via the dermal route in the rat (Section 6.2.2 and 6.3.3) there is no requirement to provide data on absorption, distribution, metabolism and excretion following dermal exposure.

Additional considerations**(i) *Lack of a toxicokinetic study following repeated administration using radiolabeling***

HSE noted that although a pharmacokinetics study following a repeated oral administration of SYN545974 is available, this study was performed without radiolabeling. Thus, this study provides PK information on the parent SYN545974 only and not on the numerous related metabolites. Information is lacking regarding the bioaccumulation of metabolites, and in particular 2,4,6 TCP, after a repeated administration of the test substance. In addition, there is also a gap regarding the absorption of the tested substance SYN545974 after a repeated administration. Thus, a toxicokinetics study following a repeated oral administration (14 days) of the test substance with radiolabeling or a robust argumentation regarding the fate of metabolites, including 2,4,6-TCP, following a repeated administration of SYN545974 should have been submitted.

The Applicant answered to HSE that a toxicokinetic study following a repeated oral administration of radiolabelled SYN545974 was considered not necessary, based on the following criteria:

- In the single dose tissue distribution study ([REDACTED], 2015b) there was no indication of accumulation of any radioactive residue in any tissues. The depletion profile (half-life) from tissues mirrored that in blood/plasma. Radioactivity was widely distributed, with the highest concentrations of radioactivity observed in the liver and kidney at all sampling time points between 0.5 h and 120 h, consistent with the excretion profile of [14C]-SYN545974.

As noted by EFSA during the commenting period (Dec 2017), high levels of radioactivity were reported in tissues other than liver or kidney. The applicant has proposed the text below to reflect this request:

At some sampling time points, tissues such as the adrenals, renal fat, pancreas, thyroid and lungs had levels of radioactivity above that observed in blood. For the adrenals and renal fat, this could be related to the high levels observed in the kidney. At 96 h post dose, no tissue, except liver and kidney had radioactivity levels higher than in blood. Therefore, as the depletion of radioactivity mirrored that in the blood, this indicates that tissue levels of radioactivity are a function of distribution with no evidence of accumulation in these tissues.

- In the long term toxicity studies ([REDACTED], 2015a, b); there were no signs of specific organ toxicity attributed to accumulation of test item or metabolites.
- After repeat administration of SYN545974, induction of metabolism was observed with accumulation ratios of less than 0.4 at doses greater than 10 mg/kg, based on parent AUC ratios ([REDACTED], [REDACTED], 2014a). For SYN545974, this means that the parent molecule was metabolised at a faster rate, as seen with the lower systemic exposure. However, the extent of metabolism would remain similar, as once absorbed SYN545974 was completely metabolised at all doses, as indicated by the absence of parent and/or conjugates in urine or bile from bile duct cannulated animals.
- Induction of metabolism only takes place at doses ≥ 30 mg/kg/day, after repeat administration ([REDACTED], [REDACTED], 2014a). The highest estimate of human exposure taking account of primary crops, animal commodities and rotated crops, according to the proposed label in Europe is 0.0018 mg/kg bw/day for a child exposed to Dutch diet or 0.0014 mg/kg bw/day for an adult. Therefore, the maximum consumed concentration of parent will not be sufficient to induce metabolism. As such the single dose study is appropriate to determine the ADME properties of SYN545974.

The major metabolite observed in rodents (rats/mice) is 2, 4, 6-trichlorophenol (TCP). Previous studies demonstrate that once absorbed, TCP is rapidly conjugated and excreted, and does not accumulate upon repeat administration ([REDACTED] et al, 1981; [REDACTED] et al 1986: see Section 6.8.1). Ninety per cent of a 25 mg/kg ip dose of TCP was excreted in 4-6 h predominantly as conjugated material. At 10 h only trace amounts of TCP related material were found in the blood and tissues. Thirty minutes after the TCP dose, 70% of the administered trichlorophenol in blood was identified as conjugates, with the conjugated fraction increasing with time, facilitating excretion. The half-life of trichlorophenol related material was very similar in all the tissues studied, blood, brain, fat, kidney, liver and muscle, at 1.4-1.8 h.

In both the preliminary and main 14C studies ([REDACTED]; [REDACTED], 2015; [REDACTED], [REDACTED], 2015) the kinetics of SYN545974 dose related material (parent and metabolites) were shown to be non-proportional with respect to dose. The blood and excretion data for SYN545974 provide evidence that the dose non-proportionality is related to oral absorption. Following oral administration of [14C]-SYN545974, systemic exposure to total radioactivity increased in a sub proportional manner between the 5 mg/kg (lowest dose tested) and higher dose levels in whole blood and plasma for both males and females. This indicates that less compound was being absorbed into blood. Therefore, as the dose increased the fraction or amount of dose absorbed approaches equivalence and so even if the nominal dose was increased the systemic dose was effectively the same. Absorption was at least 85-90% of a 5 mg/kg oral gavage dose of [14C]-SYN545974. The excretion profile showed that less than 8% of the oral dose was excreted unchanged in faeces with no SYN545974 present in bile. As the dose increased, absorption became limited, with <55% of a 100 mg/kg dose to females and <24% of a 300 mg/kg dose to males absorbed. At these doses, unchanged SYN545974 was the major component in faeces, with up to 63% of the dose, but with less than 0.2% in bile. This demonstrates that as the dose increased the amount of SYN545974 absorbed decreased

and therefore systemic levels of metabolites would also not increase. The kinetic studies show that systemic exposure to SYN545974 increased in an approximately proportional manner after both single and repeat dose up to 30 mg/kg. Therefore, oral absorption is constant (85-90%) below 30 mg/kg. Systemic exposure starts to increase less than proportionally to the dose from between 30 and 100 mg/kg, indicating the fraction of dose absorbed decreases as the dose is increased above 30 mg/kg. The absorption following repeat administration should not change, as at the doses administered absorption is a passive process as evidenced by the increased parent in faeces and should not be influenced by repeat administration and therefore systemic levels of metabolites would not increase.

HSE is of the opinion that the justification provided by the applicant is sufficient.

- Bioaccumulation of parent/metabolites was not demonstrated in any tissues after a single oral dose of radiolabeled pydiflumetofen.
- Absorption and systemic exposure to the parent (and therefore its metabolites) is limited above doses of 30 mg/kg, as demonstrated in single and repeat dose studies.
- The major metabolite of pydiflumetofen observed in rodents (rats/mice) is 2,4,6-trichlorophenol (TCP). Specific kinetic studies performed with this compound demonstrate that once absorbed, 2,4,6-TCP is rapidly conjugated and excreted, and does not accumulate upon repeat administration (██████ et al, 1981; ██████ et al 1986: see Section 6.8.1).

Therefore, HSE agrees that the ADME dataset submitted for pydiflumetofen is sufficient.

(ii) *Comparative metabolism*

An *in vitro* comparative metabolism study of SYN545974 (Adepidyn) has been performed by the applicant and is summarised below.

Report: K-CA 5.1.1/10 ██████ (2017). Adepidyn – In Vitro Comparative Metabolism of [Phenyl-U-14C]Adepidyn and [Pyrazole-5-14C] Adepidyn in Human and Rat Liver Microsomes. Final Report Amendment 1. Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland Report No. 20160346. Issue date 03 April 2017. Unpublished. Syngenta File No. SYN545974_10511.

Guidelines: There are no specific testing regulations or guidelines applicable for this study. The data requirement was based on the Commission Regulation (EU) No 283/2013, 5.1.1, in accordance with Regulation (EC) No 1107/2009.

GLP: Signed and dated GLP and Quality Assurance statements were provided. There were no amendments and no deviations to the study plan.

HSE Comment: the study is considered acceptable.

EXECUTIVE SUMMARY

Study Design

In vitro metabolic profiling of Adepidyn was carried out by incubating human and rat liver microsomes (0.5 mg protein/mL) with 5 µM of [14C]Adepidyn and a NADPH-regenerating system for 60 minutes at 37°C. The study was conducted with two distinct 14C-labelled Adepidyn molecules, *i.e.* [phenyl-U-14C]Adepidyn and [pyrazole-5-14C]Adepidyn, a mixed gender pool of human liver microsomes and separated pools of male and female Wistar rat liver microsomes.

Time zero and negative controls without microsomes and without NADPH-regenerating system were also performed in order to determine the stability of Adepidyn in the incubation medium and to validate NADPH-dependent metabolism.

Positive controls, *i.e.* testosterone 6β-hydroxylation and 7-ethoxycoumarin *O*-dealkylation enzymatic activities, were used to check the metabolic competences of human and rat liver microsomes, respectively.

Results

- Positive control enzymatic activities showed that human and rat liver microsomes possessed metabolic competences in agreement with the acceptance criteria.
- Negative controls indicated that Adepidyn did not degrade in the incubation medium and that no metabolism of Adepidyn occurred without NADPH.
- An extensive metabolism of Adepidyn was observed in human and male rat liver microsomes. Adepidyn was metabolised to a lesser extent in female rat. Remaining parent compound accounted for < 10% of the dose in human, for < 5% in male rat and for approximately 60% in female after 60 minutes of incubation.
- Up to 15 radio-HPLC peaks (P1 to P15) were observed in human liver microsomes. P15 corresponded to unchanged Adepidyn. P2, P6, P12, P13 and P14 were the main metabolites. Metabolism of Adepidyn was NADPH-dependent and involved a cleavage pathway forming P2, P3, P4 and P13
- The metabolic pattern of Adepidyn in rat liver microsomes was qualitatively similar to human. All the human metabolites formed (P1 to P14) were detected in male rat, but only 9 in the female, due to the slower metabolism in the female.

In a validated *in vitro* test system, Adepidyn was extensively metabolised in human and male rat liver microsomes, with slower metabolism in female rat. All the metabolites observed in human liver microsomes were detected in male rat liver microsomes, and 9 out of 15 observed in female

MATERIALS AND METHODS

Materials

- | | |
|---|---|
| 1. Test item – phenyl label: | [Phenyl-U-¹⁴C]SYN545974 (company code) |
| Lot/Batch #: | 5447GAR001-6 |
| CAS #: | 1228284-64-7 |
| Purity: | 97.0% as per CoA; 98.5% as determined before use. |
| 2. Test item – pyrazole label: | [Pyrazole-5-¹⁴C]SYN545974 (company code) |
| Lot/Batch #: | 5446GAR001-4 |
| CAS #: | 1228284-64-7 |
| Purity: | 97.4% as per CoA; 99.1% as determined before use. |
| 3. Marker substrate 1: | Testosterone (for human liver microsomes) |
| Description: | Mixture of [4- ¹⁴ C]Testosterone and unlabelled Testosterone |
| Lot/Batch #: | 130812 for [¹⁴ C]Testosterone and BCBL3419V for Testosterone |
| CAS #: | 58-22-0 |
| Purity: | Radiochemical purity of 98.9% (determined before used) |
| Marker substrate 2: | 7-Ethoxycoumarin (for rat liver microsomes) |
| Description: | Mixture of [3- ¹⁴ C]7-Ethoxycoumarin and unlabelled 7-Ethoxycoumarin |
| Lot/Batch #: | 150514 for [3- ¹⁴ C]7-Ethoxycoumarin and 10707DJV for 7-Ethoxycoumarin |
| CAS #: | 31005-02-4 |
| Purity: | Radiochemical purity of 99.7% (determined before used) |
| 3. Reference item 1: | 6β-Hydroxytestosterone |
| Description: | Metabolite of Testosterone |
| Lot/Batch #: | FN04141413 |
| Purity: | 99.3 % |
| Reference item 2: | 7-Hydroxycoumarin |
| Description: | Metabolite of 7-Ethoxycoumarin |
| Lot/Batch #: | 1693700 |
| Purity: | 99.5 % |
| 4. <i>In vitro</i> test systems: | |
| Human liver microsomes: (HLM) | Pool of mixed gender human liver microsomes (100 male and 100 female) |
| Male rat liver microsomes: | Pool of male Wistar rat liver microsomes (200 animals) |

(Male RLM)

Female rat liver microsomes:

(Female RLM)

Pool of female Wistar rat liver microsomes (100 animals)

5. Reagents:

Incubation Buffer

NADPH-Regenerating System

(NADPH-GS)

100 mM potassium phosphate buffer system (pH 7.4; 3 mM MgCl₂)

1 mM NADP, 5 mM G6P and 1 Unit/mL G6PDH as final concentrations in the incubation medium

STUDY DESIGN AND METHODS

Study dates:

Study Initiation Date: January 03, 2017

Experimental Starting Date: January 06, 2017

Experimental Completion Date: January 27, 2017

Study Completion Date: March 09, 2017

Preparation of dosing solutions

Adepidyn dosing solutions were prepared at a target concentration of 500 µM by diluting [phenyl-U-¹⁴C]SYN545974 (Batch No. 5447GAR001-6) and [pyrazole-5-¹⁴C]SYN545974 (Batch No. 5446GAR001-4) in acetonitrile. Marker substrate dosing solutions (both testosterone and 7-ethoxycoumarin) were prepared by mixing ¹⁴C-labelled marker substrate and unlabelled marker substrate in acetonitrile. Target concentrations of [¹⁴C]testosterone and [¹⁴C]7-ethoxycoumarin dosing solutions were 25 mM and 50 mM, respectively.

In the microsomal assays, a 5 µL aliquot of Adepidyn or marker substrate dosing solution was added to the incubation medium which final volume was 0.5 mL. The final target concentration Adepidyn in microsomal assays was 5 µM. The final target concentration of testosterone and 7-ethoxycoumarin in microsomal assays was 250 µM and 500 µM, respectively. Final concentration of acetonitrile in microsomal assays was 1%, avoiding inhibition of metabolism enzymes.

Preparation of microsomal suspension

After thawing, HLM and RLM (20 mg protein/mL) were diluted at a ratio of 1:1 with a solution containing cryoprotectant substance (sucrose) to a concentration of 10 mg protein/mL.

In the microsomal assays, an aliquot of 25 µL of diluted microsomal suspension (10 mg protein/mL) was added to the incubation medium (final volume of 0.5 mL) in order to obtain a final microsomal protein concentration of 0.5 mg/mL.

Microsomal assays

Metabolic profiling of Adepidyn

In vitro metabolic profiling of Adepidyn was performed with a target concentration of test item of 5 µM. The same incubations conditions were used for both [phenyl-U-¹⁴C]Adepidyn and [pyrazole-5-¹⁴C]Adepidyn.

HLM and RLM (0.5 mg protein/mL) were pre-incubated with 5 µM of Adepidyn in 100 mM potassium phosphate buffer (pH 7.4; 3 mM MgCl₂) for 3 to 5 minutes in a shaking water bath at 37°C. Incubations were initiated by the addition of NADPH-GS. The samples were then incubated for 60 minutes in a shaking water bath at 37°C.

Time zero controls were performed for HLM and RLM incubated with Adepidyn. Microsomes, potassium phosphate buffer, NADPH-GS and [¹⁴C] Adepidyn were added to an equal volume of ice-cold solvent in order to stop the reaction immediately.

Negative controls

Control incubations without microsomes were performed to check the chemical stability of Adepidyn under incubation conditions. Microsomal suspension was replaced by an equal volume of potassium phosphate buffer.

Control incubations without cofactor (NADPH-GS) were performed to validate NADPH-dependent metabolism. Incubations were initiated after addition of potassium phosphate buffer instead of NADPH-GS.

Positive controls

Testosterone 6 β -hydroxylation, which is a marker substrate reaction for human CYP3A4/5 activity, was used as positive control to check the metabolic competences of HLM. HLM were incubated with 250 μ M of testosterone and NADPH-GS in 100 mM potassium phosphate buffer (pH 7.4; 3 mM MgCl₂) for 20 minutes in a shaking water bath at 37°C.

7-Ethoxycoumarin *O*-dealkylation, which is a marker substrate reaction for multiple cytochrome P450 activities, was used as positive controls to check the metabolic competences of RLM. RLM were incubated with 500 μ M 7-ethoxycoumarin and NADPH-GS in 100 mM potassium phosphate buffer (pH 7.4; 3 mM MgCl₂) for 20 minutes in a shaking water bath at 37°C.

Sample analysis

After termination of incubations by solvent protein precipitation and centrifugation, the radioactivity present in the resulting supernatant was measured by liquid scintillation counting (LSC) of 10 μ L aliquots in duplicate. Radioactivity remaining in the microsomal pellet was measured after solubilisation of the pellet. Solubilisation was achieved by incubating the pellet in 1 mL of Solvable (Perkin Elmer, Shelton, CT, USA) overnight at room temperature. The LSC measurement was performed on the whole solubilised pellet.

Supernatants were analysed by radio-HPLC. HPLC peak corresponding to parent [¹⁴C]Adepidyn was confirmed by co-chromatography with unlabelled Adepidyn. Radio-HPLC peaks corresponding to 6 β -hydroxytestosterone and 7-hydroxycoumarin (marker substrate metabolites used for determination of positive control enzymatic activities) were attributed by co-chromatography with the corresponding reference items.

Data analysis

The radioactivity recovery of each sample incubated with [¹⁴C] Adepidyn and marker substrate was determined by the sum of radioactivity measured in the supernatant and the microsomal pellet. Radioactivity recovery was considered valid if ranging between 90-110% of [¹⁴C] Adepidyn or marker substrate amount applied to the *in vitro* system.

Radio-HPLC quantification was done by integrating the area under the radio-chromatographic peaks. Quantification was done by using ROI's (regions of interest), i.e. background regions between radio-chromatographic peaks were not considered in the quantification.

The rates of testosterone 6 β -hydroxylation and 7-ethoxycoumarin *O*-dealkylation were calculated by quantifying 6 β -hydroxytestosterone and 7-hydroxycoumarin produced in human and rat liver microsomal incubates, respectively. Testosterone 6 β -hydroxylation and 7-ethoxycoumarin *O*-dealkylation activities were calculated in units of picomoles of metabolite formed per minute per milligram of microsomal protein (pmol/min/mg). Metabolic competences of liver microsomes were considered acceptable if measured enzymatic rate reached, at least, 80% of the rate given by the supplier for each lot of microsomes.

RESULTS

Radiochemical purity of ¹⁴C-labelled Adepidyn and marker substrates

Radiochemical purity of the [phenyl- ^{14}C]Adepidyn and [pyrazole-5- ^{14}C]Adepidyn was determined in the respective dosing solutions prior to use. It was 98.5% for [phenyl- ^{14}C]Adepidyn and 99.1% for [pyrazole-5- ^{14}C]Adepidyn.

Radiochemical purity of ^{14}C -labelled marker substrates was determined in the dosing solution prior to use. The radiochemical purity of [^{14}C]testosterone and [^{14}C]7-ethoxycoumarin was 98.9% and 99.7%, respectively.

Concentration of Adepidyn and marker substrates in microsomal assays

Concentration of Adepidyn and marker substrate was measured in the dosing solutions by counting the radioactivity prior to each test performed. Actual concentration of Adepidyn and marker substrates in microsomal assays was calculated according to those radioactivity measurements. Results are presented in the Table below.

Substrate	Microsomal assays			
	Concentration	HLM	Male RLM	Female RLM
[phenyl- ^{14}C]Adepidyn	Target	5.0 μM	5.0 μM	5.0 μM
	Actual	5.1 μM	5.1 μM	5.3 μM
[pyrazole-5- ^{14}C]Adepidyn	Target	5.0 μM	5.0 μM	5.0 μM
	Actual	5.1 μM	5.2 μM	5.3 μM
Testosterone	Target	250.0 μM	n.a.	n.a.
	Actual	256.5 μM		
7-Ethoxycoumarin	Target	n.a.	500.0 μM	500.0 μM
	Actual		505.2 μM	515.3 μM

n.a.: not applicable

Positive control enzymatic activities

Testosterone 6 β -hydroxylation rates observed in HLM and 7-ethoxycoumarin *O*-dealkylation rates observed in RLM were higher than 80% of the rate reported by the supplier for the corresponding lot of microsomes. The positive control enzymatic activities showed that HLM and RLM used in the present study possessed metabolic competences in agreement with the acceptance criteria.

Adepidyn metabolism in human and rat liver microsomes

Radioactivity recovery

The recovery ranged between 91.4% and 107.4% in HLM, from 97.8% to 106.3% in male RLM and from 94.0% to 103.1% in female RLM. In all incubates, more than 93% of the recovered radioactivity was located in the supernatant, therefore no further extraction of the microsomal pellet was performed.

Adepidyn stability in the incubation medium

In time zero controls the percentage of parent [phenyl- ^{14}C]Adepidyn and [pyrazole-5- ^{14}C]Adepidyn was not significantly different from the radio-purity determined in the dosing solution (98.5% and 99.1% respectively), showing no degradation of [^{14}C]Adepidyn.

In negative controls without microsomes, after 60 minutes of incubation in the buffer system no degradation of Adepidyn was observed, the percentage of parent compound remained unchanged compared to time zero. This result indicated that Adepidyn did not degrade in the incubation medium.

Adepidyn metabolism

In HLM and RLM incubated without NADPH-GS for 60 minutes, the percentage of remaining unchanged parent compound did not significantly differ from time zero. This result showed that no NADPH-independent metabolism of Adepidyn occurred in HLM and RLM.

In both HLM and male RLM incubated with NADPH-GS for 60 minutes, Adepidyn was extensively metabolised. In HLM, remaining parent Adepidyn accounted for 5.8% to 8.2% of the dose and for 4.4% to 4.9% of the dose in male RLM. Adepidyn was metabolised to a lesser extent in female RLM, remaining parent Adepidyn accounting for 58.9% to 60.4% of the dose. This result also indicates that the metabolism of Adepidyn was NADPH-dependent in both HLM and RLM.

Up to 12 radio-HPLC peaks were observed in the incubations performed with [phenyl- ^{14}C]Adepidyn, namely P1 and P5 to P15. In the incubations performed with [pyrazole-5- ^{14}C]Adepidyn, up to 14 radio-HPLC peaks were observed, namely P1 to P12, P14 and P15. P15 was assigned to parent Adepidyn. In time zero samples and negative controls small amounts of P12, P13 and P14 were detected. They corresponded to impurities already observed in the dosing solutions. On the other hand, incubations with microsomes and NADPH-GS showed that P1 to P14 were clearly produced by NADPH-dependent metabolism of Adepidyn. P2, P3 and P4 were detected only when microsomes were incubated with [pyrazole-5- ^{14}C]Adepidyn and P13 was detected only in microsomes incubated with [phenyl- ^{14}C]Adepidyn. This suggests that P2, P3, P4 and P13 result from the cleavage of Adepidyn; P2, P3 and P4 corresponding to breakdown products from pyrazole moiety and P13 a breakdown product from phenyl moiety.

P6, P12, P13 and P14 were the main metabolites observed in HLM. P12 was the major metabolite, accounting for up to 29.1% of the dose after 60 minutes of incubation. P13, P6 and P14 accounted for up to 14.3%, 12.8% and 11.6%, respectively. P2 accounted for 8.8% and P8 to P11 were slightly higher than 5% of the dose. The other metabolic peaks, namely P1, P3, P4, P5 and P7, were below 5% of the dose.

The metabolic pattern of Adepidyn observed in male RLM was qualitatively similar to HLM. All the human metabolites formed (P1 to P14) were detected in male rat. Quantitative differences were observed between human and male rat. P9 was the major metabolite in male RLM, accounting for up to 27.7% of the dose after 60 minutes of incubation. P8 was also produced in higher amount in male RLM compared to HLM. The other metabolites were produced at similar level or to a lesser extent in male RLM. All the other metabolites which were clearly above 5% of the dose in human were also above 5% in male rat (P2, P6, P12, P13 and P14).

The metabolic pattern of Adepidyn observed in female RLM was qualitatively and quantitatively different from HLM and male RLM. Female rat produced P2, P3, P6, P8, P9, P11, P12, P13 and P14 metabolites. P1, P4, P5, P7 and P10 were not formed. Moreover, P2, P3, P6, P8, P11 and P13 were well below the level observed in human and male rat.

CONCLUSION:

In vitro metabolic profiling of pydiflumetofen was carried out in a GLP study by incubating human and rat liver microsomes with 5 μM radiolabelled pydiflumetofen and a NADPH-regenerating system for 60 minutes at 37°C. The study was conducted with two distinct ^{14}C -labelled pydiflumetofen, *i.e.* [phenyl- ^{14}C]pydiflumetofen and [pyrazole-5- ^{14}C]pydiflumetofen, a mixed gender pool of human liver microsomes and separated pools of male and female Wistar rat liver microsomes. Time zero and negative controls without microsomes and without NADPH-regenerating system were also performed in order to determine the stability of the test substance in the incubation medium and to validate NADPH-dependent metabolism. Positive controls, *i.e.* testosterone 6 β -hydroxylation and 7-ethoxycoumarin *O*-dealkylation enzymatic activities, were used to check the metabolic competences of human and rat liver microsomes, respectively.

Positive control enzymatic activities showed that human and rat liver microsomes possessed metabolic competences in agreement with the acceptance criteria. Negative controls indicated that pydiflumetofen did not degrade in the incubation medium and that no metabolism of pydiflumetofen occurred without NADPH. An extensive metabolism of pydiflumetofen was observed in human and male rat liver microsomes. The

test substance was metabolised to a lesser extent in the female rat. Remaining parent compound accounted for < 10% of the applied radioactivity in humans, for < 5% in the male rat and for approximately 60% in the female rat after 60 minutes of incubation.

Pydiflumetofen was metabolised into 14 different metabolites (P1-14). Metabolites were qualitatively similar between human and male rat microsomes. Fewer metabolites and higher levels of unchanged pydiflumetofen (P15) in female rat microsomes suggested metabolism occurred at a slower rate in the female rat. Some quantitative differences in metabolites were observed between human and rat microsomes which suggests the rates of metabolism may be different between the two species; however no unique human metabolites were observed.

HSE notes that there is no current guideline for the completion of in vitro comparative metabolism studies.

(██████████, 2017)

(i) ***Dose level selection to be apply for toxicity studies***

HSE had some reservations regarding the dose level selection which has been proposed by the applicant on the basis of pharmacokinetic data. Indeed, several pharmacokinetics studies were performed to determine the TK profile of SYN545974 following repeated dose by gavage, dietary or capsule in rat, mice, rabbits and dogs. However, all of these additional studies were performed with a non-radiolabeled method which did not permit to follow the fate of the metabolites. Thus, the dose selection argumentation proposed by the applicant is valid only for the parent SYN545974. It is highlighted that as SYN545974 is extensively metabolized in rat and mouse, measured blood concentrations of parent SYN545974 are extremely low compared to those of metabolites and especially 2,4,6 TCP⁶. Indeed, 2,4,6 TCP is the major circulating metabolite after administration of SYN545974 in rat and mouse with plasma concentration which largely exceeds that of the parent. It would have been appropriate to investigate also the pharmacokinetics of 2,4,6 TCP following repeated or single oral administration of SYN545974 especially since this metabolite is of toxicological concern. Indeed, 2,4,6 TCP has been classified as carcinogen by several international bodies: Carcinogen Category 2 H351 by the European Union (ATP0); carcinogen group 2B by IARC or carcinogen group B2 by US-EPA. The applicant considered that the non-proportionality of SYN545974 kinetics with increasing dose (due to dose limited absorption) will be reflected by non-proportionality in the formation of all metabolites. However, the non-proportionality of SYN545974 kinetics means that systemic exposure (measured by AUC(0-t)) stops increasing linearly with the dose but it doesn't mean that systemic exposure does not continue to increase at all with dose higher than the maximal dose levels selected by the applicant for some of the long term and reproductive toxicity studies (100-300 mg/kg/day (female-male) in rat studies and 300 mg/kg/day in mouse studies). This is confirmed both by the available toxicokinetic and toxicity studies performed with SYN545974. Table 6.1-3 presents a comparison between the plasma AUCs measured after an oral administration in rat of phenyl radiolabeled SYN545974 (permitting a follow-up of SYN545974 and its phenyl metabolites including 2,4,6 TCP) at the dose levels of either 5, 100 (female), 300 (male) or 1000 mg/kg bw/d. These toxicokinetic data showed that systemic exposure still increases beyond 100 or 300 mg/kg bw/day in rat: by 4-fold between 100 mg/kg bw/day and 1000 mg/kg bw/d and by 1,7-fold between 300 mg/kg bw/d and 1000 mg/kg bw/d. This is also confirmed by the short-term repeated studies in rat where an increase in toxicity (liver and body weight effects) was observed with increasing doses beyond the highest doses selected by the applicant for the rat long-term study (300-100 mg/kg bw/day (male-female)).

Table 6.1-3: Comparison of AUC_{0-t} for Total Radioactivity in Plasma Following a single Oral Administration of [phenyl-U-14C]-SYN545974 to Male and Female Rat

Dose (mg/kg)	Fold increase		PLASMA	
			AUC(0-t)(ng equiv.h/g)	Fold increase
5 ^{!!}		Male	8830	
		Female	10200	

⁶ From here on in 2,4,6-TCP refers to “2,4,6-TCP and its related metabolites particularly hydroxyl TCP sulphate and 2,4,6-TCP sulphate”, due to the rapid conjugation of 2,4,6-TCP *in vivo*.

5 †		Male	8705	
		Female	7890	
100 †	20	Female	113000	14
300 †	60	Male	316000	36
1000 !!	200	Male	546000	62
		Female	450000	44

!! Data extracted from [REDACTED] and [REDACTED] (2015) study (Report SGA-64; K-CA 5.1.1/01)

† Data extracted from [REDACTED] and [REDACTED] (2015) study (Report 34107; K-CA 5.1.1/04)

Taking into account these elements, HSE questioned on the possibility that the doses of SYN545974 selected by the applicant for the long-term studies might be not sufficiently high to cover the carcinogenic potential of TCP, especially in rat. Indeed, no tumors were observed in rat following a 2-year administration of SYN545974 in male rats at dose up to 300 mg/kg/day ([REDACTED] 2015; section B.6.5) whereas leukemias were observed from the dose of 250 mg/kg/day of 2,4,6 TCP in male rat in a long-term toxicity study from the NTP (NCI 1979; section B.6.8.1).

2,4,6-TCP shows equivocal evidence of carcinogenicity in F344 rats (leukemia) and has harmonised classification with Carc Cat 2 under the CLP Regulation. HSE is of the view that the question whether the dose levels used in the pydiflumetofen carcinogenicity studies were sufficient to investigate the carcinogenic potential of its major metabolite 2,4,6-TCP is irrelevant for two reasons:

- 1) Dose levels should be appropriate to maximise the potential of detecting a carcinogenic response of the tested substance (pydiflumetofen in this case). The MTD was reached in both carcinogenicity studies and higher levels did not need to be used to try and investigate the carcinogenic potential of 2,4,6-TCP.
- 2) As specific and extensive toxicological data exist on 2,4,6-TCP, these should take priority in characterising its toxicity profile over and above the predictive approach of using the parent's data just because the substance is a major rat metabolite of the parent.

B.6.1.3. ADME summary

The ADME properties of pydiflumetofen were investigated by the oral route in several rat studies. The excretion and metabolism of pydiflumetofen were also investigated in mice by the oral route. The blood pharmacokinetic profile of pydiflumetofen following repeat oral dosing of non-radiolabelled test material was determined in rats, mice, rabbits and dogs (for the dog, the data are presented in the short-term toxicity section). These blood kinetic data were used to support dose level selection for some toxicity studies based on linear versus non-linear kinetics of the parent substance. In addition, intravenous (iv) administration of the radiolabelled test substance and measurement of radioactivity in blood and/or excreta were used to establish the oral bioavailability of pydiflumetofen in rats.

In the rat, preliminary ADME studies using [pyrazole-5-¹⁴C]- and [phenyl-U-¹⁴C]- radiolabelled pydiflumetofen indicated that pydiflumetofen was metabolically cleaved between the pyrazole and phenyl moieties. Therefore, subsequent ADME studies used both radiolabels. Bile duct cannulated rats were used in the main ADME study, as the preliminary study showed that greater than 20% of the administered dose was excreted in faeces. Additionally, metabolism was investigated in human and rat microsomes in vitro.

The table below provides an overview of the available studies.

Title	Reference
A Preliminary Study of Pharmacokinetics, Absorption, Metabolism and Excretion in Rats Following Single Oral and Intravenous Administration of ¹⁴ C-SYN545974	[REDACTED], [REDACTED] (2015). SYN545974_10188

The Absorption and Excretion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C] SYN545974 Following Single Oral Administration in the Rat	██████ (2015).
Tissue Depletion of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C] SYN545974 Following Single Oral Administration in the Rat	██████ (2015a).
The Pharmacokinetics of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral and Intravenous Administration in the Rat	██████, ██████ (2015).
Biotransformation of [¹⁴ C]-SYN545974 in Rat	██████, ██████ (2015).
Pharmacokinetics of SYN545974 in the Rat Following Multiple Oral and Single Intravenous Administration	██████, ██████ (2014).
Pharmacokinetics of SYN545974 in the Mouse Following Multiple Oral and Single Intravenous Administration	██████, ██████ (2014a).
The Excretion and Biotransformation of [Phenyl-U- ¹⁴ C] and [Pyrazole-5- ¹⁴ C]-SYN545974 Following Single Oral Administration in the Mouse	██████, ██████, ██████ (2015).
SYN545974 - Oral (Gavage) Toxicokinetic Study in the Pregnant Rabbit.	██████ (2015).
Adepidyn – In Vitro Comparative Metabolism of [Phenyl-U- ¹⁴ C]Adepidyn and [Pyrazole-5- ¹⁴ C] Adepidyn in Human and Rat Liver Microsomes.	██████ (2017)

Absorption

After a single gavage dose of 5 mg/kg bw pydiflumetofen, **oral absorption** was **85-90%** (sum of material excreted in urine, bile, cage wash and remaining carcass, excluding GI tract) in rats. Absorption decreased to 50-55% as dose increased to 100 mg/kg bw and further decreased to 19-24% at 300 mg/kg bw, as also shown by the increased amount of unchanged parent in faeces. Bile was the major route of excretion, indicating a significant first-pass effect. Indeed, post-hepatic systemic **bioavailability (F)** following oral dosing was approximately **50%**. Bioavailability may be a more appropriate parameter than oral absorption in adjusting the AOEL and AAOEL.

Tissue distribution

Tissue distribution after a single oral dose of pydiflumetofen (5, 100 and 300 mg/kg bw) was similar, irrespective of radiolabel ([¹⁴C]-phenyl or pyrazole), sex or dose. The highest levels of radioactivity were observed in the liver, kidneys, adrenals and renal fat. After seven days, levels of radioactivity were only higher in the liver and kidneys compared to blood. The depletion profile of all tissues appeared to be similar to that of blood/plasma. No plateaus were observed in any tissue, suggesting that accumulation in tissues is unlikely. At termination, total tissue and carcass residues accounted for ≤ 3.0% of the administered dose.

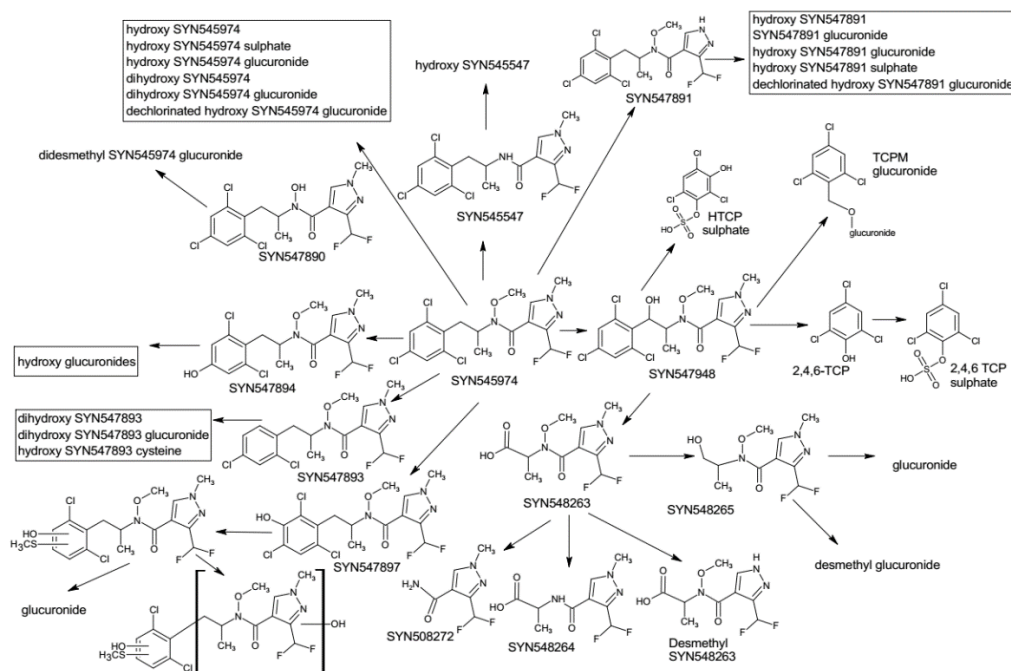
Metabolism

After a single oral low dose of 5 mg/kg bw, pydiflumetofen was extensively metabolised (> 95% of administered dose) in the rat mainly via first pass metabolism. Generally, metabolite profiles were similar irrespective of radiolabel apart from a few radiolabel-specific metabolites, dose (except for faeces) or sex. At 5 mg/kg bw, unchanged pydiflumetofen was a minor component only present in faeces up to 3.9% of the administered dose. However, at the higher doses (100-300 mg/kg bw) unchanged pydiflumetofen was a major component in faeces present up to 48.2% of the administered dose.

Only two metabolites (2,4,6 TCP sulphate in urine and plasma and SYN548272 in plasma) individually accounted for >10% of the administered dose. Numerous other metabolites were detected as cleavage products and as molecules that retained both the phenyl and pyrazole ring moieties. The intact metabolites detected were qualitatively and quantitatively similar between the two labels. The cleavage of the parent molecule occurred following hydroxylation of pydiflumetofen on the carbon adjacent to the trichlorophenyl ring to give SYN547948. This then cleaved to yield the 2,4,6 trichlorophenol (2,4,6-TCP) and SYN548263.

2,4,6-TCP was then sulphated, which accounted for the largest % of dose excreted at up to 14.9% of the administered dose. Other metabolites that retained the phenyl ring were hydroxyl 2,4,6-TCP (HTCP) sulphate and 2,4,6-TCP methanol (TCPM) glucuronide. SYN548263 was further metabolised by demethoxylation to SYN548264 and by N-demethylation. Amide hydrolysis of the pyrazole half molecules gave the pyrazole amide, SYN508272. Reduction of SYN548263 yielded the alcohol SYN548265. SYN548265 was further metabolised via demethylation and glucuronidation.

Numerous metabolites that retained both the phenyl and pyrazole moieties were observed. The primary metabolic routes for these metabolites included demethoxylation to SYN545547, N-dealkylation to SYN547891, single and di-hydroxylation, O-demethylation to SYN547890, oxidative dechlorination to SYN547894 and reductive dechlorination to SYN547893. The majority of these metabolites were also mono and di-hydroxylated and in many cases conjugated with glucuronide or in the case of SYN547894, glutathione. The proposed metabolic pathway of pydiflumetofen in the rat is shown below:



In the mouse, no major urinary metabolites (>10% of the administered dose) were identified. However, the most abundant metabolites in the mouse were qualitatively similar to those in the rat suggesting metabolism is similar between the two species.

In a GLP in vitro comparative metabolism study using human and rat liver microsomes, pydiflumetofen was metabolised into 14 different metabolites (P1-14). Metabolites were qualitatively similar between human and male rat microsomes. Fewer metabolites and higher levels of unchanged pydiflumetofen (P15) in female rat microsomes suggested metabolism occurred at a slower rate in the female rat. Some quantitative differences in metabolites were observed between human and rat microsomes which suggests the rates of metabolism may be different between the two species; however no unique human metabolites were observed.

Excretion

Irrespective of radiolabel, dose or sex, following a single oral administration of [^{14}C]-pydiflumetofen in rats, the majority of the radioactivity (> 91%) was eliminated by 48 hours post dose and excretion was essentially complete by 168 h (as indicated by the low levels of radioactivity in the carcass). Absorption was limited by dose. The majority of the absorbed dose was excreted in faeces via bile elimination.

The main route of excretion was in the faeces via the bile; urinary excretion was a minor route. After an oral dose of 5 mg/kg bw, 81% of the administered dose was excreted via bile compared to 15% via the faeces. However at higher doses, excretion decreased in bile to 41% of the administered dose at 100 mg/kg bw (females) and 18% at 300 mg/kg bw (males) but increased in faeces.

After a single oral dose in the mouse, excretion was essentially complete after 7 days. The major route of excretion was via faeces (bile duct cannulated mice were not investigated); urinary excretion was a minor route. Approximately 63-79% of the administered dose was excreted via faeces at 10 mg/kg bw; however this increased to 76-94% at 300 mg/kg bw suggesting oral absorption may also be limited by dose in mice.

Pharmacokinetics

Parent and metabolites (total radioactivity)

In the rat, after a single oral dose of 5 mg/kg bw, blood/plasma C_{max} was reached from 0.5-2 h post dose, whereas at higher doses (100 or 300 mg/kg bw), C_{max} was reached at 8 h. Systemic exposure was comparable between blood and plasma, irrespective of dose or radiolabel. After a single oral exposure, systemic exposure increased in a sub-proportional manner between 5 and 300 mg/kg bw. Additional pharmacokinetic parameters for total radioactivity in the rat are presented in the table below.

	Pharmacokinetic parameters for total radioactivity in rat plasma after oral administration with pyrazole-labelled pydiflumetofen			
	5 mg/kg bw		300 mg/kg bw	100 mg/kg bw
	Male	Female	Male	Female
C_{max} (µg	0.49	0.67	7.1	3.1
C_{max}/D	0.0969	0.131	0.0259	0.0365
t_{max} (hours) ¹	2	0.5	8	2
$t_{1/2}$ (hours)	56.6*	30.4*	18.6*	10.6
AUC(0-t) (µg equiv.h/mL)	6.43	5.37	195	55.9
AUC(0-t)/D	1.26	1.05	0.705	0.653
AUC(0-inf) (µg equiv.h/mL)	7.45*	5.81*	197*	56.2
AUC(0-inf)/D	1.47*	1.13*	0.712*	0.658
AUC % Extrapolation	13.7*	7.56*	1.02*	0.678

* = 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.

¹ = Median reported for t_{max}

Parent only (non-radiolabelled)

In the rat, systemic exposure to pydiflumetofen after repeat oral dosing increased in a proportional manner from 3 to 10 mg/kg bw/d, however became sub-proportional at concentrations above 300 and 100 mg/kg bw/d in males and females respectively. Systemic exposure was higher in females compared to male rats. Linearity for males below 30 mg/kg bw/d could not be determined due to low systemic exposure. HSE notes that although non-linear kinetics were observed in the rat from approximately 300 mg/kg bw/d in males and from 100 mg/kg bw/d in females, systemic exposure continued to increase and no plateau was observed up to the highest dose of 1000 mg/kg bw/d.

In the mouse, after repeat oral dosing, systemic exposure (AUC) increased sub-proportionally in relation to dose above 100 mg/kg bw/d. HSE notes that although non-linear kinetics were observed in the mouse from approximately 100 mg/kg bw/d in both sexes, systemic exposure continued to increase and no plateau was observed up to the highest dose of 1000 mg/kg bw/d.

In the pregnant rabbit, after repeat oral dosing from gestation day 6-27, systemic exposure to pydiflumetofen was sub-proportional in relation to dose above 300 mg/kg bw. Inter-individual variability was high between tested animals which decreases the reliability of these findings.

In the dog, after repeat oral dosing of pydiflumetofen for 90-days inter-individual variability was high. However, systemic exposure appeared to increase approximately proportionally (sometimes supra-proportionally) with dose and was generally higher in males compared to females.

HSE notes that no repeat dose ADME studies were conducted using radiolabelled pydiflumetofen. However, an adequate justification was provided by the applicant.

Residue definition for body fluids and tissues

Based on the main ADME studies in the rat, the residue definition for body fluids (blood) and tissues (liver) was set as pydiflumetofen and 2,4,6-TCP (free + conjugates).

B.6.2. ACUTE TOXICITY

The acute toxicity, skin and eye irritation and skin sensitisation of SYN545974 have been investigated in standard in vivo studies. An in vitro phototoxicity study is also available.

B.6.2.1. Oral

Report: K-CA 5.2.1/01 [REDACTED] (2012). SYN545974 – Acute Oral Toxicity Study in the Rat (Up and Down Procedure). [REDACTED]
[REDACTED]. Laboratory Report No. 12/344-001P, issue date 20 December 2012. Unpublished. Syngenta File No. SYN545974_10043.

Guidelines: Acute Oral Toxicity (rat): OECD Test Guideline 425 (2008); EPA OPPTS 870.1100 (2002)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In an acute oral toxicity study, 3 female [REDACTED]:(WI) rats were given a single oral (gavage) dose SYN545974, prepared in 0.5% carboxymethyl cellulose at the limit dose of 5000 mg/kg bodyweight (bw). The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. Animals were observed individually for up to 14 days thereafter and necropsies were performed on all surviving animals at the end of the study.

No deaths occurred during the study.

Treatment with SYN545974 at the dose level of 5000 mg/kg bw caused slight decreased activity in one animal. All animals were symptom free from 4 hours after the treatment.

There were no treatment related body weight changes. Body weights were within the range commonly recorded for this strain and age.

No treatment related macroscopic observations were recorded in any animals dosed at 5000 mg/kg bw.

Under the conditions of this study, the acute oral median lethal dose (LD50) of the test item, SYN545974, was greater than 5000 mg/kg bw (limit dose) in female [REDACTED]:(WI) rats.

Materials:

Test Material:	SYN545974
Description:	off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Product Code:	SYN545974 technical
Stability of test compound:	Protected from light and humidity, room temperature
Recertification date:	End of June 2016

Test Animals:	
Species	Rat
Strain	Wistar-Kyoto (WKY)
Age/weight at dosing	Young adult rats, 8-9 weeks old. /174-189 g
Source	Charles River
Housing	Individual caging
Acclimatisation period	At least 5 days
Diet	Animals received Wistar-Kyoto SM R/M "Autoclavable complete diet for rats and rats – breeding and maintenance" produced by Charles River ad libitum
Water	Tap water from municipal supply, provided in 500 mL bottles ad libitum
Environmental conditions	Temperature: 20.3 – 24.3°C Humidity: 33 – 64 % Air changes: 15-20 air exchanges/hour Photoperiod: 12 hours daily, from 6.00 a.m. to 6.00 p.m.

In-life dates: Start: 18 September 2012 End: 09 October 2012

Animal assignment and treatment:

In an acute oral toxicity study, 3 female [REDACTED]:(WI) rats were given a single oral (gavage) dose SYN545974, prepared in 0.5% carboxymethyl cellulose at the limit dose of 5000 mg/kg bodyweight (bw) using an application volume of 10 mL/kg bw. The animals were fasted overnight prior to treatment and food was returned 3 hours after dosing.

Single animals were dosed sequentially at no less than approximately 48 hour intervals. The time intervals between dosing were determined by the onset, duration and severity of clinical signs. The first animal was treated at a dose level of 5000 mg/kg bw. This animal survived, therefore 2 additional animals were sequentially dosed at 5000 mg/kg bw according to the instructions of AOT425StatPgm program such that a total of 3 animals were tested.

Animals were observed individually after dosing at 30 minutes, then 1, 2, 3, 4 and 6 hours post treatment and once each day for 14 days thereafter. Body weight was measured on Day -1, just before dosing and weekly thereafter. All animals were examined macroscopically at the end of the study.

Surviving animals were euthanised at the end of the observation period by exsanguination under pentobarbital anaesthesia (Euthasol® 40%, Lot No.: 11H15 8, Expiry Date: July 2014, Produced by: Produlab Pharma B.V.).

All animals were subjected to gross macroscopic evaluation. The cranial, thoracic and abdominal cavities were opened and the appearance of the tissues and organs were observed. All gross pathological changes were recorded for each animal on the post mortem record sheets and the animals were discarded.

Statistics: The LD₅₀ was calculated using the AOT425StatPgm program. This program was prepared for the US Environmental Protection Agency by Westat, May 2001 and updated by the US EPA June 2003. This programme was constructed using the most appropriate method to estimate the LD₅₀.

RESULTS

Mortality: No mortality was observed during the study.

Table 6.2.1-1: Acute oral toxicity of SYN545974 in the female rat, application scheme and mortality data

Animal Number	Dosage [mg/kg body weight]	Dose volume [mL/animals]	Viability/Mortality
6453	5000	1.7	Survived
6454	5000	1.8	Survived
6455	5000	1.9	Survived

Clinical observations: Treatment with SYN545974 at the dose level of 5000 mg/kg bw caused slight decreased activity in one animal. All animals were symptom free from 4 hours after the treatment.

Bodyweight: There were no treatment related effects on body weight or body weight gain.

Necropsy: No treatment related macroscopic observations were recorded in any animals dosed at 5000 mg/kg bw.

CONCLUSION:

The potential acute oral toxicity of pydiflumetofen was investigated in a GLP compliant study carried out in accordance with the most recent (2008) OECD 425 guideline. Three female XXXXWI rats were administered the 5000 mg/kg bw limit dose of the test item dissolved in 0.5% carboxymethyl cellulose by oral gavage. Paragraph 26 of the OECD 425 guideline states that the 5000 mg/kg bw limit dose should only be considered in exceptional circumstances. Initially one rat was tested at 5000 mg/kg bw, and when no symptoms were observed, the two other rats were tested sequentially at the same dose. Animals were observed 30 minutes after dosing, as well as 1, 2, 3, 4 and 6 hours post treatment, and then daily thereafter for 14 days. Animals were then euthanised and subject to macroscopic evaluation. The LD₅₀ was calculated based on the AOT425StatPgm program.

Only one major symptom was observed during the study; a slight decrease in activity in a single animal which reversed within 4 hours. No moribundity or mortality was observed throughout the study, nor were there any treatment related macroscopic observations at necropsy. Body weight and body weight gain were also unaffected by treatment. As a result, HSE agrees with the EU evaluation, that the acute oral LD₅₀ of pydiflutofen was greater than 5000 mg/kg bw.

In conclusion, the estimated acute oral median lethal dose (estimated LD₅₀) of pydiflumetofen was found to be greater than 5000 mg/kg bw (Limit Dose) in female XXXXWI Wistar rats. As a result, in accordance with Regulation 1272/2008, pydiflumetofen does not meet the criteria for acute oral toxicity classification.

B.6.2.2. Dermal

Report: K-CA 5.2.2/01 [REDACTED] (2013). SYN545974 - Acute Dermal Toxicity Study in Rats. [REDACTED]. Laboratory Report No. 12/344-002P, issue date 14 January 2013. Unpublished. Syngenta File No. SYN545974_10046.

Guidelines: Acute Dermal Toxicity (rat) OECD 402 (1987); United States Environmental Protection Agency Health Effects Division Test Guidelines, OPPTS 870.1200 Acute Dermal Toxicity EPA 712-C-98-192 (1998); Commission Regulation (EC) No 440/2008, B.3 (L 142, 30 May 2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

A group of 5 male and 5 female [REDACTED]:(WI) Wistar rats were treated with a single dermal application of SYN545974 at 5000 mg/kg body weight (bw). The application period was 24 hours, followed by a 14-day observation period. No mortality occurred during the 14-day observation period. The test item was moistened with approximately 1mL of tap water to ensure good contact with the skin and applied uniformly over the skin throughout a 24- hour exposure period.

Clinical observations along with a check of viability and mortality were performed on all animals at 1 and 5 hours after dosing and daily for 14 days thereafter. Body weight was measured prior to dosing on Day 0 and on Days 7 and 14. Rats were euthanized and subjected to a gross macroscopic examination at the end of the 2-week observation period (Day 14).

No mortality occurred during the study.

Dermal administration of the test item at a dose level of 5000 mg/kg bw caused decreased activity in 10/10 animals on Day 1. From Day 2, all animals were symptom free.

There were no treatment related effects on body weight or body weight gain during the observation period.

There was no evidence of the test item-related observations at a dose level of 5000 mg/kg bw at necropsy.

No treatment related macroscopic observations were recorded in any animals dosed at 5000 mg/kg bw.

The median lethal dose of SYN545974 after a single dermal administration was found to be greater than 5000 mg/kg bw in male and female [REDACTED]:(WI) Wistar rats.

Materials:

Test Material:	SYN545974
Description:	off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Product Code:	SYN545974 technical
Stability of test compound:	Protected from light and humidity, room temperature
Recertification date:	End of June 2016

Vehicle and/or positive control: None

Test Animals:	
Species	Rat
Strain	Wistar-Kyoto (WKY) (WI)
Age/weight at dosing	Young adult rats, between 215 g and 254 g
Source	Charles River Laboratories (CRL)
Housing	Individual cages Type II, polypropylene/polycarbonate. Laboratory bedding (Lignocel Hygienic Animal Bedding produced by J. Rettenmaier & Söhne GmbH+Co.KG (Holzmühle 1, 73494 Rosenberger, Germany Bedding was available to animals during the study))
Acclimatisation period	6 days
Diet	SM R/M-Z+H "Autoclavable complete feed for rats and mice – breeding and maintenance" produced by CRL ad libitum (Lot number: 601 7197, expiry date: September 2012)
Water	Tap water from municipal supply ad libitum
Environmental conditions	Temperature: 20.3 – 24.4°C Humidity: 36 - 67 % Air changes: 15-20 air exchanges/hour Photoperiod: 12 hours daily, from 6.00 a.m. to 6.00 p.m.

In-life dates: Start: 12 September 2012 End: 26 September 2012

Animal assignment and treatment: A single administration of SYN545974 at a dose of 5000 mg/kg body weight was applied dermally to 5 male and 5 female (WI) rats, followed by a 14-day observation period. The test item was applied as supplied. The application period was 24 hours. A limit test was carried out at 5000 mg/kg body weight (bw) in both sexes (5 rats/sex).

The backs of the animals were shaven (approximately 10% area of the total body surface) approximately 24 hours prior to treatment. Only those animals without injury or irritation on the skin were used in the test. On test day 0, the test item was applied at a single dose of 5000 mg/kg body weight applied uniformly over the skin and remained on the skin throughout a 24- hour exposure period. Sterile gauze pads were placed on the skin of rats at the site of application. These gauze pads were kept in contact with the skin by a patch with adhesive hypoallergenic plaster. The entire trunk of the animal was then wrapped with semi occlusive plastic wrap for 24 hours. At the end of the exposure period, residual test item was removed, using body temperature water.

A clinical examination was performed on the day of treatment, at 1 and 5 hours after the application of the test item, and once each day for 14 days thereafter.

Observations included the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, and somatomotor activity and behaviour pattern. Particular attention was directed to the observation of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The body weights were recorded on Day 0 (beginning of the experiment) and on Days 7 and 14.

Statistics: There was no mortality in the study therefore statistical analysis was not required.

RESULTS

Mortality: No mortality occurred after the 24-hour dermal exposure to SYN545974 in (WI) rats.

Table 6.2.2-1: Acute dermal toxicity of SYN545974 in the rat, application scheme and mortality data

Dose Level (mg/kg)	Day Number	Number of Deaths	
		Male	Female
5000	1	0	0
	Total at day 14	0/5	0/5

Clinical observations: Dermal administration of the test item at a dose level of 5000 mg/kg bw caused decreased activity in 10/10 animals on Day 1. From Day 2, all animals were symptom free.

Bodyweight: There were no treatment related effects on body weight or body weight gain during the observation period.

Necropsy: There was no evidence of the test item-related observations at a dose level of 5000 mg/kg bw at necropsy.

CONCLUSION:

The potential acute dermal toxicity of pydiflumetofen was investigated in a GLP compliant study, carried out in accordance with the 1987 OECD 402 guideline. The study is considered to be in accordance with the most recent (2017) version of the guideline, with only minor deviations that did not impact the validity of the study. There is a waiver criterion in the new version that states that acute dermal toxicity testing is not needed where acute oral toxicity is >2000 mg/kg bw; however this did not exist at the time the study was conducted (2013). Considering that acute dermal toxicity is very rarely more severe than the acute oral toxicity of a compound, and that pydiflumetofen was found (, 2012) to have an acute oral toxicity greater than 5000 mg/kg bw, the same LD₅₀ would have been considered acceptable for the acute dermal toxicity of the substance.

A single dermal dose of the test item was applied at 5000 mg/kg bw to the skin of 5 male and 5 female (WI) rats, after which there was a 14 day observation period. All 10 of the tested animals exhibited decreased activity on the first day, but all were symptom free by Day 2. No treatment related effect on body weight or body weight changes were observed, and likewise no test item related macroscopic observations were noted at necropsy. Considering the lack of symptoms, as well as the lack of any mortality and moribundity throughout the study, HSE agrees with the EU conclusion that the median lethal dose of the test item was greater than 5000 mg/kg bw in male and female (WI) rats.

In conclusion, under the conditions of this GLP and OECD guideline compliant study, the acute dermal median lethal dose (LD₅₀) of pydiflumetofen was found to be greater than 5000 mg/kg bw in male and female (WI) rats. As a result in accordance with Regulation 1272/2008, the compound does not meet the classification criteria for acute dermal toxicity.

(██████████, 2013)

B.6.2.3. Inhalation

Report:	K-CA 5.2.3/01 ██████████ (2013). SYN545974 - Acute Inhalation Toxicity Study (Nose-Only) in the Rat. ██████████. Report number 12/334-004P, issue date 29 January 2013. Unpublished. Syngenta File No. SYN545974_10051.
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Guidelines: Acute Inhalation Toxicity Study (Nose-Only) in the Rat: OECD Test Guideline 403 (2009); EPA OPPTS 870.1300 (1998); EC 440/2008, Annex Part B, B.2 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

The aim of this study was to assess the acute inhalation toxicity of SYN545974. Due to insufficient information about the test item's inhalation toxicity, a single sighting exposure for 4 hours using 2 male and 2 female rats was performed at a target aerosol concentration of 5 mg/L before the main study.

The main study group, consisting of 10 (5 male and 5 female) ██████████: (WI) Wistar strain rats, was exposed to a target aerosol concentration of 5 mg/L SYN545974. The animals were exposed for 4 hours using a nose-only exposure system, followed by a 14 day observation period. The day of exposure was designated Day 0. Aerosol concentrations were measured gravimetrically. The particle size distribution of the test aerosol was determined regularly during the exposure period. Clinical observations and bodyweights were recorded throughout the study and at the end of the scheduled period the animals were euthanised and subjected to a gross examination post mortem.

Sighting Exposure:

The mean achieved atmosphere concentration was 5.14 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was $3.48 \mu\text{m} \pm 2.35$ (GSD [Geometric Standard Deviation]).

Wet fur, ruffled coat and fur staining were commonly recorded on the day of exposure and several days after exposure in the sighting group. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be of toxicological relevance. Laboured and noisy respiration, sneezing, decreased activity and ataxia were recorded for the exposed animals on day of exposure. Only laboured respiration was recorded for two females on day after exposure. No significant clinical signs were noted from Day 2.

Normal bodyweight gain was noted for the exposed animals, with the exception of one female where slight bodyweight loss was noted during the first week of observation period.

There was no mortality in the sighting study.

Main Study:

The mean achieved atmosphere concentration was 5.11 mg/L. The MMAD (Mean Mass Aerodynamic Diameter) was $3.54 \mu\text{m} \pm 2.32$ (GSD [Geometric Standard Deviation]).

Wet fur, ruffled coat and fur staining were commonly recorded on the day of exposure and several days after exposure in the main group. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be of toxicological relevance. Laboured,

gasping and noisy respiration, sneezing, decreased activity, prostration and ataxia were recorded for the exposed animals on day of exposure. One female was found dead after exposure. Noisy respiration or weak condition were recorded in 3 males and 3 females the day after exposure, weakened condition was noted in 2 males and 1 female 2 days after exposure and no significant clinical signs were observed from Day 3.

Normal bodyweight gain was noted for the exposed animals in the main study phase.

A single four hours nose-only exposure of SYN545974 to [REDACTED]: (WI) Wistar rats led to the death of one animal dosed at 5.11 mg/L during main study phase. Diffuse, dark red discoloration of the non collapsed lungs were seen in this rat at necropsy, the relationship of these findings to treatment of these findings are considered uncertain.

No macroscopic changes were observed in the lungs at dose level of 5.14 and 5.11 mg/L in neither the sighting, or the main study phase.

Under the experimental conditions of this study, one death occurred in a group of 10 rats exposed to a mean achieved atmosphere of 5.11 mg/L for 4 hours. The acute inhalation median lethal concentration of SYN545974, in [REDACTED]: (WI) Wistar strain rats is therefore considered to be greater than 5.11 mg/L.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Product Code:	SYN545974 technical
Stability of test compound:	Protected from light and humidity, room temperature
Recertification date:	End of June 2016

Vehicle and/or positive control: None

Test Animals:	
Species	Rat
Strain	[REDACTED]: (WI) Wistar rats
Age/weight at dosing	Young adult rats 9-11 weeks old and in the weight range of 227 to 499 g (males: 405-499g; females: 227-284g)
Source	[REDACTED]
Housing	Group caging
Acclimatisation period	At least 5 days
Diet	[REDACTED] "Autoclavable Complete Feed for Rats and Mice – Breeding and Maintenance" ad libitum
Water	Tap water ad libitum
Environmental conditions	Temperature: 22 ± 3 °C Humidity: 30 - 70 % Air changes: 15-20 air exchanges/hour Photoperiod: 12 hours daily, from 6.00 a.m. to 6.00 p.m.

Study Design and Methods:

In-life dates: Start: 25 September 2012

End: 16 October 2012

Exposure conditions: Trial generations were carried out prior to the start of the main study in order to establish generation procedures to achieve, to the extent possible, the desired chamber concentration and desired particle size distribution (mass median aerodynamic diameter between 1 and 4 µm).

Exposure conditions during the study are given later in a table of the test atmosphere characteristics of SYN545974.

Generation of the test atmosphere / chamber description: Due to the granular nature of the material, suitable test atmospheres could not be produced from the material as supplied. Prior to use, the test item was ground using a Ball Mill - "*Mixer Mill MM 400*".

The test item was aerosolised using two Wright's Dust Feed Systems (TSE Systems GmbH, Bad Homburg, Germany) located at the top of the exposure chamber. Compressed air was supplied by means of an oil-free compressor and passed through a suitable filter system prior to introduction to the dust generator.

The animals were exposed, nose-only, to an atmosphere of the test item using a TSE Rodent Exposure System (TSE Systems GmbH, Bad Homburg, Germany). This system comprises of 2, concentric anodised aluminium chambers and a computer control system incorporating pressure detectors and mass flow controllers.

Fresh aerosol from the generation system was constantly supplied to the inner plenum (distribution chamber) of the exposure system from where, under positive pressure, it was distributed to the individual exposure ports. The animals were held in polycarbonate restraint tubes located around the chamber which allowed only the animal's nares to enter the exposure port. After passing through the animal's breathing zone, used aerosol entered the outer cylinder from where it was exhausted through a suitable filter system. Atmosphere generation was therefore dynamic.

Airflows and relative pressures within the system were constantly monitored and controlled by the computer system thus ensuring a uniform distribution and constant flow of fresh aerosol to each exposure port (breathing zone). The flow of air through each port was at least 0.7 L/min. This flow rate was considered adequate to minimise re-breathing of the test atmosphere as it is about twice the respiratory minute volume of a rat.

Homogeneity of the test atmosphere within the test chamber and amongst the exposure ports was not specifically determined during this study. However, chambers of this design have been fully validated and have shown to produce evenly distributed atmospheres in the animals' breathing zones (██████, 1994).

Test atmosphere concentration: The test atmosphere was sampled at regular intervals during each exposure period. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone) by pulling a suitable, known volume of test atmosphere through weighed GF10 glass fibre filters (Whatman GmbH, Hahnstraße 3 – D-37586 Dassel, Germany). The difference in the pre- and post-sampling weights, divided by the volume of atmosphere sampled, was equal to the actual achieved test atmosphere concentration.

The nominal concentration was calculated by dividing the mass of test material disseminated into the chamber by the total volume of air that went through the chamber during the same period.

Particle size determination: The particle size of the test atmosphere was determined three times during the exposure period using a 7-stage impactor of Mercer style (TSE Systems GmbH, Bad Homburg, Germany). Such devices employ an inertial separation technique to isolate particles in the discrete aerodynamic size ranges. Samples were taken from an unoccupied exposure port (representing the animal's breathing zone).

The collection substrates and the backup filter were weighed before and after sampling and the weight of test item, collected at each stage, calculated by this difference.

The total amount collected for each stage was used to determine the cumulative amount below each cut-off point size. In this way, the proportion (%) of aerosol less than 0.55, 0.96, 1.55, 2.11, 3.56, 6.66 and 10.55 µm was calculated.

From these data, using software supplied with the impactor (TSE Systems GmbH, Bad Homburg, Germany), the Mass Median Aerodynamic Diameter (MMAD), and Geometric Standard Deviation (GSD) were calculated. In addition, the proportion (%) of aerosol less than 4µm (considered to be the inhalable portion) was determined.

Summary of acute study test atmosphere characteristics

GROUP 1					
Parameter	Maximum attainable concentration				
Gravimetric concentration	5.11 ±0.18 mg/L				
Particle size MMAD; GSD	MMAD: 3.54 µm GSD: 2.32				
Particle size distribution	mg/stage				
Effective cutoff diameter (µm)	Impactor 1	Impactor 2	Impactor 3	Total Mass/stage	Cumulative Mass (%)
10.55	0.45	0.56	0.43	1.44	100.00
6.66	0.73	0.82	0.87	2.42	92.17
3.56	1.75	1.73	1.72	5.20	79.01
2.11	1.93	1.63	1.87	5.43	50.73
1.55	0.68	0.55	0.59	1.82	21.21
0.96	0.41	0.37	0.37	1.15	11.31
0.55	0.16	0.09	0.16	0.41	5.06
< 0.55	0.13	0.17	0.22	0.52	2.83
Flow rate (whole system)	17.7-21.0 (mean = 30.3) L/min				
Temperature	23.5-24.8°C (exposure tube); 22±3°C (room)				
Humidity	30-70% (room)				

Animal assignment and treatment: Prior to the start of the study the animals were examined to ensure that they were physically normal and exhibited normal activity. All animals were observed for mortality during the exposure period. The animals were examined for signs of gross toxicity, and behavioural changes upon removal from the exposure tube and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhoea and coma.

Individual bodyweights were recorded prior to treatment on the day of exposure (Day 0) and on Days 1, 3, 7 and 14.

At the end of the 14-day observation period, the animals were sacrificed by exsanguination under anaesthesia (intra-peritoneal injection of pentobarbital solution - 'Euthasol® 40%; Lot No.: 11H15 8; Expiry: 07-2014; Produced by AST Beheer B.V. Oudewater Netherlands (Produlab Pharma, Raamsdonksveer)) and gross macroscopic examination was performed. All animals were subject to a gross necropsy which included a detailed examination of the abdominal and thoracic cavities. Special attention was given to the respiratory tract for macroscopic signs of irritancy or local toxicity.

Statistics: The acute inhalation LC50 was not estimated.

RESULTS

Mortality: There was one death observed in the main study.

Clinical observations Wet fur, ruffled coat and fur staining were commonly recorded on the day of and several days after exposure. These observations were considered to be related to the restraint and exposure procedures and, in isolation, were considered not to be toxicologically relevant.

Sighting exposure: Laboured and noisy respiration, sneezing, decreased activity and ataxia were recorded for the exposed animals on day of exposure. Laboured respiration was recorded for the two females on the day after exposure. No significant clinical signs were noted from Day 2. Thin fur was observed in one female on days 10-14 and fur loss was observed in another female on days 11-14, but was considered not to be related to the test substance.

Main study: Laboured, gasping and noisy respiration, sneezing, decreased activity, prostration and ataxia were recorded for the exposed animals on day of exposure. One female was found dead after exposure. Noisy respiration or weak condition were recorded in 3 males and 3 females the day after exposure, weakened condition was noted in 2 males and 1 female 2 days after exposure and no significant clinical signs were observed from Day 3. Thin fur was also observed in two females, on days 1 to 3 and days 1 to 10 respectively, but was considered not to be related to the test substance.

Bodyweight: Normal bodyweight gain was noted for the exposed animals, with the exception of one female from the sighting group where slight bodyweight loss was noted during the first week of observation period.

Necropsy: A single four hours nose-only exposure of SYN545974 to [REDACTED]: (WI) Wistar rats led to the death of one animal dosed at 5.11 mg/L during Main study phase. Diffuse, dark red discoloration of the non collapsed lungs were seen in this rat at necropsy, the relationship of these findings to treatment of these findings are considered uncertain.

The necropsy examination after the recovery period indicated no macroscopic changes in the lungs at dose levels of 5.14 and 5.11 mg/L for the sighting and main study phase respectively.

CONCLUSION:

In a GLP compliant study carried out in accordance with the most recent OECD 403 test guideline (2009), pydiflumetofen was assessed for potential inhalation toxicity by exposure of 5 male and 5 female [REDACTED] WI rats through a nose-only exposure system, to a target aerosol concentration of 5 mg/L of test item for 4 hours followed by a 14 day observation period.

Due to insufficient information on the inhalation toxicity potential of the test substance, an initial sighting exposure experiment in 2 male and 2 female [REDACTED]:WI rats to a target concentration of 5 mg/L (with an MMAD of $3.48 \mu\text{m} \pm 2.35$ Geometric standard deviation (GSD)) was performed, resulting in a range of symptoms. Some symptoms (wet fur, ruffled coat and fur staining) were considered to be the result of the study restraint and exposure procedures and therefore not of toxicological relevance. All other significant clinical signs (sneezing, ataxia, laboured and noisy respiration and decreased activity) had reversed by day 2 of the study, besides thin fur in one female on days 10-14 and fur loss in one female on days 11-14. However, these symptoms were considered to be incidental, given that they did not occur near the treatment time. Considering the reversibility of symptoms, in addition to the lack of mortality and moribundity, the target concentration of this study was considered acceptable for use in the main study.

In the main study, a mean atmosphere concentration of 5.11 mg/L was achieved (MMAD: $3.54 \mu\text{m} \pm 2.32$ (GSD)). Wet fur, ruffled coat and fur staining were observed solely on the day of exposure and were considered to be the result of the restraint and exposure procedures and therefore not of toxicological relevance. Symptoms considered to be of toxicological relevance included laboured gasping, noisy respiration, sneezing, decreased activity, prostration and ataxia on the day of exposure. All of these symptoms had reversed within three days and thin fur was observed in two females (days 1 to 3 and days 1-10 respectively). However, all of these symptoms were considered incidental, as in the sighting study. One female was found dead after exposure, with diffuse, dark red discoloration of the (non-collapsed) lungs observed at necropsy; however it was not considered to be treatment-related, in view of the fact that this was the only mortality of the study, and no similar lung findings were seen at necropsy in any other animal. The achieved MMAD ($3.54 \mu\text{m}$) was within the target range (between 1 and $4 \mu\text{m}$). No other macroscopic

observations were observed nor were any treatment related effects on body weight and body weight gain. Considering the single, isolated mortality, lack of macroscopic observations or treatment related changes to body weight or body weight changes, HSE agrees with the EU conclusion, that the inhalation 4-hr-LC₅₀ of pydiflumetofen aerosol is greater than 5.11 mg/L.

In conclusion, under the conditions of this GLP and OECD guideline compliant study, the acute inhalation median lethal concentration (4-hr-LC₅₀) of pydiflumetofen aerosol was found to be greater than 5.11 mg/L in male and female [REDACTED]:WI rats. As a result in accordance with Regulation 1272/2008, the compound does not meet the classification criteria for acute inhalation toxicity.

([REDACTED], 2013)

B.6.2.4. Skin irritation

Report:	K-CA 5.2.4/01 [REDACTED] (2012). SYN545974 – Primary Skin Irritation Study in Rabbits. [REDACTED] Laboratory Report No. 12/344-006N, issue date 26 November 2012. Unpublished. Syngenta File No. SYN545974_10035.
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Guidelines: Acute Skin Irritation (rabbit) OECD 404 (2002); OPPTS 870.2500 (1998); EC No 440/2008, B.4 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In a primary dermal irritation study, young adult New Zealand white rabbits (3 males) were exposed to 0.5 g SYN545974, applied to the intact shaved flank under a semi-occlusive dressing, for 4 hours. Skin reactions were scored at 1, 24, 48 and 72 hours after removal of the dressings. Irritation was scored by a numerical scoring system and the Primary Irritation Index (PII) was calculated.

No local dermal signs were observed in the treated animals throughout the study

The primary irritation index was 0.00.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred. The body weights of all rabbits were considered to be within the normal range of variability. The study was terminated after the 72 hours observation period.

According to the Draize classification criteria SYN545974 is considered to be “Not-Irritant” to rabbit skin (P.I.I. = 0.00).

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Product Code:	SYN545974 technical
Stability of test compound:	Protected from light and humidity, room temperature
Recertification date:	End of June 2016

Vehicle and/or positive control: None

Test Animals:	
Species	Rabbit
Strain	Young Adult New Zealand White Rabbit
Age at dosing	~11 weeks,
Source	
Housing	Individual caging
Acclimatisation period	At least 5 days
Diet	UNI diet for rabbits () ad libitum
Water	Tap water, from an automatic system, ad libitum
Environmental conditions	Temperature: 17.5-22.0 °C Humidity: 38-78 % Air changes: 15-20 air exchanges/hour Photoperiod: 12 hours daily, from 6.00 a.m. to 6.00 p.m.

Study Design and Methods:

In-life dates: Start: 13 September 2012 End: 18 September 2012

Animal assignment and treatment: A weight of 0.5 g (per animal) of SYN545974 was measured and applied undiluted as supplied by the Sponsor.

According to EC 2004/73, B.4. and OECD Guidelines 404, a test item does not need to be tested if the pH-value is less than 2 or greater than 11.5, owing to its predictable corrosive properties. The pH of the test item was measured before the study initiation date and was found to be 5.5.

Approximately 24 hours prior to the test the hair was clipped from the back and flanks of the animals with an electric clipper, exposing an area of approximately 100 cm² (10 cm x 10cm). Animals with overt signs of skin injury or marked irritation, which may have interfered with the interpretation of the results, were not used in the test.

On the day of treatment, 0.5 g SYN545974 was placed on a surgical gauze pad (*ca.* 2.5 cm x 2.5 cm). This gauze pad was applied to the intact skin of the clipped area and was kept in contact with the skin by a patch with a surrounding adhesive hypoallergenic plaster. The entire trunk of the animals was then wrapped with plastic wrap held in place with an elastic stocking. The test item was moistened with the smallest of water sufficient to ensure good skin contact.

The duration of treatment was 4 hours. The dressing was then removed.

Initially, a single animal was treated. As neither a corrosive effect nor a severe irritant effect were observed after the 24-hour after the start of the sentinel animal treatment, the test was completed using the 2 remaining animals with an exposure period of 4 hours.

The viability/mortality was recorded daily from the day of application of the animals to the termination of test. Clinical signs were recorded daily and body weights were recorded on the day of application and at termination of observation.

The primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours for all animals and then dividing by the number of data points.

RESULTS

No local dermal signs were observed in the treated animals throughout the study. The primary irritation index was 0.00.

No clinical signs were observed in the animals during the study and no mortality occurred.

As no clinical signs were observed up to 72 hours after patch removal, the study was terminated after the 72 hour observation.

The body weights of all rabbits were considered to be within the normal range of variability

The primary irritation index was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours for all animals and then dividing by the number of data points. The primary irritation index was 0.00 (out of a maximum score of 8.0). No corrosive effects were noted on the treated skin of any animal at any of the observation intervals.

Table 6.2.4-1: Individual and mean skin irritation scores of SYN545974 according to the Draize scheme

Time	Erythema			Oedema		
Animal number	2061	2060	2008	2061	2060	2008
after 1 hour	0	0	0	0	0	0
after 24 hours	0	0	0	0	0	0
after 48 hours	0	0	0	0	0	0
after 72 hours	0	0	0	0	0	0
after 1 week	0	0	0	0	0	0
after 2 weeks	0	0	0	0	0	0
mean score 24-72 h	0	0	0	0	0	0

CONCLUSION:

The potential of pydiflumetofen to induce skin irritation in 3 young adult New Zealand White (NZW) Rabbits was tested in a GLP and OECD 404 (2002) compliant study. Although the study was performed under the old 2002 version of the guideline, it was also found to comply with the newest guideline (2015). No stepwise procedure was followed to minimise *in vivo* testing, with the justification being that the study was performed to support registration in other regulatory authorities where *in vivo* testing is a requirement. The pH of 5.5 was also cited as a reason for testing, as the pH is not less than 2 or more than 11.5, in which case irritation/corrosivity can be assumed. This justification is not considered valid by HSE, meaning that this study is considered to be in breach of article 62 of Regulation 1107/2009.

Rabbits were exposed to 0.5 g of the test item, on a shaved flank for 4 hours, and were then assessed for irritation after an hour, and subsequently each day for 3 days, with irritation scored by the primary irritation index (PII). A single animal was treated, and when no irritation or corrosivity was observed, the test was completed in the 2 remaining animals.

No local dermal signs were observed in any of the treated animals in the study (Primary Irritation index: 0.00 out of a maximum of 8.00.). Likewise, there were no clinical signs or mortality observed during the test period, with body weight parameters also found to lie within the normal range of variability. Given the lack of clinical signs up to 72 hours after patch removal the study was terminated after this period. Given the lack of any irritation/corrosivity or other treatment related effects, HSE agrees with the EU evaluation that the test item is not a skin irritant and does not require classification under Regulation 1272/2008 for skin irritancy, as the mean values of erythema and oedema (0.00 and 0.00 respectively) did not exceed the threshold for classification (≥ 2.3 for oedema or erythema.)

In conclusion, pydiflumetofen was not a skin irritant in male NZW rabbits, therefore it does not need to be classified for skin irritation, in accordance with Regulation 1272/2008.

(██████████ 2012)

B.6.2.5. Eye irritation

Report:	K-CA 5.2.5/01 ██████████ (2012a). SYN545974 - Acute Eye Irritation Study in Rabbits. ██████████. Laboratory Report No. 12/344-005N, issue date 26 November 2012. Unpublished. Syngenta File No. SYN545974_10033.
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Guidelines: Acute Eye Irritation (rabbit) OECD 405 (2002); EPA OPPTS 870.2400 (1998); EC No 440/2008, B.5 (2008); Directive 2004/73/EC B.5 (L 152 2004 29th April)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

In a primary eye irritation study, 0.1 g SYN545974 was instilled into the conjunctival sac of the left eye of each of 3 adult male New Zealand White rabbits. The untreated right eyes served as the control. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours after test material instillation. Observations with fluorescein staining were made approximately 24 hours before treatment and then 24, 48 and 72 hours after treatment.

No mortality occurred during the study.

An Initial Pain Reaction (IPR) score of 2 was observed in all animals. At the 1 hour after treatment observation, discharge was observed in one rabbit (score 1) and conjunctival redness was seen in all rabbits, two with a score 2 and one rabbit had a score 1. Conjunctival redness (score 1) was seen in one rabbit at 24 and 48 hours after treatment. All symptoms had fully reversed in all animals at the 72 hour observation. Fluorescein staining was negative in all animals at any time point.

No clinical signs of systemic toxicity were observed in the animals during the study.

SYN545974 was graded as a mild irritant (Class 4 on a 1 to 8 scale) to the rabbit eye according to the modified Kay and Calandra classification system.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Product Code:	SYN545974 tech.
Stability of test compound:	Protected from light and humidity, room temperature
Recertification date:	End of June 2016

Vehicle and/or positive control: Test material was used as supplied.

Test Animals:	
Species	Rabbits
Strain	New Zealand white rabbits
Age of animals at dosing	Young adult rabbits, ~ 12 weeks old
Source	
Housing	Individual caging
Acclimatisation period	at least 5 days
Diet	UNI diet for rabbit),ad libitum
Water	Tap water from the automatic system ad libitum
Environmental conditions	Temperature: 17.0 – 22.0 °C Humidity: 35– 78 % Air changes: 15-20 air exchanges/hour Photoperiod: 12 hours daily, from 6.00 a.m. to 6.00 p.m.

Study Design and Methods:

In-life dates: Start: 19 September 2012 End: 23 September 2012

Animal assignment and treatment: Approximately 24 hours before the start of the test, the treated eyes of the provisionally selected test rabbits were examined for evidence of ocular irritation or defect using a hand-held slit-lamp and one drop of 2% fluorescein solution. Only animals free of ocular damage were used.

Initially, a single rabbit was treated. A single amount of 0.1 g of the test material was placed into the conjunctival sac of the left eye, formed by gently pulling the lower lid away from the eyeball. The upper and lower eyelids were held together for several seconds immediately after treatment, to prevent loss of the test material, and then released. The right eye remained untreated and was used for control purposes. Immediately after administration of the test material, an assessment of the initial pain reaction was made.

Following the 1- and 24-hour examinations and a review of the ocular responses produced in the first treated animal, 2 additional animals were treated.

An assessment of eye irritation was made according to a 6 point scale at approximately 1, 24, 48 and 72 hours after instillation. The ocular reaction (i.e. corneal opacity, iridic effects, conjunctivae and chemosis) was assessed according to the Draize numerical evaluation (Draize J H 1977). The eyes were further examined using 2% fluorescein solution at least 24 hours before treatment and then 24, 48 and 72 hours after treatment.

RESULTS

Mortality: No intercurrent mortality occurred during the study.

Clinical observations: An Initial Pain Reaction (IPR) score of 2 was observed in all animals. At the 1 hour after treatment observation, discharge was observed in one rabbit (score 1) and conjunctival redness was seen in all rabbits, two with a score 2 and one rabbit had a score 1. Conjunctival redness (score 1) was seen in one rabbit at 24 and 48 hours after treatment. All symptoms had fully reversed in all animals at the 72 hour observation. Fluorescein staining was negative in all animals at any time point.

No clinical signs of systemic toxicity were observed in the animals during the study and no mortality occurred.

During the study, the control eye of all animals was symptom-free.

Table 6.2.5-1: Eye irritation scores of SYN545974 according to the Draize scheme

Time	Cornea			Iris			Conjunctiva					
							Redness			Chemosis		
Animal number	2067	2063	2062	2067	2063	2062	2067	2063	2062	2067	2063	2062
after 1 hour	0	0	0	0	0	0	2	1	2	0	0	0
after 24 hours	0	0	0	0	0	0	0	0	1	0	0	0
after 48 hours	0	0	0	0	0	0	0	0	1	0	0	0
after 72 hours	0	0	0	0	0	0	0	0	0	0	0	0
mean scores 24-72h	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00

The study was terminated after a period of 72 hours observation since all signs of eye irritation had reversed.

Bodyweight: All animals showed an expected gain in bodyweight by the end of the study.

Necropsy: No necropsy was performed in the study.

CONCLUSION:

The potential of pydiflumetofen to induce eye irritation in 3 young adult New Zealand White (NZW) Rabbits was tested in a GLP and OECD 405 (2002) guideline compliant study. Although the study was performed under the old 2002 version of the guideline, it was also found to comply with the newest guideline (adopted 2017, updated 2021). No stepwise procedure was followed to minimise *in vivo* testing, with the justification being that the study was performed to support registration in other regulatory authorities where *in vivo* testing is a requirement. This justification is not considered valid by HSE, meaning that this study is considered to be in breach of article 62 of Regulation 1107/2009.

Rabbits were exposed to 0.1 g of the test item, through instillation into the conjunctival sac of the left eye of the animals, with the untreated eye serving as a control. Observations were made according to a 6 point scale after an hour, and subsequently each day for 3 days after instillation according to Draize evaluation. Eyes were also examined with 2% fluorescein solution at least 24 hours before and 24, 48 and 72 hours after treatment.

An initial pain reaction (IPR) score of 2 was observed in all three animals; discharge was observed in one rabbit (score 1) and conjunctival redness was seen in all three rabbits (2 with score 2 and 1 with score 1, mean of 0.67 in one rabbit, 0 in the other two). One rabbit was observed to exhibit conjunctival redness 24 and 48 hours after treatment, but all symptoms had fully reversed by the 72 hour observation. Fluorescein staining produced a negative response at all points tested, and the control eye of all three animals remained symptom-free at all points. Bodyweight parameters remained in the expected range; no necropsy was performed. Based on these signs of irritancy the test item was graded as a mild irritant (Class 4 on a 1 to 8 scale). Pydiflumetofen doesn't meet the current criteria for serious eye irritation (Regulation 1272/2008), as

the only observed symptom of irritation (conjunctival redness), had a mean score of 0.67 in one rabbit, below the threshold of 2 in 2 of 3 rabbits required for classification. Consequently, HSE agrees with the EU evaluation that the test item does not require classification for eye irritancy.

In conclusion, pydiflumetofen exhibited only mild eye irritant effects (a mean of 0.67 for conjunctival redness) in male NZW rabbits, so did not meet the threshold of classification to be considered an eye irritant. Therefore, pydiflumetofen does not require classification as an eye irritant in accordance with Regulation 1272/2008.

([REDACTED], 2012a)

B.6.2.6. Skin sensitization

Report: K-CA 5.2.6/01 [REDACTED] (2013). SYN545974 - Local Lymph Node Assay in the Mouse. [REDACTED] Laboratory Report No. 12/344-037E, issue date 31 January 2013. Unpublished. Syngenta Fiole No. SYN545974_10052.

Guidelines: Dermal Sensitisation Local Lymph Node Assay OECD 429 (2010); OPPTS 870.2600 (2003); EC No 440/2008 of 30 May 2008, B.42, EPA Health Effects Test Guidelines, OPPTS 870.2600, “Skin Sensitization“, EPA 712-C-03-197, March 2003

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

SYN545974 was assessed for its skin sensitisation potential using the mouse Local Lymph Node Assay. The assay determines the level of T lymphocyte proliferation in the lymph nodes draining the site of chemical application, by measuring the amount of radiolabelled thymidine incorporated into the dividing cells. The criterion for a positive response is that one or more of the concentrations tested should elicit a 3-fold or greater increase in isotope incorporation relative to the vehicle control group. The test substance was applied as 50, 25 and 10 % (w/v) SYN545974 preparations in acetone:olive oil 4:1 (v:v).

The test item solutions were applied on the dorsal surface of ears of experimental animals (25 µL/ear) for 3 consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. On Day 6, 5 hours prior to termination, animals were intravenously injected via the tail vein with tritiated methyl thymidine (3HTdR). Cell proliferation in the local lymph nodes was measured by incorporation of 3HTdR and the values obtained were used to calculate stimulation indices (SI).

Groups of five female CBA/J Rj mice were treated with:

- 50, 25 or 10 % (w/v) SYN545974 (formulated in acetone:olive oil 4:1 (v:v) mixture, AOO)
- the negative (vehicle) control group received the vehicle (AOO)
- the positive control group received 25 % (w/v) α -Hexylcinnamaldehyde (HCA) in AOO

No mortality or signs of systemic toxicity was observed during the study. There were no indications of any irritancy at the site of application. No marked body weight loss was observed in the experimental animals. Test item precipitate was observed on the ear of the animals after treatment in the 50 % (w/v) dose group on Days 1-3 and in the 25 % (w/v) dose group on Days 2-3.

Stimulation index values of the test item were 1.0, 1.1 and 1.1 at concentrations of 50, 25 and 10 % (w/v), respectively.

Under the conditions of the present assay, SYN545974, when tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitizer) in the Local Lymph Node Assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off-white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 %
CAS#:	1228284-64-7
Product Code:	SYN545974 technical
Other product code:	CSCD678790
Recertification date:	30 June 2016
Storage conditions	< 30°C, protected from light and humidity

Vehicle and/or positive control: The vehicle for the test substance was AOO (acetone:olive oil 4:1 (v:v) mixture) and 25% (w/v) α -Hexyl-cinnamaldehyde solution dissolved in AOO for the positive control.

Test Animals:	
Species	Mouse
Strain	CBA/J Rj
Age/weight at dosing	12 weeks old
Source	
Housing	Individual caging / mice were provided with glass tunnel-tubes
Acclimatisation period	34 days
Diet	"Autoclavable complete diet for rats and mice, ad libitum
Water	tap water from the municipal supply, ad libitum
Environmental conditions	Temperature: 19.4 – 25.0 °C Humidity: 30 - 70% Air changes: 15-20 air exchange/hour Photoperiod: 12 hours daily

Study Design and Methods:

In-life dates: Start: 03 October 2012

End: 08 October 2012

Preliminary irritation/toxicity test: A Preliminary Irritation/Toxicity Test was performed on CBA/J Rj mice using two doses (2 animals/dose), at test item concentrations of 50 and 25 % (w/v) in AOO. The 50% formulation was considered to be a suitable maximum dose level for the study. The application of the material and the local effects on the animals were considered acceptable for a valid LLNA.

Animal assignment and treatment: Groups of five female CBA/J Rj mice were treated with 50, 25 and 10 % (w/v) SYN545974. The negative control group received AOO and the positive control group received 25 % α -Hexylcinnamaldehyde (HCA) in AOO.

The test item solutions were applied on the dorsal surface of the ears (25 μ L/ear) for 3 consecutive days (Days 1, 2 and 3). There was no treatment on Days 4, 5 and 6. On Day 6, animals were killed and cell proliferation in the local lymph nodes was measured by incorporation of tritiated methyl thymidine (3HTdR). The values obtained were used to calculate stimulation indices (SI).

Terminal procedures: On Day 6, animals were intravenously injected with 250 μ L of sterile PBS (phosphate buffered saline) containing approximately 20 μ Ci of 3HTdR. Five hours after intravenous injection, the mice were humanely killed and the draining auricular lymph nodes were excised, and placed in separate Petri dishes containing PBS to keep the nodes wet before processing. The nodes of each animal were processed individually.

Preparation of Lymph Node Cells: A single cell suspension (SCS) of lymph node cells (LNCs) was prepared and collected in disposable tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer. LNCs for each mouse were pelleted by centrifugation after which supernatants were discarded. Pellets were gently resuspended and 10 mL of PBS was added to the tubes. The washing step was repeated twice. This procedure was repeated for the lymph nodes of each individual animal.

After the final washing step, supernatants were removed. Pellets were gently agitated resuspended and 3 mL of 5 % (w/v) TCA solution was added to the tubes for precipitation of macromolecules.

After incubation with 5% TCA at 2-8 °C for approximately 42 hours), precipitate was recovered by centrifugation at 190 x g for 10 minutes at 4 °C, and supernatants were removed. Pellets were resuspended in 1 mL of 5% (w/v) TCA solution and dispersed using an ultrasonic water bath. Each precipitate was transferred to a suitable sized scintillation vial with 10 mL of scintillation liquid and thoroughly mixed. The vials were loaded into a β -scintillation counter and $^3\text{HTdR}$ incorporation was measured (10-minute measurement per sample).

The β -counter expresses the $^3\text{HTdR}$ incorporation as the number of radioactive disintegrations per minute (DPM). Background level was also measured in duplicates by adding 1 mL of 5 % (w/v) TCA solution into a scintillation vial filled with 10 mL of scintillation liquid.

Statistics / Data Evaluation: Disintegrations per minute (DPM) were measured for each animal of nodes (correcting for background radioactivity). The results were expressed as disintegrations per node (DPN) by dividing the DPM by the number of lymph nodes.

Stimulation index (SI = mean DPN of treated group divided by mean DPN of the appropriate control group) for each treatment group was calculated. A stimulation index of 3 or greater is the criteria for defining a positive result.

A stimulation index of 3 or greater is the criteria for defining a positive result.

Results were analyzed for homogeneity by a specific statistical method (Grubb's test). The outcome of a statistical method was taken into consideration in result interpretation.

RESULTS

No mortality or signs of systemic toxicity was observed during the study. There were no indications of any irritancy at the site of application. Test item precipitate was observed on the ear of the animals after treatment in the 50 % (w/v) dose group on Days 1-3 and in the 25 % (w/v) dose group on Days 2-3.

The stimulation index values were 1.0, 1.1 and 1.1 at concentrations of 50, 25 and 10 % (w/v), respectively.

Table 6.2.6-1: Skin sensitisation potential of SYN545974

Concentration of test substance (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test : control ratio (SI)
0 (AOO)	2	166.0	83.0	N/A
	2	27.0	13.5	
	2	208.0	104.0	
	2	127.0	63.5	
	2	272.0	136.0	
50 % (w/v)	2	127.0	63.5	1.0
in AOO	2	143.0	71.5	
	2	121.0	60.5	
	2	191.0	95.5	
	2	223.0	111.5	

Concentration of test substance (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test : control ratio (SI)
25 % (w/v)	2	240.0	120.0	1.1
in AOO	2	132.0	66.0	
	2	86.0	43.0	
	2	165.0	82.5	
	2	269.0	134.5	
10 % (w/v)	2	239.0	119.5	1.1
in AOO	2	2729.0	1364.5 [#]	
	2	65.0	32.5	
	2	229.0	114.5	
	2	146.0	73.0	

N/A = not applicable

[#]: The indicated DPN value is a significant outlier at $p < 0.05$ level (Grubbs' test). Data are not used for calculation of the group DPN value, therefore excluded from the SI calculation

In the positive control group, α -Hexylcinnamaldehyde induced positive responses when applied as 25% (w/v) preparations in AOO, confirming the validity of the protocol used in this study.

Table 6.2.6-2: Radiolabel incorporation into lymph-nodes of mice treated with the positive control substance (α -Hexylcinnamaldehyde)

Concentration of hexylcinnamaldehyde (%w/v)	Number of lymph nodes assayed	Disintegrations per minute (dpm)	dpm per lymph node	Test : control ratio (SI)
0 (AOO)	2	166.0	83.0	N/A
	2	27.0	13.5	
	2	208.0	104.0	
	2	127.0	63.5	
	2	272.0	136.0	
25 % (w/v) HCA	2	1136.0	568.0	7.4
in AOO	2	1312.0	656.0	
	2	1220.0	610.0	
	2	2805.0	1402.5 [#]	
	2	1082.0	541.0	

N/A = not applicable

[#]: The indicated DPN value is a significant outlier at $p < 0.05$ level (Grubbs' test). Data are not used for calculation of the group DPN value, therefore excluded from the SI calculation

CONCLUSION:

The potential of pydiflumetofen to induce skin sensitisation was investigated in a GLP and OECD 429 (2010) compliant LLNA study. A preliminary irritation/ toxicity test was carried out, on two groups of 2 mice dosed with 50 and 25% (w/v) concentrations of the test item. As no irritation or toxicity was noted in either dose group, the 50% formulation was considered to be the suitable maximum dose level for the main study.

In the main experiment, 5 groups of 5 female CBA/J Rj mice were tested, including groups treated with 50, 25 and 10% (w/v) solutions of the test item respectively, a negative control group treated with just the vehicle, acetone : olive oil (4:1 v:v, AOO) and a positive control group treated with of 25% α -Hexylcinnamaldehyde (HCA) in AOO. A volume of 25 μ L of the respective solutions for each group was applied to the dorsal surface of the ears for 3 consecutive days, after which animals were euthanised following an additional period of 3 days (Study day 6). Before euthanasia on day 6, animals were injected with 250 μ L of sterile phosphate buffered saline (PBS) containing 20 μ Ci of triated methyl thymidine (3HTdR) and stimulation indices (SI) were calculated by measuring proliferation of local lymph nodes

from incorporation of the 3HTdR. Test item precipitate was observed on the ear of animals of the 50% w/v dose group on days 1-3 and in the 25% w/v dose group on days 2-3. This means the experiment can be considered to have been performed up to the limit of solubility.

SI values in the 50, 25 and 10 % (w/v) test groups were found to be 1.0, 1.1 and 1.1 respectively, whilst the positive control group of 25% HCA in AOO induced an SI of 7.4. According to Regulation 1272/2008, an SI greater than 3 can be considered as the threshold for defining a positive result, so therefore the test item does not meet the criteria to be considered a skin sensitizer, whilst the positive control instigated a clear sensitising response, demonstrating the efficacy of the test system. Consequently, HSE agree with the conclusion of the EU evaluation, that pydiflumetofen is not a skin sensitizer.

In conclusion, pydiflumetofen is not a skin sensitizer up to the limit of solubility in this OECD and GLP compliant LLNA in female CBA/J Rj mice and therefore does not meet the criteria for classification under Regulation 1272/2008.

(██████████, 2013)

B.6.2.7. Phototoxicity

Report:	K-CA 5.2.7/01 ██████████ (2015). SYN545974: <i>In Vitro</i> 3T3 NRU Phototoxicity Test. Eurofins BioPharma, Product Testing Munich GmbH, Behringstrasse 6/8, 82152 Planegg, Germany. Laboratory Report No. 151200, 2 September 2015. Unpublished. Syngenta File No. SYN545974_10297.
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Guidelines: Phototoxicity – *in vitro* 3T3 NRU test; OECD 432 (2004); OECD 101 (1981); 440/2008/EC (2008).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

EXECUTIVE SUMMARY

The phototoxic potential of SYN545974 was analysed. SYN545974 was dissolved in DMSO and diluted in a 1:100 ratio in Earle's balanced salt solution. BALB/c 3T3 cells were treated for 1 h with different concentrations of the test solution at $37 \pm 1^\circ\text{C}$ and further 50 min in absence and in presence of a non-cytotoxic dose of UVA light, respectively. One day after treatment cytotoxicity was analysed as a measure of reduction of neutral red uptake and compared to the controls.

In this study under the given conditions SYN545974 showed a cytotoxic effect with and without irradiation. With irradiation the viability of the cells was reduced to 17.1% and without irradiation to 36.7%.

- UVA: 41.66 µg/mL

+ UVA: 24.56 µg/mL

PIF = 1.7

SYN545974 is classified as “non phototoxic”.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	White solid
Lot/Batch number:	SMU2EP12007
Supplied by:	Syngenta Crop Protection
Purity:	98.5%
Contaminants:	Not reported
CAS#:	1228284-64-7
Stability of test compound:	30 June 2016 (stored at room temperature)

Media:

Cell culture medium: Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L D-glucose. The medium supplemented with the following items:

10% Calf Serum (CS; Biochrom)

1% Penicillin/Streptomycin (final concentration: 100 IU/100 µg/mL)

Neutral Red (NR) Stock Solution

0.4 g neutral red

100 mL H₂O

NR Medium

1 mL NR stock solution

79 mL cell culture medium (without NCS)

NR Desorb

1% acetic acid, glacial

50% ethanol

49% H₂O

Controls: 1% DMSO in EBSS was tested.

Positive: Chlorpromazine (2-Chloro-10-[3-dimethylaminopropyl] phenothiazine).

Study Design and Methods:

Experimental dates: Start: 6 May 2015 End: 22 July 2015

Preparation of test item: SYN545974 was dissolved in DMSO (Applichem) and diluted in EBSS (Sigma) in a 1:100 ratio to a highest final concentration of 125 µg/mL (stock solution).

Cells: The test was carried out with BALB/c 3T3 cells. Cells from frozen stock cultures, tested routinely for mycoplasma, were seeded in culture medium at an appropriate density and subcultured at least once before they were used in the *in vitro* 3T3 NRU phototoxicity test. Cells at passage number 79 were used. Cells were precultured in 75 cm² culture flasks in DMEM with 10% new born calf serum at 37 ± 1°C and 5% CO₂.

The UVA-sensitivity of the cells was determined and found to be acceptable. Six microtiter plates with cells were irradiated with the UVA-doses 0 (dark control), 3, 5, 7, 9 and 11 J/cm². The cells meet acceptance criteria if their viability after irradiation with 5 J/cm² is not less than 80% and after irradiation with 9 J/cm² is not less than 50%.

Doses: 125.00; 39.53; 12.50; 3.95, 1.25; 0.40; 0.13 and 0.04 µg/mL, plus negative control (1% DMSO in EBSS), blank (EBSS) and positive control (Chlorpromazine 100; 31.6, 10.0; 3.16; 1.00; 0.316; 0.100 and 0.0316 µg/mL without UVA and 10; 3.16; 1.00; 0.316; 0.100, 0.0316, 0.0100, and 0.00316 µg/mL with UVA).

UV Irradiation: For the phototoxicity test the solar simulator SOL-500 equipped with a H1-filter (Dr. Hönle) was used. The light source was filtered by the filter H1 (315 to 400 nm) to attenuate the highly toxic UVB wavelength (280 to 315 nm). The UVA irradiance was measured by a UVA meter with a spectral sensitivity in a range from 315 to 400 nm and a measuring range between 0 – 199.9 mW/cm². The UVA radiometer (Dr. Hönle) was calibrated by the supplier using a calibrated UV meter.

Experimental procedure: A cell suspension of 1×10^5 cells/mL in culture medium was prepared. 100 µL culture medium were dispensed into the peripheral wells of a 96-well tissue culture microtiter plate (blanks). In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL (1×10^4 cells/well) were dispensed. For each test item two plates were prepared: one for determination of cytotoxicity (without UVA), and the other for determination of photocytotoxicity (with UVA).

The cells were incubated for 24 ± 2 h (5% CO₂, $37 \pm 1^\circ\text{C}$) until they formed a half-confluent monolayer. This incubation period allowed for cell recovery and adherence, and for exponential growth.

After incubation, cells were washed with 150 µL EBSS per well.

The solutions of the test item and the positive control were diluted seven times at a ratio of $\sqrt[7]{10}$. The positive control was tested in a full scale phototoxicity test on two plates in parallel to the test item. 8 different concentrations were applied to 6 parallel cultures each.

100 µL of the appropriate concentration of test item or solvent only (negative control) were added to the cells. The cells were then incubated in the dark for 60 minutes (5% CO₂, $37 \pm 1^\circ\text{C}$).

The cells were irradiated for 50 min through the lid of the 96-well plate with 1.5-1.8 mW/cm² UVA (= 4.5-5.4 J/cm²). The positions of the plates were exchanged after half time of the irradiation (25 min.). The distance light source - test system was 64 cm. The temperature during irradiation was 28.6°C (+ UVA) and 23.4°C (- UVA). Duplicate plates (- UVA) were kept at room temperature in a dark box for 50 min (= - UVA exposure time).

After exposition the cells were washed with 150 µL EBSS. EBSS was replaced with culture medium and the plates were incubated (5% CO₂, $37 \pm 1^\circ\text{C}$) overnight (18-22 hours). Following the incubation the cells were washed with 150 µL EBSS. 100 µL neutral red (NR) medium were added and the plates were incubated at $37 \pm 1^\circ\text{C}$, in 5.0% CO₂ / 95% air, for 3 h.

After incubation, the NR medium was removed and the cells were washed with 150 µL EBSS. 150 µL NR desorb solution (freshly prepared ethanol/acetic acid) was added. The microtiter plate was shaken rapidly on a microtiter plate shaker for 10 min, until the NR had been extracted from the cells and had formed a homogeneous solution. Then the optical density of the NR extract was measured at 540 nm in a micro plate auto reader, using blanks as a reference.

Data analysis: Relative cell viability, expressed as percentage of untreated controls, was calculated for each of the eight test concentrations. To predict the phototoxic potential, the concentration responses obtained in the presence (+UVA) and in the absence (-UVA) of irradiation were compared at the EC₅₀ level, i.e. at the concentration inhibiting cell viability by 50% in comparison with untreated controls.

If complete concentration response curves are obtained, both in the presence (+UVA) and in the absence (-UVA) of light, a photo-irritation-factor (PIF) is calculated by means of the following formula:

$$\text{PIF} = \frac{\text{EC}_{50}(-\text{UVA})}{\text{EC}_{50}(+\text{UVA})}$$

$$\text{EC}_{50}(+\text{UVA})$$

Interpretation:

$$\text{PIF} < 2: \text{“no phototoxicity”},$$

PIF > 2 and < 5: “probable phototoxicity”,

PIF > 5: “phototoxicity”.

If both EC₅₀ (-UVA) and EC₅₀ (+UVA) cannot be calculated due to the fact that a test item does not show any cytotoxicity up to the highest concentration, this indicates no phototoxic potential.

If a test item is only cytotoxic + UVA and is not cytotoxic when tested - UVA, the PIF cannot be calculated, although this result indicates a phototoxic potential of the test item. In such cases the mean photo effect (MPE) is analysed. The MPE is based on comparison of the complete concentration response curves and is defined as the weighted average across a representative set of photo effect values

$$\text{MPE} = \frac{\sum_{i=1}^n w_i \text{PE}_{Ci}}{\sum_{i=1}^n w_i}$$

The photo effect PEC at any concentration C is defined as the product of the response effect REC and the dose effect DEC i.e. PEC = REC x DEC. The response effect REC is the difference between the responses observed in the absence and presence of light, i.e. REC = RC (- UVA) - RC (+ UVA). The dose effect is given by:

$$\text{DEC} = \frac{C/C^* - 1}{C/C^* + 1}$$

where C* represents the equivalence concentration, i.e. the concentration at which the +UVA response equals the - UVA response at concentration C. If C* cannot be determined because the response values of the + UVA curve are systematically higher or lower than RC (- UVA) the dose effect is set to 1. The weighting factors w_i are given by the highest response value, i.e. w_i = MAX {R_i (+ UVA), R_i (- UVA)}. The concentration grid C_i is chosen such that the same number of points falls into each of the concentration intervals defined by the concentration values used in the experiment. The calculation of MPE is restricted to the maximum concentration value at which at least one of the two curves still exhibits a response value of at least 10%. If this maximum concentration is higher than the highest concentration used in the + UVA experiment the residual part of the + UVA curve is set to the response value “0”.

Interpretation of results:

MPE < 0.1: “no phototoxicity”

MPE > 0.1 and < 0.15: “probable phototoxicity”

MPE > 0.15: “phototoxicity”

Statistics: Data was analysed using the Phototox Version 2.0 Software (*Peters B. and Holzhütter HG, 2002 and Bundesinstitut für Risikobewertung {Federal Institute for Risk Assessment, BfR}*).

RESULTS

An UV/VIS absorption spectrum was measured at a wavelength from 200 nm to 750 nm with a freshly prepared 125 µg/mL solution of SYN545974 in 1% DMSO in EBSS versus 1% DMSO in EBSS. One peak was measured with 0.431 AU at 252 nm wavelength. A molar absorption coefficient of 1471.3 L x mol⁻¹ x cm⁻¹ was calculated, based on 426.7 g/mol as the molecular weight of SYN545974.

SYN545974 showed a cytotoxic effect with and without irradiation. With irradiation the viability of the cells was reduced to 17.1% and without irradiation to 36.7%.

- UVA: 41.66 µg/mL

+ UVA: 24.56 µg/mL

PIF = 1.7

The positive control showed cytotoxic and phototoxic effects. With the highest concentration of the positive control in the non-irradiated part of the experiment (100 µg/mL), viability of the cells was reduced to 0.0% relative to the untreated negative controls. The EC₅₀ value was calculated to 12.9 µg/mL. In the irradiated part of the experiment (highest test item concentration: 10 µg/mL), the EC₅₀ value was calculated to 0.76 µg/mL.

Table 6.2.7-1: Mean OD540 and cell viability (%) with and without UVA irradiation

Compound	Without UVA			With UVA		
	Concentration (µg/mL)	Mean value OD _{540nm}	% viability	Concentration (µg/mL)	Mean value OD _{540nm}	% viability
SYN545974	125.00	0.251	36.7	125.00	0.112	17.1
	39.53	0.373	54.6	39.53	0.327	49.9
	12.50	0.482	70.7	12.50	0.430	65.5
	3.95	0.669	98.0	3.95	0.607	92.4
	1.25	0.697	102.0	1.25	0.685	104.4
	0.395	0.697	102.1	0.395	0.668	101.9
	0.125	0.681	99.7	0.125	0.667	101.7
	0.040	0.686	100.4	0.040	0.669	101.9
Chlorpromazine	100.00	0.000	0.0	10.00	0.008	1.2
	31.62	-0.002	-0.3	3.16	0.000	0.0
	10.00	0.461	68.9	1.00	0.103	15.8
	3.16	0.663	99.1	0.316	0.630	96.7
	1.00	0.678	101.5	0.100	0.639	97.9
	0.316	0.692	103.5	0.032	0.653	100.2
	0.100	0.676	101.2	0.010	0.656	100.6
	0.032	0.656	98.1	0.003	0.653	100.2

CONCLUSION:

A phototoxicity test was required according to Reg 283/2013 as pydiflumetofen absorbs electromagnetic radiation in the range 290-700 nm and the ultraviolet/visible molar extinction/absorption coefficient of the substance is $< 10 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$. The phototoxic potential of pydiflumetofen was tested in a GLP and OECD 432 (2004) compliant *in vitro* 3T3 NRU study. Although the study was performed under the old 2004 guideline, it was also found to comply with the newest OECD 432 guideline (adopted 2019). The test item was dissolved in DMSO and diluted in EBSS to create a 125 µg/mL stock solution, which was then further diluted to create the following concentrations of the test item: 125.00, 39.53, 12.50, 3.95, 1.25, 0.40, 0.13, 0.04 µg/mL. Negative controls were also formulated (1% DMSO in EBSS), as well as blanks (EBSS) and positive controls (chlorpromazine 100, 31.6, 10.0, 3.16, 1.00, 0.316, 0.100 and 0.0316 µg/mL without UVA and 10, 3.16, 1.00, 0.316, 0.100, 0.0316, 0.0100 µg/mL with UVA). Two plates of cell suspensions of BALB/c 3T3 (one to determine cytotoxicity, without UVA and one to determine phototoxicity, with UVA) were treated for 1 hour with each of the above concentrations and then for another 50 minutes in the presence and absence of UVA light depending on the group. Neutral red medium was administered to each plate which were incubated for three hours at 37°C. Cytotoxicity and phototoxicity were then measured as a reduction in uptake of neutral red (recorded as changes in the OD of the extract at 540 nm wavelength) with and without irradiation when compared to the controls. This was measured using a micro plate auto reader, using the blanks as a reference.

A cytotoxic effect was seen both with and without irradiation. With irradiation, the cell viability was reduced to 17.1% at the highest test item concentration (125.0 µg/mL), whilst without UVA, cell viability was reduced to 36.7% at the highest concentration. In comparison the positive control showed a clear phototoxic effect, reducing cell viability to 0.00% in comparison to the negative control when exposed to UVA. The IC₅₀ value of the positive control was 12.9 µg/mL, much higher than the IC₅₀ calculated for the test item when irradiated, which was 0.76 µg/mL. The Photo Irritation Factor (PIF) was 1.7, based on the non-irradiated IC₅₀ (41.66 µg/mL) divided by the irradiated IC₅₀ (24.56 µg/mL). As the PIF calculated was less than 2, the test item does not require classification for phototoxicity, in accordance with OECD 432 (2019). Consequently HSE agrees with the EU evaluation, that induced pydiflumetofen is not phototoxic.

In conclusion a cytotoxic, but not phototoxic effect was observed from pydiflumetofen in this OECD and GLP compliant *in vitro* 3T3 NRU study.

REFERENCES:

Peters B. and Holzhütter HG (2002), *In Vitro* Phototoxicity Testing: Development and Validation of a New Concentration Response Analysis Software and Biostatistical Analyses Related to the Use of Various Prediction Models, *ATLA* **30**, pp. 415-432.

Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment, BfR), Zentralstelle zur Erfassung und Bewertung von Ersatz- und Ergänzungsmethoden zum Tierversuch (Centre for the Documentation and Evaluation of Alternatives to Animal Experiments, ZEBET), Phototox Version 2.0, “How to get started”.

(██████████, 2015)

B.6.2.8. Summary of acute toxicity

The acute toxicity of pydiflumetofen was investigated in standard studies conducted via the oral, dermal and inhalation routes. Studies investigating skin and eye irritation/corrosion, as well as skin sensitisation were also conducted. An *in vitro* 3T3 NRU study was also performed to investigate the potential phototoxicity of pydiflumetofen. As no phototoxic effect was observed, photomutagenicity tests are not required, in accordance with the data requirements of Regulation 283/2013.

Based upon the results of these studies, pydiflumetofen is of low acute toxicity via the oral (LD₅₀ >5000 mg/kg bw), dermal (LD₅₀ >5000 mg/kg bw) or inhalation (4-hr-LC₅₀ aerosol >5.11 mg/mL) routes. Pydiflumetofen was also found to be non-irritating to the skin and eye of rabbits. The *in vivo* skin and eye irritation studies are regarded by HSE to be in breach of Art 62 of Reg 1107/2009 as *in vitro* alternatives should have been employed in the first instance. Pydiflumetofen was also non-sensitising to the skin in a LLNA when tested up to limit of solubility. The *in vitro* phototoxicity study produced a PIF of 1.7, meeting the threshold of non-phototoxicity (< 2), and thus pydiflumetofen is not phototoxic.

The following conclusions have been made in terms of the classification of pydiflumetofen:

- No acute toxicity classification is proposed
- The data requirements of regulation 283/2013 have been met.

The table below summarises the studies carried out as part of the acute toxicity investigations of pydiflumetofen:

Study and Acceptability	Species/ Strain	Sex	Acceptable	Result	Classification according to Reg (EC) No. 1272/2008
Acute oral	Rat (Wistar)	M & F	Y	LD ₅₀ >5000	No

toxicity study (OECD 425) [REDACTED] [REDACTED] (2012) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon</i>				mg/kg bw	Classification
Acute dermal toxicity study (OECD 402) [REDACTED] [REDACTED] (2013) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon</i>	Rat (Wistar)	M & F	Y	LD ₅₀ >5000 mg/kg bw	No Classification
Acute inhalation toxicity study (OECD 403) [REDACTED] [REDACTED] (2013) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon</i>	Rat (Wistar)	M & F	Y	4hr-LC ₅₀ > 5.11 mg/L (aerosol)	No Classification
Skin irritation study (OECD 404) [REDACTED] [REDACTED] (2012) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon but in breach of Art 62 of Reg 1107/2009</i>	Rabbit (New Zealand White, NZW)	M	Y	Not Irritating	No Classification

Eye irritation study (OECD 405) [REDACTED], (2012a) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon but in breach of Art 62 of Reg 1107/2009</i>	Rabbit (New Zealand White, NZW)	M	Y	Not Irritating	No Classification
Skin Sensitisation study (LLNA) (OECD 429) [REDACTED], (2013) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon</i>	Mice (CBA/J Rj)	F	Y	Not Sensitising (SI < 3)	No Classification
Phototoxicity study (OECD 432) [REDACTED], (2015) Batch: 1228284-64-7 Purity %: 98.5 <i>Acceptable, relied upon</i>	BALB/c 3T3 Cells	n/a	Y	Not phototoxic (PIF <2)	Not applicable

B.6.3. SHORT-TERM TOXICITY

The short-term toxicity of SYN545974 has been evaluated via the oral route in rats, dogs and mice and via the dermal route in a 28-day study in the rat. To establish the NOAELs in mice, rat and dog repeated toxicity studies, the EU RMS (FR) has considered as adverse an increase above 15% of the liver weight (absolute and relative) compared with controls and associated with histopathological findings (hepatocellular hypertrophy) and/or changes in clinical parameters. HSE has also adopted this approach in the evaluation.

B.6.3.1. Oral 28-day study

B.6.3.1.1 Rat

During the commenting period, EFSA requested the following additional information regarding the 28-day rat study:

- Applicant to provide additional information regarding relative liver weight and amend the statistical analysis excluding the outlier in the control group regarding GLDH measurements in the 28-day rat study”

The additional information was provided by the applicant and reviewed by the EU RMS (FR) within the summary of the 28-day rat study ([REDACTED] 2012) below.

Report:	K-CA 5.3.1/01 [REDACTED] (2012). SYN545974, SYN546022: 28 Day Dietary Toxicity Study in Rats. [REDACTED]. Laboratory Report No. 32168, issue date 20 December 2012. Report Amendment 1, 27 July 2017. Unpublished. Syngenta File No. SYN545974_10044.
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Guidelines: Repeat dose oral toxicity study in rodents, OECD guideline reference 407 (2008): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.3050 712-C-00-366, July 2000: EC Directive 96/54/EC, B.7 (1996).

GLP: This study has not been subjected to any study specific Quality Assurance procedures. GLP regulations are not applicable to studies of this nature and, therefore, no claim of GLP compliance is made. However, the study was conducted in a GLP compliant facility and the practices and procedures adopted during its conduct were consistent with the OECD Principles of Good Laboratory Practice.

This study was conducted on 2 Active Ingredients, only data on SYN545974 are presented.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a 28 day toxicity study Han Wistar rats ([REDACTED]:WI(Han)) rats were allocated to groups and treated as shown in the table below.

Animals were dosed continuously by the diet ad libitum. Toxicity study animals were dosed for a period of 28 days. Six Satellite study animals/group were treated for 3 or 7 days. Control animals received blank diet only i.e. diet not containing the test materials.

The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. The following were assessed at pre-determined intervals from pretrial until study completion for all Toxicity study animals: clinical observations, body weight, food consumption, haematology, coagulation, clinical chemistry, urinalysis and organ weights. Blood samples were obtained from Toxicity study animals for determination of plasma levels of SYN545974 (Groups 1 to 5) on Days 4 and 28 of the study.

The Satellite study animals were used for cell proliferation investigations. Routine observations, body weights, food consumption and organ weight data were assessed.

All animals were terminated and subjected to a detailed necropsy examination after the completion of either 3, 7 or 28 days of treatment. Tissues from Toxicity study animals were processed to slides and those from

animals in the Control and High dose groups were examined histologically. Additionally, histopathological evaluation of the liver tissue was undertaken for Toxicity study animals in the Low and Intermediate groups treated with SYN545974 (Groups 2, 3 and 4). Selected tissues from Satellite animals were processed to slides, however these were not examined histologically.

Group	Treatment			Sex	Numbers of animals		
	Test substance	Group name	Dose (ppm)		Toxicity study	Interim kill animals (satellite study)	
						28 days	3 days
1	Blank diet	Control	0	M	6	6	6
				F	6	6	6
2	SYN545974	Low	500	M	6		
				F	6		
3		Intermediate 1	4000	M	6		
				F	6		
4		Intermediate 2	8000	M	6		
				F	6		
5		High	16000	M	6	6	6
				F	6	6	6

There were no mortalities. There were no treatment-related clinical observations noted during treatment. Bioanalysis data of blood was highly variable and test compound was observed in both treated and control groups at similar levels. However, it appeared that the exposure in males were generally higher than females at each treatment level on Day 4 and females were generally higher than males at each treatment level on Day 28.

Treatment with 16000 ppm SYN545974 resulted in a lower group mean body weight gain for males when compared to control animals.

Decreased group mean glutamate dehydrogenase was observed in all female groups treated with SYN545974. Additionally, a statistically significant decrease in ALT was observed in females treated at ≥ 8000 ppm.

Increased liver weights were observed in males treated with ≥ 4000 ppm and in females treated with ≥ 500 ppm. With the exception of females treated with 500 ppm, this was accompanied by centrilobular hepatocellular hypertrophy.

Dietary administration of SYN545974 for 28 days was associated with lower body weight gain in males and increases in liver weights in males and females, accompanied by centrilobular hepatocellular hypertrophy. Under the conditions of the study a no observed adverse effect level (NOAEL) is considered to be 500 ppm SYN545974, equivalent to 43 mg/kg/day and 40 mg/kg/day for males and females, respectively.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	White powder
Lot/Batch number:	LOT 2491-DC/110
Purity:	98.6 % w/w
CAS#:	Not reported
Stability of test compound:	Stable until end October 2011 (stored at <30°C)

Vehicle and/or positive control: The test substance was administered via Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) supplied by [REDACTED].

Test Animals:	
Species	Rat
Strain	Han Wistar rats ([REDACTED]:WI(Han))
Age/weight at dosing	7 weeks / 198-230 g (males), 152-191 g (females)
Source	[REDACTED]
Housing	3 per cage by sex in suspended polycarbonate cages (overall dimensions 61 x 43.5 x 24 cm) with stainless steel grid tops and solid bottoms with a separate stainless steel food hopper.
Acclimatisation period	9 days
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) Diet ([REDACTED]) ad libitum
Water	Water from the public water supply ad libitum
Environmental conditions	Temperature: 21-22°C Humidity: 43-64% Air changes: minimum of 15 per hour Photoperiod: 12 hours light, 12 hours dark

Study Design and Methods:

Study dates: Start: 27 January 2011, End: 19 April 2011

In a 28 day toxicity study, groups of male and female Han Wistar rats ([REDACTED]:WI(Han)) rats were allocated to groups and dosed continuously by the diet ad libitum. Toxicity study animals were dosed for a period of 28 days. Six Satellite study animals/group were treated for 3 or 7 days. Control animals received blank diet only i.e. diet not containing the test materials.

Animal assignment: From 93 males and 93 females supplied, 90 males and 90 females were assigned to the study (18 males and 18 females were assigned to control groups; 36 males and 36 females were assigned to groups administered diet with SYN545974, remaining animals were assigned to dose groups with another active ingredient). On arrival from the suppliers, the animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Control animals were housed on the same rack as treated groups. During pretrial, group mean body weights were checked to ensure all groups were within $\pm 20\%$ of the mean weight/per sex.

Study design

Group	Treatment			Sex	Numbers of animals		
	Test substance	Group name	Dose (ppm)		Toxicity study	Interim kill animals (satellite study)	
					28 days	3 days	7 days

1	Blank diet	Control	0	M	6	6	6
				F	6	6	6
2	SYN545974	Low	500	M	6		
				F	6		
3		Intermediate 1	4000	M	6		
				F	6		
4		Intermediate 2	8000	M	6		
				F	6		
5		High	16000	M	6	6	6
				F	6	6	6

Diet preparation and analysis: A 200 g concentrated premix of each test material was prepared by mixing the test material with the required amount of untreated control diet in an automated mortar and pestle until visibly homogeneous.

Diet for the high dose groups at a concentration of 16000 ppm SYN545974, was prepared by adding the appropriate 200 g concentrated premix to a suitably sized diet bin before adding an appropriate amount of untreated diet and blending for approximately 20 minutes in a Winkworth change drum diet mixer. Diets at lower concentrations were prepared, as above, as a serial dilution from the respective high dose group.

No correction factor was applied to the concentrations.

Blank diet i.e. diet containing no test substance, was prepared for control animals.

SYN545974 diets were prepared three times, with sufficient diet prepared for 7 days of dosing in first two weeks and for 14 days of dosing in Week 3. Immediately after preparation, the diets were split so that 2 containers per week for each diet were available.

All containers were stored in a freezer at -20°C until required. On removal from the freezer the diets were allowed to acclimatise to room temperature before feeding to the animals. Diets were not analysed for stability, homogeneity or achieved concentration.

Observations: All Toxicity and Satellite study animals were checked early morning and as late as possible each day for viability. Once each week, starting pretrial, all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. In addition, all animals were examined for reaction to treatment at least once each day.

Body weight: Body weights for Toxicity and Satellite study animals were recorded twice during pretrial, daily during the first week of treatment and twice weekly thereafter until the completion of the designated treatments.

Food consumption and achieved dose: The quantity of food consumed by each cage of animals from the Toxicity and Satellite study was measured and recorded twice during pretrial, daily during the first week of treatment and twice weekly thereafter until the completion of the designated treatments. Achieved dose was calculated for each cage of Toxicity study animals over every period of food consumption during treatment.

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study, commencing pretrial.

Haematology and coagulation: Blood was collected from all Toxicity study animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. The rats were not starved overnight prior to termination. The following parameters were examined:

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	white blood cell count
mean cell volume	differential white blood cell count
mean cell haemoglobin	red cell distribution width
reticulocytes	activated partial thromboplastin time
prothrombin time	

Clinical chemistry: Blood was collected from all Toxicity study animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. The rats were not starved overnight prior to termination. The following parameters were evaluated:

urea	aspartate aminotransferase activity
creatinine	alanine aminotransferase activity
glucose	gamma glutamyltransferase activity
albumin	calcium
total protein	inorganic phosphate
cholesterol	sodium
triglycerides	potassium
total bilirubin	chloride
creatine phosphokinase activity	globulin
glutamate dehydrogenase activity	A/G ratio
alkaline phosphatase activity	

Urinalysis: Urine samples from all Toxicity study animals were collected over an approximate 4 h period during Week 4. The animals were housed individually in metabolism collection cages and were deprived of food and water. The following parameters were evaluated:

volume	glucose
colour	ketones
turbidity	protein
specific gravity	bilirubin
pH	blood pigments
urobilinogen	leukocytes
microscopy of spun deposit	

Toxicokinetic analysis: Toxicity study animals were used for toxicokinetic blood sampling on Days 4 and 28 of the study. Blood samples (approximately 0.1 mL) were obtained from each animal, in each sex group to determine the blood levels of SYN545974 (Groups 1 to 5) at the following times: 0700-0830h, 1100-1230h, 1400-1530h and 1700-1830h.

The blood samples were collected from the tail vein using disposable plastic syringes and vygon microflex infusion sets into BD Microtainers containing K₂EDTA. Following collection, 0.05 mL of whole blood was accurately measured into a Data Matrix tube containing exactly 0.05 mL of Milli Q water. The samples were gently mixed and placed directly onto dry ice. The samples were then stored, at -80°C until analysed.

Concentrations of SYN545974 (Groups 1-5) in each sample were determined using a research grade 3 assay (RGA 3). The assay accuracy and precision was within 100 ± 20% and ≤ 20%, respectively.

Investigations post mortem:

Termination: After completion of 3 days of treatment (Day 4) the first 6 Satellite study animals from each sex were necropsied. After the completion of 7 days of treatment (Day 8), the remaining Satellite study animals from each sex were necropsied. After completion of 28 days of treatment (Day 29), all Toxicity study animals were necropsied.

Two hours before the designated termination time, each Toxicity and Satellite study animal had 5 mL/kg of 5-bromo-2'-deoxyuridine (BrdU) at a concentration of 15 mg/mL, administered subcutaneously in the scapular region, in order to achieve a dose of 75 mg BrdU/kg body weight. The rats were killed by exposure to carbon dioxide and necropsied in a randomised order, exactly 2 hours after the injection of BrdU. Immediately after death each animal was weighed followed by severance of major blood vessels.

Macroscopic examination: All animals were examined post mortem. This involved an external observation and an internal examination of all organs and structures.

Organ weights: The organs below were weighed from Toxicity study animals at termination after at least 28 days treatment before sampling:

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	thymus
kidneys	uterus with cervix & oviducts
liver	lung
pituitary gland	prostate + seminal vesicles & coagulating glands
submaxillary and sublingual salivary glands	thyroids with parathyroids

The organs below were weighed from Satellite study animals at termination, after 3 or 7 days treatment, before sampling.

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	thymus
kidneys	uterus with cervix & oviducts
liver	thyroids with parathyroids

Tissue submission: The following tissues from Toxicity study animals were examined in situ, removed and examined and fixed in an appropriate fixative:

abnormal tissue	nerve - sciatic
adrenal gland	oesophagus
aortic arch	optic nerve
brain (forebrain, midbrain, cerebellum and pons)	ovary
cervical lymph node	pancreas
caecum	pituitary gland
colon	prostate + seminal vesicle + coagulating gland

abnormal tissue	nerve - sciatic
duodenum	skin + mammary gland
epididymis	submaxillary and sublingual salivary gland
eye	skeletal muscle
femur (bone marrow smear)	spinal cord (cervical, midthoracic, lumbar)
femur (including stifle joint)	spleen
Harderian gland	sternum + rib
heart	stomach
ileum	testis
jejunum	thymus
kidney	thyroid with parathyroid
liver	tongue
lung	trachea
lymph node - mandibular	urinary bladder
lymph node - mesenteric	uterus + cervix + oviduct
	vagina

The following tissues from all Satellite study animals were examined *in situ* removed and examined and fixed in an appropriate fixative:

abnormal tissue	liver
adrenal gland	ovary
brain (forebrain, midbrain, cerebellum and pons)	spleen
duodenum	testis
epididymis	thymus
femur (including stifle joint)	thyroid with parathyroid
heart	urinary bladder
kidney	uterus + cervix + oviduct

Microscopic examination: All processed tissues on all Control (Group 1) and High dose (Group 5) Toxicity study animals were examined by light microscopy together with the liver samples from male groups 2, 3 and 4 and female groups 3 and 4.

Tissues from the satellite study animals were not examined histopathologically.

Statistics: Body weights, cumulative body weight gain, food consumption (males only), absolute organ weights and clinical chemistry parameters were analysed for Toxicity and Satellite study animals using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test distribution. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances.

Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate.

Analyses of variance and covariance were carried out using the MIXED procedure in SAS (9.1.3). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-

squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Dunnett's t-test, based on the error mean square in the analysis. The following pairwise comparisons were performed: Control Group 1 vs Group 2, 3, 4, 5, 6, 7, 8 and 9. All statistical tests were two-sided and performed at the 5% and 1% significance level using in-house software.

Micropathology incidence data was analysed using Fisher's Exact Test. Findings with multiple severities were analysed using a Mann-Whitney U-test.

RESULTS

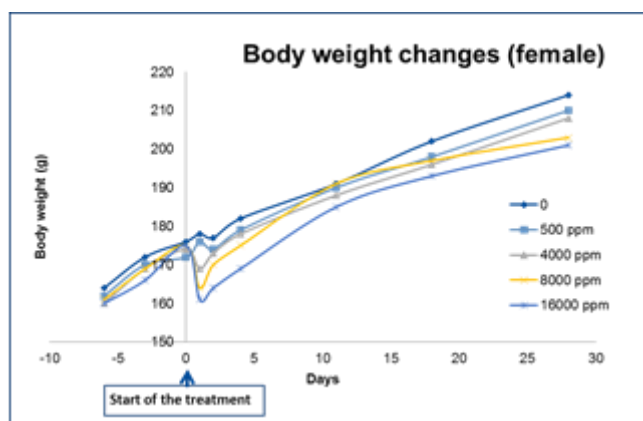
Mortality: There were no mortalities.

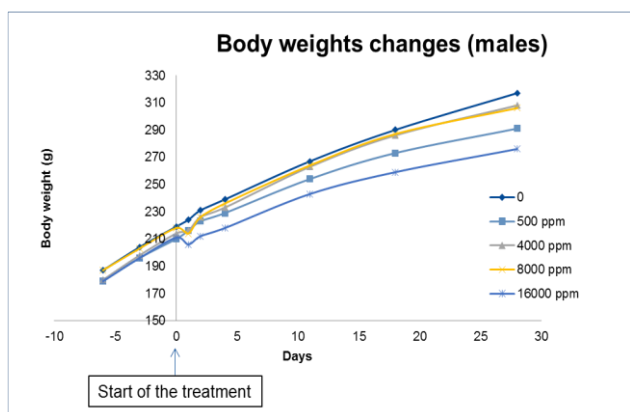
Clinical observations: There were no treatment related clinical signs noted in any of the animals treated with SYN545974.

Body weight: Reduced group mean body weight gain was observed in animals treated with 16000 ppm. In males, this reduction was statistically significant throughout the treatment period when compared with the controls and resulted in a statistically significantly lower group mean body weight at termination. Females lost weight on days 0-1 but took until day 7 to return to their starting weight and this resulted in a non-statistically significant lower group mean body weight at termination compared with controls.

Table 6.3.1-1: Intergroup comparison of body weights and body weight gain (g) toxicity study- selected timepoints

days	Dietary Concentration (ppm)									
	Males					Females				
	0	500	4000	8000	16000	0	500	4000	8000	16000
0	219	210	214	218	211	176	172	174	175	175
1	224	216	216	214	206**	178	176	169	164	161*
2	231	223	226	226	212**	177	174	173	170	164
4	239	229	233	236	218*	182	179	178	175	169
11	267	254	263	264	243*	191	190	188	191	185
18	290	273	286	287	259*	202	198	196	197	193
28	317	291	308	306	276**	214	210	208	203	201
0-28	98±19	81±8	94±22	88±18	65**±17	39±5	38±5	35±7	29±10	27±10
* Statistically significant difference from control group mean, p<0.05										
** Statistically significant difference from control group mean, p<0.01										





A statistically significant reduction in group mean body weight gain was also observed in all male satellite animals treated with 16000 ppm for 3 or 7 days.

Table 6.3.1-2: Intergroup comparison of body weights and body weight gain (g) satellite study- selected timepoints

days	Dietary Concentration (ppm)			
	Males		Females	
	0	16000	0	1600
0	223	224	170	172
1	230	218*	172	161**
2	234	226	176	166*
4	245	237	175	175
7	257	248	178	179
0-3	18	7**	7	-1**
0-7	36	22**	11	8

* Statistically significant difference from control group mean, $p < 0.05$
 ** Statistically significant difference from control group mean, $p < 0.01$

Food consumption: Food consumption was markedly lower in animals treated with 16000 ppm for the first day of treatment for males and for the first 1 to 2 days of treatment for females. Food consumption was lower in females treated with 4000 or 8000 ppm for the first 1 to 2 days of treatment. Food consumption recorded in satellite animals was comparable with control, with the exception of Day 1.

Table 6.3.1-3: Intergroup comparison of food consumption (g/animal/day) toxicity study- selected timepoints

day	Dietary Concentration (ppm)									
	Males					Females				
	0	500	4000	8000	16000	0	500	4000	8000	16000
0	21.1	20.1	21.1	21.3	20.8	14.5	14.6	14.6	14.2	13.9
1	21.7	21.4	16.2	11.6	11.5	15.4	15.7	8.9	3.8	6.6
2	22.9	21.3	22.6	21.4	18.8	15.4	14.4	13.8	12.7	6.2

Table 6.3.1-4: Intergroup comparison of food consumption (g/animal/day) satellite study- selected timepoints

day	Dietary Concentration (ppm)			
	Males		Females	
	0	16000	0	16000

0	21.8	21.3	14.4	15.0
1	20.7	6.8	14.4	1.4
2	22.4	21.0	17.2	11.9

Estimated achieved dose: The estimated dose received was calculated.

Table 6.3.1-5: Intergroup comparison of achieved dosage (mg/kg bw/day) – overall means

Group	Treatment			Sex	Estimated achieved dose (mg/kg bw/day)		
	Test substance	Group name	Dose (ppm)		Toxicity study	Interim kill animals (satellite study)	
					28 days	3 days	7 days
2	SYN545974	Low	500	M	43		
				F	40		
3		Intermediate 1	4000	M	343		
				F	322		
4		Intermediate 2	8000	M	677		
				F	619		
5		High	16000	M	1322	1142	1321
				F	1174	947	1182

Water consumption: There were no treatment related effects on water consumption.

Haematology and coagulation: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: SYN545974: Statistically significant lower group mean glutamate dehydrogenase was observed in all female groups. Following exclusion of the large value seen for control animal number 56 (measured value 28.9 iu/L) as a clear outlier, statistical significance was attained at 8000 and 16000 ppm only. However, HSE points out that the biological relevance of a decrease (in contrast to an increase) of GLDH maybe questionable. Additionally, statistically significant lower ALT was observed in females treated at ≥ 8000 ppm. All other changes are considered to represent normal biological variation and are considered not to be treatment-related.

Table 6.3.1-6: Intergroup comparison of selected clinical chemistry parameters

Parameter	Dietary Concentration (ppm)									
	Males					Females				
	0	500	4000	8000	16000	0	500	4000	8000	16000
GGT (iu/L)	3	3	3	3	4**	3	3	3	4	3
GLDH (iu/L) (including the outlier (28.9) in female control group)	5.5	6.1	9.0	5.3	6.8	9.6	4.0**	3.9**	3.0**	3.0**
GLDH (iu/L) (excluding the outlier (28.9) in female control group)	5.5	6.1	9.0	5.3	6.8	5.7	4.0	3.9	3.0**	3.0**
ALT (iu/L)	53	52	43	45	38	40	37	34	29*	28*

* Statistically significant difference from control group mean, $p < 0.05$
 ** Statistically significant difference from control group mean, $p < 0.01$

	Individual values for the female control group					
GLDH (m/L)	6.4	28.9	4.6	7.5	4.8	5.3

Urinalysis: Specific gravity was generally lower and volume was higher in all female groups treated with SYN545974.

Table 6.3.1-7: Intergroup comparison of selected urinalysis parameters

Parameter	Dietary Concentration (ppm)									
	Males					Females				
	0	500	4000	8000	16000	0	500	4000	8000	16000
Specific gravity	1.059	1.063	1.046	1.061	1.063	1.055	1.036	1.031*	1.033	1.029*
Urinary volume (mL)	1.0	0.9	1.2	0.8	0.8	0.7	1.1	1.1	1.1	1.5
* Statistically significant difference from control group mean, p<0.05										

Toxicokinetic analysis: The data were very variable, but it appeared that the exposure in females was generally higher than males at each treatment level and that there was no apparent increase in exposure at any treatment level for males or females between Days 4 and 28.

Sacrifice and pathology: Macroscopic findings: There were no macroscopic findings considered to be related to the administration of SYN545974.

Organ weights: After 3 days and 7 days of treatment, increases in group mean covariate liver weight were observed at 16000 ppm in satellite animals, when compared with controls.

After 28 days of treatment, statistically significant increased group mean absolute and covariate liver weights were observed for males and females treated with ≥ 4000 ppm (relative liver weight was increase by 21% in males and 24% in females at 4000 ppm). In addition, a statistically significant increase in covariant liver weight was also noted in females treated with 500 ppm. At this dose in female, absolute liver weight was increased by 11% (not statistically significant), covariate liver weight was increased by 13% (statistically significant) and relative liver weight was increased by 12% (no statistical analysis performed).

All other changes were considered to represent normal biological variation and were not related to treatment.

Table 6.3.1-8: Intergroup comparison of liver weights (g) toxicity study

	Dietary Concentration (ppm)									
	Males					Females				
	0	500	4000	8000	16000	0	500	4000	8000	16000
Absolute (g)	11.51	11.18	13.68**	14.51**	13.65**	7.31	8.10	8.80*	8.90**	9.22**
Covariate (g)	10.89	11.47	13.31**	14.25**	14.64**	6.50	7.36*	8.34**	8.68**	9.16**
Relative (% body weight)	3.68	3.85	4.46	4.78	5.01	3.45	3.86	4.29	4.46	4.68
* Statistically significant difference from control group mean, p<0.05										
** Statistically significant difference from control group mean, p<0.01										

Table 6.3.1-9: Intergroup comparison of liver weights (g) satellite study

		Dietary Concentration (ppm)			
		Males		Females	
		0	16000	0	16000
Day 4	Absolute	10.84	12.02	7.32	8.02
	Covariate	10.24	12.05**	6.88	8.05*

Day 8	Absolute	11.42	13.35**	6.62	8.58**
	Covariate	10.66	13.09**	6.03	8.05**
* Statistically significant difference from control group mean, p<0.05					
** Statistically significant difference from control group mean, p<0.01					

Microscopic findings: In all males receiving 16000 ppm, there was minimal to mild centrilobular hepatocellular hypertrophy in the liver. This was also present at a minimal grade in 5/6 and 4/6 males receiving 8000 and 4000 ppm, respectively. Minimal centrilobular hepatocellular hypertrophy was recorded in 5/6 and 3/6 females receiving 16000 and 8000 ppm, respectively. All other histology findings were typical of spontaneously occurring background pathology in rats of this age on this type of study at [REDACTED].

Table 6.3.1-10: Intergroup comparison of selected microscopic findings SYN545974 toxicity study

		Dietary Concentration (ppm)									
		Males					Females				
		0	500	4000	8000	16000	0	500	4000	8000	16000
LIVER (number examined)		6	6	6	6	6	6	0	6	6	6
No abnormality detected		5	5	1	0*	0*	5	-	5	2	0*
Centrilobular hypertrophy	minimal	0	0	4	5*	4	0	-	0	3	5*
	mild	0	0	0	0	2	0	-	0	0	0
	total	0	0	4	5*	6**	0	-	0	3	5*
Inflammatory cell foci		1	1	3	3	3	1	-	1	2	2
Clear cell focus		0	0	0	0	1	0	-	0	0	0
Mineralisation, periportal, focal		0	0	0	0	0	0	-	0	1	0
* Statistically significant difference from control group mean, p<0.05											
** Statistically significant difference from control group mean, p<0.01											

CONCLUSION:

In a 28-day repeated dose toxicity study, conducted in a GLP compliant facility and in accordance with OECD TG 407 (2008), Han Wistar rats (6/sex/dose) were administered dietary concentrations of 0, 500, 4000, 8000 or 16000 ppm, which equated to mean estimated doses of 0, 43, 343, 677 and 1322 mg/kg bw/d in males and 0, 40, 322, 619 and 1174 mg/kg bw/d in females. All study animals were used for toxicokinetic blood sampling on Days 4 and 28 of the study. Blood samples (approximately 0.1 mL) were obtained from each animal, in each sex group to determine the blood levels of pydiflumetofen.

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

In the kinetics investigations, the data were very variable, but it appeared that the exposure in females was generally higher than in males at each treatment level and that there was no apparent increase in exposure at any treatment level for males or females between Days 4 and 28.

In males, body weight gain was reduced at the high dose of 16000 ppm (-34% at termination compared with controls). Liver weights were increased from 4000 ppm (relative liver weights were increased in comparison with controls by 21%, 30% and 36% at 4000, 8000 and 16000 ppm respectively, whilst absolute weights were increased by 18%, 26% and 19% at the same respective doses); liver weight increases in males were associated with centrilobular hepatocellular hypertrophy from 4000 ppm.

In females there was no effect on body weight. Liver weights were increased from 4000 ppm (relative liver weights were increased in comparison with controls by 24%, 29% and 36% at 4000, 8000 and 16000 ppm respectively, whilst absolute liver weights were increased by 20%, 22% and 26% at the same respective doses). Increased liver weights in females were associated with centrilobular hepatocellular hypertrophy from 4000 ppm and statistically significant lower ALT from 8000 ppm.

Therefore, a **NOAEL of 500 ppm (equivalent to 43 and 40 mg/kg bw/d in males and females respectively)** was identified. At the LOAEL of 4000 ppm (equivalent to 343 and 322 mg/kg bw/d in males and females respectively), liver weights were increased in males and females with histopathological correlates (centrilobular hepatocellular hypertrophy).

(██████████, 2012)

B.6.3.1.2 Mouse

Report:	K-CA 5.3.1/02 ██████████ (2012a). SYN545974, SYN546022: 28 Day Dietary Toxicity Study in Mice. ██████████. Laboratory Report No. 32153, issue date 20 December 2012. Unpublished. Syngenta File No. SYN545974_10042.
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Guidelines: Repeat dose oral toxicity study in rodents, OECD guideline reference 407 (2008): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.3050 712-C-00- 366, July 2000: EC Directive 96/54/EC, B.7 (1996).

GLP: This study has not been subjected to any study specific Quality Assurance procedures. GLP regulations are not applicable to studies of this nature and, therefore, no claim of GLP compliance is made. However, the study was conducted in a GLP compliant facility and the practices and procedures adopted during its conduct were consistent with the OECD Principles of Good Laboratory Practice.

This study was conducted on 2 Active Ingredients, only data on SYN545974 are presented.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a 28 day toxicity study CD-1 mice (██████████CD-1 (ICR)) were allocated to groups and treated as shown in the table below.

Animals were dosed continuously by the diet ad libitum. Toxicity study animals were dosed for a period of 28 days. Six Satellite study animals/group were treated for 3 or 7 days. Control animals received blank diet only i.e. diet not containing the test materials.

The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. The following were assessed at pre-determined intervals from pretrial until study completion for all Toxicity study animals: clinical observations, body weight, food consumption, haematology, clinical chemistry and organ weights. Blood samples were obtained from Toxicity study animals for determination of plasma levels of SYN545974 (Groups 1 to 5) on Days 4 and 28 of the study.

The Satellite study animals were used for cell proliferation investigations. Routine observations, body weights, food consumption and organ weight data were assessed.

All animals were terminated and subjected to a detailed necropsy examination after the completion of either 3, 7 or 28 days of treatment. Tissues from Toxicity study animals in the Control and High dose group were examined histologically. Selected tissues from Satellite animals were processed to slides, however these were not examined histologically.

Group	Treatment			Sex	Numbers of animals	
		Group name			Toxicity study	Interim kill animals (satellite study)

	Test		Dose		28 days	3 days	7 days
1	Blank diet	Control	0	M	6	6	6
				F	6	6	6
2	SYN545974	Low	500	M	6		
				F	6		
3		Intermediate 1	1500	M	6		
				F	6		
4		Intermediate 2	4000	M	6		
				F	6		
5		High	7000	M	6	6	6
				F	6	6	6

There were no mortalities. There were no treatment-related clinical observations noted during treatment.

Body weight gain for all groups of males were lower than controls over the first 11 days of the study, thereafter the gain for males receiving 500, 1500 and 4000 ppm improved but overall body weight and body weight gain was still lower than control over the 28 days of treatment. Males receiving 7000 ppm continued to show lower weight gain until the end of the treatment period. There were no changes observed in the female treated groups.

There was no effect of treatment on food consumption.

There were no haematology findings that were considered to be due to SYN545974.

Triglycerides were higher and phosphate lower in males treated with 7000 ppm, but these differences are considered to be of doubtful toxicological significance in the absence of supporting findings.

The bioanalysis data were very variable, but it appeared that the exposure in females was generally higher than males at each treatment level and that there was no apparent increase in exposure at any treatment level for males or females between Days 4 and 28.

Liver weights were higher after 3 and 7 days of treatment, at 7000 ppm in males and females. After 28 days of treatment, dose-related increases were seen in absolute and covariate or relative liver weights, in males and females, at all dose levels.

There were no necropsy or histology findings considered to be related to administration of SYN545974.

Treatment with SYN545974 up to and including 7000 ppm resulted in effects on body weight in all groups of males and increased liver weights in males and females. As body weight gain in the low dose male group (500 ppm) was affected a no observed effect level (NOAEL) was not obtained in this study.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	White powder
Lot/Batch number:	LOT 2491-DC/110
Purity:	98.6 % w/w
CAS#:	Not reported

Stability of test compound:

Stable until end October 2011 (stored at <30°C)

Vehicle and/or positive control: The test substance was administered via Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) supplied by [REDACTED].

Test Animals:	
Species	Mouse
Strain	CD-1 mice ([REDACTED]CD-1 (ICR))
Age/weight at dosing	Approximately 7 weeks / 32.0-41.1 g (males), 19.3-30.6 g (females)
Source	[REDACTED]
Housing	Males were singly housed and females were housed 2 or 3 per cage in suspended polypropylene cages (overall dimensions 48 x 15 x 13 cm) with stainless steel grid tops and solid bottoms with a separate stainless steel food hopper. Sterilised white wood shavings supplied by Datesand, Manchester, UK were used as bedding.
Acclimatisation period	Up to 8 days
Diet	Rat and Mouse (modified) No. 1 Diet SQC Expanded (Ground) Diet ([REDACTED]) ad libitum
Water	Water from the public water supply ad libitum
Environmental conditions	Temperature: 7-22°C (temperature was below the target range of 19-23°C on 7 occasions, with the lowest temperature recorded as 7°C on one day in pretrial, but the average temperature over this day was recorded as 19°C). Humidity: 31-75% Air changes: Minimum of 15 per hour Photoperiod: 12 hours light, 12 hours dark

Study Design and Methods:

Study dates: Start: 21 January 2011, End: 20 March 2012

In a 28 day toxicity study, groups of male and female CD-1 mice ([REDACTED]CD-1 (ICR)) were allocated to groups and dosed continuously by the diet ad libitum. Toxicity study animals were dosed for a period of 28 days. Six Satellite study animals/group were treated for 3 or 7 days. Control animals received blank diet only i.e. diet not containing the test materials.

Animal assignment: From 93 males and 93 females supplied (18 males and 18 females were assigned to control groups; 36 males and 36 females were assigned to groups administered diet with SYN545974, remaining animals were assigned to dose groups with another active ingredient). On arrival from the suppliers, the animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Control animals were housed on the same rack as treated groups. During pretrial, group mean body weights were checked to ensure all groups were within $\pm 20\%$ of the mean weight/per sex.

Study design

Group	Treatment			Sex	Numbers of animals		
	Test substance	Group name	Dose (ppm)		Toxicity study	Interim kill animals (satellite study)	
					28 days	3 days	7 days
1	Blank diet	Control	0	M	6	6	6
				F	6	6	6
2	SYN545974	Low	500	M	6		
				F	6		
3		Intermediate 1	1500	M	6		
				F	6		

4		Intermediate 2	4000	M	6		
				F	6		
5		High	7000	M	6	6	6
				F	6	6	6

Diet preparation and analysis: A 200 g concentrated premix of each test substance was prepared by mixing the test substance with the required amount of untreated control diet in an automated mortar and pestle until visibly homogeneous.

Diet for the high dose group at a concentration of 7000 ppm SYN545974 was prepared by adding the appropriate 200 g concentrated premix to a suitably sized diet bin before adding an appropriate amount of untreated diet and blending for approximately 20 minutes in a Winkworth change drum diet mixer. Diets at lower concentrations were prepared, as above, as a serial dilution from the respective high dose group.

No correction factor was applied to the concentrations.

Blank diet i.e. diet containing no test substance, was prepared for control animals.

Diets were prepared three times, twice with diet intended for 14 days of dosing at each preparation for Week 1 and Week 3, and third time for smaller additional weights required for filling the hoppers during Week 2. Immediately after preparation, the diets were split so that 2 containers per week for each diet were available. All containers were stored in a freezer set to maintain -20°C until required. On removal from the freezer the diets were allowed to acclimatise to room temperature before feeding to the animals.

Diets were not analysed for stability, homogeneity or achieved concentration.

Observations: All Toxicity and Satellite study animals were checked early morning and as late as possible each day for viability. Once each week, starting pretrial, all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. In addition, all animals were examined for reaction to treatment at least once each day.

Body weight: Body weights for Toxicity and Satellite study animals were recorded twice during pretrial, daily during the first week of treatment and twice weekly thereafter until the completion of the designated treatments.

Food consumption and achieved dose: The quantity of food consumed by each cage of animals from the Toxicity and Satellite study was measured and recorded twice during pretrial, daily during the first week of treatment and twice weekly thereafter until the completion of the designated treatments. Achieved dose was calculated for each cage of Toxicity study animals over every period of food consumption during treatment.

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study, commencing pretrial.

Haematology: Blood was collected from 3 mice/sex/group in the Toxicity study. Samples were collected prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. Mice were not starved overnight prior to termination. The following parameters were evaluated:

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelets
red blood cell count	white blood cell count
mean cell volume	differential white blood cell count
mean cell haemoglobin	red cell distribution width
reticulocytes	

Clinical chemistry: Blood was collected from 3 mice/sex/group in the Toxicity study (different animals than those used for haematology) prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. Mice were not starved overnight prior to termination. The following parameters were evaluated:

urea	creatine phosphokinase activity
creatinine	alkaline phosphatase activity
glucose	aspartate aminotransferase activity
albumin	alanine aminotransferase activity
total protein	gamma-glutamyl transferase activity
cholesterol	calcium
triglycerides	phosphate
total bilirubin	

Toxicokinetic analysis: All Toxicity study animals were used for toxicokinetic blood sampling on Days 4 and 28 of the study. Blood samples (approximately 0.1 mL) were obtained from each animal, in each sex group to determine the blood levels of SYN545974 (Groups 1 to 5) at the following times: 0700-0830, 1100-1230, 1400-1530, 1700-1830.

The blood samples were collected from the saphenous vein without anaesthesia using glass capillary tubes into BD Microtainers containing K2EDTA. Following collection, 0.05 mL of whole blood was accurately measured into a Data Matrix tube containing exactly 0.05 mL of Milli Q water. The samples were gently mixed and placed directly onto dry ice. The samples were then stored, at -80°C until analysed.

Concentrations of SYN545974 (Groups 1-5) in each sample were determined using a research grade 3 assay (RGA 3). The assay accuracy and precision was within $100 \pm 20\%$ and $\leq 20\%$, respectively.

Investigations post mortem:

Termination: After completion of 3 days of treatment (Day 4) the first 6 Satellite study animals from each sex were necropsied. After the completion of 7 days of treatment (Day 8), the remaining Satellite study animals from each sex were necropsied. After completion of 28 days of treatment (Day 29), all Toxicity study animals were necropsied.

Two hours before the designated termination time, each Toxicity and Satellite study animal had 5 mL/kg of BrdU at a concentration of 15 mg/mL, administered subcutaneously in the scapular region, in order to achieve a dose of 75 mg BrdU/kg body weight. The mice were killed by exposure to carbon dioxide and necropsied in a randomised order, exactly 2 hours after the injection of BrdU. Immediately after death each animal was weighed followed by severance of major blood vessels.

Macroscopic examination: All animals were examined post mortem. This involved an external observation and an internal examination of all organs and structures.

Organ weights: The organs below were weighed from all Group 9 Toxicity study female premature decedents and all Toxicity study animals at termination after 28 days treatment:

adrenal glands	ovaries
brain	prostate (+ seminal vesicles & coagulating glands)
epididymides	spleen
heart	submaxillary and sublingual salivary glands

kidneys	testes
liver + gall bladder	thymus
lung	uterus (with cervix & oviducts)

The organs below were weighed from Satellite study animals at termination, after 3 or 7 days treatment, before sampling.

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	thymus
kidneys	uterus (with cervix & oviducts)
liver + gall bladder	

Tissue submission: The following tissues from Toxicity study animals were examined *in situ* removed and examined and placed in an appropriate fixative:

abnormal tissue	oesophagus
adrenal gland	ovary
abnormal tissue	oesophagus
aortic arch	optic nerve
bone marrow smear (femur)	pancreas
brain (forebrain, midbrain, cerebellum and pons)	skeletal muscle
cervical lymph node	pituitary gland
caecum	prostate + seminal vesicles + coagulating glands
colon	rectum
duodenum	submaxillary and sublingual salivary gland
epididymis	spinal cord (cervical, thoracic, lumbar)
eye	skin + mammary gland
femur (including stifle joint)	spleen
Harderian gland	sternum + rib
heart	stomach
ileum	testis
jejunum	thymus
kidney	thyroid with parathyroid
liver + gall bladder	tongue
lung	trachea
lymph node - mandibular	urinary bladder
lymph node - mesenteric	uterus + cervix + oviducts
nerve - sciatic	vagina

The following tissues from all Satellite study animals were examined *in situ* removed and examined and placed in an appropriate fixative:

abnormal tissue	kidney
adrenal gland	liver + gall bladder
aortic arch	ovary
brain (forebrain, midbrain, cerebellum and pons)	rectum
caecum	spleen
colon	stomach
duodenum	testis
epididymis	thymus
femur (including stifle joint)	thyroid with parathyroid
heart	urinary bladder
ileum	uterus + cervix + oviducts
jejunum	

Microscopic examination: All processed tissues on all Control (Group 1) and High dose (Group 5) Toxicity study animals were examined by light microscopy.

Tissues from the satellite study animals were not examined histopathologically.

Statistics:

Body weights, cumulative body weight gain, food consumption (males only), absolute organ weights and clinical chemistry parameters were analysed for Toxicity and Satellite study animals using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test distribution. If the variances were heterogeneous, log or square root transformations were used in an attempt to stabilise the variances.

Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate.

Analyses of variance and covariance were carried out using the MIXED procedure in SAS (9.1.3). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Dunnett's t-test, based on the error mean square in the analysis. The following pairwise comparisons were performed: Control Group 1 vs Groups 2, 3, 4, 5, 6, 7, 8 and 9. All statistical tests were two-sided and performed at the 5% and 1% significance level using in-house software.

Micropathology incidence data was analysed using Fisher's Exact Test. Findings with multiple severities were analysed using a Mann-Whitney U-test.

RESULTS

Mortality: There were no mortalities.

Clinical observations: There were no treatment related clinical signs noted.

Body weight: In the males, lower group mean body weight and body weight gain were noted compared with control over the first 11 days of dosing. Thereafter the males receiving 7000 ppm continued to show

statistically significant lower body weight gain through to the end of the treatment period (overall weight gain was 80% lower than control). The body weights of males receiving 500, 1500 and 4000 ppm recovered from Day 11, however, overall body weight gain by the end of treatment remained lower than the controls (by 55%, 16% and 45%, respectively). Body weight and body weight gain for all treated groups of females was considered not to be affected by treatment.

Table 6.3.1-11: Intergroup comparison of body weights and body weight gain (g) toxicity study- selected timepoints

days	Dietary Concentration (ppm)									
	Males					Females				
	0	500	1500	4000	7000	0	500	1500	4000	7000
0	37.3	36.6	35.9	37.0	36.9	26.3	25.4	25.3	25.0	26.1
1	38.4	36.6	36.3	36.5	35.0	26.9	25.6	26.8	25.1	25.9
2	39.2	36.4	36.6	36.6	36.5	27.8	26.6	27.7	26.1	26.4
4	38.8	36.3	35.7	36.5	35.3*	27.1	25.9	26.0	25.6	27.0
11	40.2	37.2	37.7	37.4	37.0	28.8	28.2	28.3	27.6	28.4
18	41.9	38.5	40.0	38.9	37.5	30.4	28.6	29.5	28.7	28.8
28	42.4	38.8	40.2	39.8	37.9	31.1	28.9	29.0	28.6	30.0
0-28	5.1	2.3	4.3	2.8	1.0*	4.8	3.5	3.7	3.6	4.0

* Statistically significant difference from control group mean, $p < 0.05$

Table 6.3.1-12: Intergroup comparison of body weights and body weight gain (g) satellite study- selected timepoints

days	Dietary Concentration (ppm)			
	Males		Females	
	0	7000	0	7000
0	36.9	37.2	23.5	26.5**
1	37.6	36.7	26.7	25.9
2	37.4	37.3	26.5	26.6
3	37.7	37.5	27.1	26.5
4	37.6	38.9	28.1	28.6
7	38.1	38.4	27.7	28.4
0-3	0.8	0.3	3.6	0.0**
0-7	1.8	1.4	6.5	0.8**

** Statistically significant difference from control group mean, $p < 0.01$

Food consumption: Food consumption was variable and was considered to be unaffected by treatment with SYN545974.

Estimated achieved dose: The estimated dose received was calculated.

Table 6.3.1-13: Intergroup comparison of achieved dosage (mg/kg bw/day) – overall means

Group	Treatment			Sex	Estimated achieved dose (mg/kg bw/day)		
	Test substance	Group name	Dose (ppm)		Toxicity study	Interim kill animals (satellite study)	
					28 days	3 days	7 days
2	SYN545974	Low	500	M	76		
				F	96		
3		Intermediate 1	1500	M	213		
				F	266		
4		Intermediate 2	4000	M	612		

				F	701		
5		High	7000	M	1115	925	1053
				F	1312	1132	1595

Water consumption: There were no treatment related effects on water consumption.

Haematology: There were no differences which were considered to be related to treatment.

Blood clinical chemistry: Triglycerides concentrations were higher and phosphate concentrations were lower in males treated with 7000 ppm, compared with controls, with statistical significance being achieved. There was no clear dose-relationship, and in the absence of associated histopathological changes these differences are considered to be of doubtful toxicological significance. Similar changes were not seen in females. All other changes are considered to represent normal biological variation and are considered not to be treatment-related.

Table 6.3.1-14: Intergroup comparison of selected clinical chemistry parameters

	Dietary Concentration (ppm)									
	Males					Females				
Parameter	0	500	1500	4000	7000	0	500	1500	4000	7000
Triglycerides (mmol/L)	1.18	1.99	2.00	1.49	2.54**	0.89	1.86	1.65	1.40	1.52
Phosphate (mmol/L)	2.37	1.92	1.89	2.40	1.56*	2.29	2.41	2.64	2.31	2.39
* Statistically significant difference from control group mean, p<0.05										
** Statistically significant difference from control group mean, p<0.01										

Toxicokinetic analysis: The data were very variable, but it appeared that the exposure in females was generally higher than males at each treatment level and that there was no apparent increase in exposure at any treatment level for males or females between Days 4 and 28.

Sacrifice and pathology:

Macroscopic findings: There were no macroscopic findings considered to be related to the administration of SYN545974.

Organ weights: After 3 and 7 days of treatment, higher absolute, covariate and relative liver weights were observed at 7000 ppm in males and females, when compared with controls. After 28 days of treatment, dose-related increases were seen in absolute and covariate or relative liver weights, in males and females at all treated doses, when compared with controls.

There were other, minor, inter-group differences in organ weights between treated groups and the controls, including some which attained statistical significance but they were considered to be related to the lower body weights seen and showed no dose relationship. Consequently, these differences were not attributed to treatment with SYN545974.

Table 6.3.1-15: Intergroup comparison of liver weights (g) toxicity study

	Dietary Concentration (ppm)									
	Males					Females				
	0	500	1500	4000	7000	0	500	1500	4000	7000
Absolute (g)	2.07	2.25	2.59**	3.02**	3.15**	1.64	1.87	2.01	2.14**	2.47**
Covariate (g)	1.92	2.24*	2.53**	2.98**	3.18**	1.45	1.85**	1.95**	2.16**	2.37**
Relative (% body weight)	5.09	5.89	6.65	7.81	8.43	5.48	6.62	7.10	7.79	8.59

* Statistically significant difference from control group mean, $p < 0.05$
 ** Statistically significant difference from control group mean, $p < 0.01$

Table 6.3.1-16: Intergroup comparison of liver weights (g) satellite study

		Dietary Concentration (ppm)			
		Males		Females	
		0	7000	0	7000
Day 4	Absolute (g)	2.38	2.96*	1.46	1.96**
	Covariate (g)	2.26	3.04**	1.47	2.19**
	Relative (% body weight)	6.36	8.09	5.75	7.79
Day 8	Absolute (g)	2.41	3.34**	1.61	2.56**
	Covariate (g)	2.27	3.15**	1.54	2.46**
	Relative (% body weight)	6.46	8.84	5.91	9.30

* Statistically significant difference from control group mean, $p < 0.05$
 ** Statistically significant difference from control group mean, $p < 0.01$

Microscopic findings: There were no histology findings considered to be related to the administration of SYN545974.

CONCLUSION:

In a 28-day repeated dose toxicity study, conducted in a GLP compliant facility and in accordance with OECD TG 407 (2008), CD-1 mice (6/sex/dose) were administered dietary concentrations of 0, 500, 1500, 4000 or 7000 ppm (0, 76, 213, 612 and 1115 mg/kg bw/d in males and 0, 96, 266, 701 and 1312 mg/kg bw/d in females). All study animals were used for toxicokinetic blood sampling on Days 4 and 28 of the study. Blood samples (approximately 0.1 mL) were obtained from each animal, in each sex group to determine the blood levels of pydiflumetofen.

There were no mortalities or treatment-related clinical signs of toxicity and food consumption was not affected.

In the toxicokinetic analyses, the data obtained were very variable, but it appeared that the exposure in females was generally higher than in males at each treatment level and that there was no apparent increase in exposure at any treatment level for males or females between Days 4 and 28.

In males, body weight and body weight gain were reduced for the first 11 days of treatment at all doses and continued to be lower throughout treatment at the high dose of 7000 ppm. As a result, overall final body weight gains were 55%, 16%, 45% and 80% lower than controls at 500, 1500, 4000 and 7000 ppm respectively. Liver weights were increased in comparison with controls in all treated groups (relative liver weights were increased by 16%, 31%, 53% and 66% at 500, 1500, 4000 and 7000 ppm respectively, whilst absolute liver weights were increased by 9%, 25%, 46% and 52% at the same respective doses). There were no histopathological correlates or treatment related clinical chemistry findings in males.

In females there was no effect on body weight. Liver weights were increased in all treated groups (relative liver weights were increased by 21%, 30%, 42% and 57% at 500, 1500, 4000 and 7000 ppm respectively, whilst absolute liver weights were increased by 14%, 23%, 30% and 51% at the same respective doses). There were no unusual microscopic findings or treatment related clinical chemistry findings.

As body weight development was affected in all treated males, and liver weights were increased in all treated males and females, including at the lowest tested dose of 500 ppm (equivalent to 76/96 mg/kg bw/d in males and females), **a NOAEL could not be determined from this study.**

(██████████, 2012a)

B.6.3.2. Oral 90- day study**B.6.3.2.1 Rat**

Report:	K-CA 5.3.2/01 [REDACTED] and [REDACTED] (2015). SYN545974: A 13 Week Toxicity Study of SYN545974 by Oral (Dietary) Administration in Rats (final report amendment 2). [REDACTED] [REDACTED]. Laboratory Report No. 33012, issue date 28 July 2015. Unpublished. Syngenta File No. SYN545974_10210.
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Report:	K-CA 5.3.2/02 [REDACTED], [REDACTED], 2016 SYN545974 - Historical Control Data for SYN545974_10210 and SYN545974_10211, [REDACTED] [REDACTED]. Syngenta File No. SYN545974_10448.
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Guidelines: Repeat dose oral toxicity study in rodents. OECD guideline reference 408 (1998): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.3100 (1998): EU Directive 96/52/EC, B.26 (2001).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

Additional Historical Control Data for some clinical chemistry parameters and urine pH and volume are provided and the information incorporated into the study summary below.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rodent diet has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

The objective of this study was to determine the potential toxicity of SYN545974 in the rat after oral administration by diet for a minimum of 91 days and to determine dose levels for a subsequent carcinogenicity study in rats. The study is part of animal toxicity experiments designed to provide information which will allow the test substance to be used safely.

Groups of 10 male and 10 female, 7-8 week old, Han Wistar ([REDACTED]WI(Han) rats were fed diets containing 0, 250, 1500, 8000 or 16000 ppm of SYN545974 (purity 99.5%) for a period of 91/92 consecutive days.

The following were assessed at pre-determined intervals from pre-trial until study completion from all animals: viability, clinical observations, body weight and food consumption. Ophthalmoscopy examinations were performed on all animal once during pre-trial and control and high dose animals during Week 13 of treatment. All animals received a detailed functional observation battery (including motor activity) during pre-trial and once during treatment (Week 12) over a 5/6 day period, respectively. Blood samples were collected from all animals at termination for haematology, coagulation and clinical chemistry investigations; urine samples were collected during Week 13 of treatment. Additional blood samples were collected on Days 2, 9, 28 and 91 (i.e. after 1, 8, 27 and 90 full days dietary exposure) from designated animals for whole blood bioanalysis.

All surviving animals were terminated after completion of 91/92 days of treatment and underwent a detailed necropsy examination with selected organs weighed. Tissues from all animals in the control and high dose groups were subjected to a comprehensive histopathological evaluation. Additionally, sections of liver and thyroid glands were also evaluated from low and intermediate I and II dose animals.

The overall mean achieved dose levels were 18.6, 111, 587 and 1187 mg SYN545974/kg/day for males and 21.6, 127, 727 and 1324 mg/kg/day for females, corresponding to dietary inclusion levels of 250, 1500, 8000 and 16000 ppm, respectively.

SYN545974 treated rats were generally continuously exposed to measurable concentrations of SYN545974 at all dietary inclusion levels.

There were no treatment-related mortalities or clinical observations noted during treatment. Body weight gain was reduced in males and females that received 8000 or 16000 ppm. Food consumption profiles at these dosages also indicated an initial reduction in food consumption over the first 2-3 days of treatment. Food utilisation was lower in animals receiving 8000 ppm and above. There were no ophthalmoscopy findings noted during examinations following 90 days treatment.

There were no differences in qualitative or quantitative functional observations or motor activity assessments noted during the neurotoxicity assessment.

There were no treatment-related differences in haematological, coagulation or urinalysis parameters. A reduction in alkaline phosphatase activity was observed in males and females that received 1500, 8000 or 16000 ppm when compared with controls. Higher cholesterol levels were also observed in females that received 8000 or 16000 ppm.

There were no macroscopic differences in treated animals noted at termination when compared with controls. In the livers of animals treated with 8000 or 16000 ppm or males treated with 1500 ppm there were increased incidences of hepatocyte hypertrophy, with a corresponding increase in liver weight. An increase in liver weight, with no correlating histopathological findings was observed in females that received 1500 ppm. Follicular cell hypertrophy was observed in the thyroid glands of males that received ≥ 1500 ppm and females at ≥ 8000 ppm; an increase in incidence and severity was observed with increasing dietary concentration.

Under the conditions of this study the No Observed Adverse Effect Level (NOAEL) for males and females following at least 91 consecutive days of dietary exposure to SYN545974 is 250 ppm, equating to 18.6 and 21.6 mg/kg/day, respectively, based on the reduced body weight, food consumption and food utilisation performance at ≥ 8000 ppm (587 and 727 mg/kg/day, respectively), follicular cell hypertrophy in the thyroid gland at ≥ 1500 ppm (111 and 127 mg/kg/day, respectively) and above, and hypertrophy observed in the livers of males treated with ≥ 1500 ppm (111 mg/kg/day) and in females that received ≥ 8000 ppm (727 mg/kg/day).

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Technical, white powder
Lot/Batch number:	2637-BA/110
Purity:	99.5 %
CAS#:	Not reported
Stability of test compound:	Stable until end July 2013 (stored at $<30^{\circ}\text{C}$)

Vehicle and/or positive control: The test substance was administered via Certified Rodent Diet No. 5CR4 (14% protein) Ground, supplied by [REDACTED] ([REDACTED]).

Test Animals:	
Species	Rat
Strain	Han Wistar rats ([REDACTED]:WI(Han))

Age/weight at dosing	Approximately 7-8 weeks / 218-278 g (males), 129-183 g (females)
Source	████████████████████
Housing	2 per cage by sex in polycarbonate cages (61 x 43.5 x 24 cm) with stainless steel grid tops, solid bottoms and a separate food hopper. Sterilised wood shavings (Datesand, Manchester, UK) were provided as bedding.
Acclimatisation period	15 days
Diet	Certified Rodent Diet No. 5CR4 (14% protein) Ground, supplied by ██████████ (██████). <i>ad libitum</i>
Water	Water from the public water supply <i>ad libitum</i> , provided in water bottles
Environmental conditions	Temperature: 21-23°C Humidity: 40-70% Air changes: 15 per hour Photoperiod: 12 hours light, 12 hours dark

Study Design and Methods:

In-life dates: Start: 12 October 2011, End: 06 May 2015

In a 13 week dietary toxicity study, Groups of 10 male and 10 female, 7-8 week old, Han Wistar (██████:WI(Han) rats were fed diets containing 0, 250, 1500, 8000 or 16000 ppm of SYN545974 (purity 99.5%) for a period of 91/92 consecutive days. Control animals received blank diet only *i.e.* diet not containing the test substances.

Dose level selection: Dose levels were set after evaluation of data from a 28 day study (████████, 2012; MCA Section 5.3.1/01) and took into account the maximum tolerated dose in the test model and other factors, such as anticipated human exposure.

Animal assignment: On arrival from the suppliers, animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Control animals were housed on a separate rack. Treated animals were housed on two racks, containing 2 groups per rack. During pre- trial, group mean body weights were checked to ensure all animals were within 20% of the mean weight of each sex. All groups were within $\pm 5\%$ indicating acceptable homogeneity of body weight.

Animals were allocated to 5 treatment groups and treated as follows:

Study design

Test group	Treatment	Dietary concentration (ppm)	# male	# female
1	control	0	10	10
2	low dose	250	10	10
3	intermediate dose I	1500	10	10
4	intermediate dose II	8000	10	10
5	high dose	16000	10	10

Diet preparation and analysis: Diet formulations were prepared from the high dose level of 16000 ppm. This was prepared from a 200 g premix containing the total weight of test substance required and untreated ████████ control diet, and was ground in an automated mortar and pestle for 5-6 min. The premix was then blended with the required amount of untreated ████████ control diet and mixed for 20 minutes in a diet mixer (Winkworth change drum mixer). The diets at the lower concentrations (250, 1500, and 8000 ppm) were prepared as a serial dilution from the higher concentration group (*i.e.* 8000 ppm prepared from 16000 ppm, 1500 ppm prepared from 8000 ppm diet and so on) by adding an appropriate amount of higher concentration diet to the appropriate weight of untreated ████████ control diet. Diets were mixed for 20 minutes in a diet mixer (Winkworth change drum mixer).

Diet formulations were prepared once every two weeks, stored and used within the conditions established during [REDACTED] Study Number 427957. Diet formulations (without the test substance SYN545974) were dispensed for the control animals.

Analysis of diets was carried out with regard to achieved concentration and homogeneity. Duplicate 20 g samples were taken from the top, middle and bottom of each formulated diet (including control) immediately after preparation for feeding on Day 1 and during Weeks 4, 7 and 13. All samples were stored in the dark at ambient temperature until analysed.

Results: Analysed concentrations of the test substance in diets formulated for use on Day 1 and in Weeks 4, 7 and 13 for concentrations of 250, 1500, 8000 and 16000 ppm were found to be -1.4 to - 8.9% of the theoretical concentrations, indicating acceptable accuracy of formulations. The coefficient of variation for triplicate samples was 3.9% or lower, indicating acceptable homogeneity. SYN545974 was not detected in the control diet samples.

Observations: All animals were generally checked early morning and as late as possible each day for viability. Once each week starting pre-trial, all animals received a detailed clinical examination including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta. At least twice daily, all animals were examined in their cages for reaction to treatment. The onset, intensity and duration of any signs were recorded.

Bodyweight: Body weights were recorded twice during Pre-trial, daily during Week 1 of treatment and once each week thereafter until the completion of treatment.

Food consumption, utilisation and achieved dose: The quantity of food consumed by each cage of animals was measured and recorded once during pre-trial, daily during Week 1 of treatment and once each week thereafter until the completion of treatment.

Food utilisation was calculated for each cage as follows: $(\text{cage mean weight gain} \times 100) / \text{cage total food consumption}$. Values were calculated for intervals of Weeks 1-4, 5-8, 9-13 and also an overall value for Weeks 1-13.

The amount of experimental diet ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{dose (ppm)} \times \text{cage mean food consumption (g/animal/day)}}{\text{cage mean body weight at the middle of the period}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Ophthalmoscopy: The eyes of all animals (including extras) were examined once during the pre-trial period using an indirect ophthalmoscope after the application of mydriatic agent (1% Tropicamide, Mydracyl®). Anterior, lenticular and fundic areas were evaluated. All control and high dose animals were also examined during Week 13 of treatment.

Detailed functional observations: Once during the pre-trial period and during the treatment period (Week 12/13), a more detailed examination was made of all animals. The observations were made by one observer who was 'blind' with respect to the animals' treatment and comprised the following:

Cageside observations

- Posture/condition on first approach (animal undisturbed), checked for: Prostration; Lethargy; Writhing; Circling; Breathing abnormalities; Gait abnormalities; Tremor; Fasciculation; Convulsions; Biting (of cage components or self mutilating); Vocalisations; Piloerection.
- Ease of removal from the cage.
- Body temperature was taken from the implanted electronic identification chip. On some occasions a rectal temperature was taken, either due to chip failure or the temperature being considered spurious (<35°C or > 39°C).

- Condition of the eyes, checked for: Pupillary function; Miosis; Mydriasis; Exophthalmos; Encrustation; Lachrymation.
- Condition of the coat.
- Presence of salivation.
- Overall ease of handling.

Observations in a standardised area (2 min observation period)

- Latency (time to first locomotory movement)
- Level of mobility.
- Rearing.
- Grooming.
- Urination/defecation.
- Arousal (level of alertness).
- Posture, tremor/convulsions, vocalisation, piloerection – recorded as for cageside observations.
- Palpebral closure.
- Gait abnormalities.
- Stereotypy (excessive repetition of behaviours) and/or unusual behaviours.

Functional tests

Once during the pre-trial period and during the treatment period (Week 12/13), the following additional functional tests were performed for all animals. Again, these assessments were performed at an approximately standardised time of day.

- Reaction to sudden sound (click above the head).
- Reaction to touch on the rump with a blunt probe.
- Grip strength. The procedure was repeated 3 times for the forelimbs and 3 times for the hindlimbs, and the mean fore and hind grip strengths calculated.
- Landing foot splay. The procedure was repeated 3 times and the average measurement recorded.
- Pain perception. This was assessed by measurement of the tail flick response
- Motor activity. Each animal was placed in an individual monitoring cage, scanned by a motion sensor utilising infra-red pyroelectric detectors. Movement was detected in 3 dimensions anywhere in the cage, and was differentiated into large and small movements. Each animal was monitored for one session of 1 h, activity counts were recorded over successive periods of 5 min.

Other physical/functional abnormalities

Any other abnormality not already recorded in the above screening battery.

Haematology and coagulation: Blood was collected from all animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. They were not starved overnight. The following parameters were examined:

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count
mean cell haemoglobin	red cell distribution width
reticulocytes	reticulocyte count
prothrombin time	blood smear (not examined)
fibrinogen	activated partial thromboplastin time

Clinical chemistry: Blood was collected from all animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. They were not starved overnight. The following parameters were examined:

urea	albumin
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glucose	globulin
aspartate aminotransferase	albumin/globulin ratio
alanine aminotransferase	cholesterol
alkaline phosphatase	creatinine
lactate dehydrogenase	creatine phosphokinase
sodium	total bilirubin
potassium	calcium
chloride	phosphate
total protein	

Urinalysis: Urine samples were collected from all animals over an approximate 4 h period during Week 13. The animals were housed individually in metabolism collection cages and were deprived of food and water. The following parameters were evaluated:

colour	glucose
turbidity	bilirubin
specific gravity	ketones
volume	leucocytes
pH	blood pigments
protein	microscopy of spun deposit

Bioanalysis evaluation: Blood samples (approximately 0.1 mL) were obtained from all animals in each group for whole blood analysis of SYN545974 on Days 2, 9, 28 and 91 at the following timepoints: 0700 h, 1100 h, 1500 h and 1800 h.

Samples were collected from the tail vein following warming of the animal in a heating cabinet (37- 40°C) for an appropriate time. The samples were then stored at -80°C until analysed.

Concentrations of SYN545974 in each sample were determined using a suitable LC-MS/MS analytical method. The assay accuracy and precision was within 100±20% and ≤20% respectively.

Investigations *post mortem*:

Termination: Following a minimum of 91 days of treatment, all surviving animals were killed in a random order by exposure to a rising concentration of carbon dioxide and had their terminal body weight recorded, followed by severance of major blood vessels.

Macroscopic examination: Each animal was subject to a detailed necropsy which consisted of a complete external and internal examination including body orifices (ears, nostrils, mouth, anus and vulva) and cranial, thoracic and abdominal organs and tissues.

Organ weights: The organs below were removed and weighed from all animals (with the exception of the premature decedent) before sampling and preservation. Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

brain	liver
epididymides	ovaries
adrenal glands	spleen
thyroid glands	testes
heart	thymus
kidneys	uterus

Tissue submission: The following tissues from all animals were examined *in situ*, removed and examined and fixed in an appropriate fixative:

aorta (from thoracic segment)	lymph nodes cervical
bone marrow smear	lymph node mandibular
bone marrow femur	lymph nodes mesenteric
bone marrow sternum	skeletal muscle (thigh)
brain (forebrain, midbrain, cerebellum and medulla oblongata)	nasal cavity
cervix	optic nerves
epididymis	sciatic nerve
eyes	oesophagus
adrenal glands	ovaries
Harderian glands	oviducts
lachrymal glands	pancreas
mammary glands (with inguinal skin (females only))	pharynx
parathyroid glands	skin
pituitary gland	duodenum
prostate gland	ileum
salivary glands (submandibular and submaxillary)	jejunum
seminal vesicles	spinal cord (cervical, thoracic and lumbar)
thyroid	spleen
gut-associated lymphoid tissue	stomach (glandular and non-glandular region)
heart	testes
kidneys	thymus
caecum	tongue
colon	trachea
rectum	ureter
larynx	urinary bladder
liver	uterus
lung	vagina

Microscopic examination: All processed tissues from all control and high dose (16000 ppm) animals (with the exception of bone marrow smears and nasal cavity) were examined by light microscopy. In addition, sections of liver and thyroid glands from all low and intermediate dose animals (250, 1500 and 8000 ppm) were processed and examined by light microscopy.

Additional Samples: Following sampling for histopathology, two additional 5mm liver sections from the remaining tissue of the left lateral lobe were evenly divided into a total of 12 samples (6 samples per 5 mm liver section), and placed in separate RNA-ase free tubes. Samples were snap frozen in liquid nitrogen as quickly as possible after collection and stored -80°C for possible future investigations. A representative section of the left lateral lobe, the right median lobe and the caudate lobe were taken and fixed in formalin for 48 hours and processed to paraffin wax block; a small piece of duodenum was incorporated into each block. The remainder of the liver was cut into approximately 2g pieces and snap frozen in liquid nitrogen for possible future analysis.

Statistics: The following statistical approaches were used in this study:

- All analyses were two-tailed for significance levels of 5% and 1%. Males and females were analysed separately.
- All means are presented with standard deviations.
- Body weights, cumulative body weight gain, food consumption, food utilisation, selected functional observation battery and motor activity data, absolute organ weights, haematology, coagulation, clinical chemistry and selected urinalysis parameters were analysed initially by a one-way analysis of variance (ANOVA).
- Organ weights were also analysed by analysis of covariance (ANCOVA) on final body weight. This statistical analysis provided Adjusted Organ Weight values.

- Summary values of organ to body weight ratios were not analysed statistically.
- For all parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant.
- Micropathology incidence data were analysed using Fisher's Exact Test. Functional observational battery parameters that yield discontinuous or descriptive data were analysed using Fisher's Exact Test.

RESULTS

Mortality: One control male was terminated prematurely on Day 9 due to convulsions prior to blood sampling. There were no treatment-related mortalities.

Clinical observations: There were no notable clinical observations, throughout the treatment period that were thought to be related to treatment with SYN545974,

Bodyweight: Group mean body weights of males that received 8000 or 16000 ppm were lower throughout the treatment period, when compared with controls, achieving statistical significance at several or all time points, respectively. Group mean body weights of females that received 8000 or 16000 ppm were lower throughout the treatment period, when compared with controls, but were not statistically significant.

Group mean body weight gain was statistically significantly lower at all time periods for animals (males and females) that received 8000 or 16000 ppm.

There were no differences of note in males or females that received 250 or 1500 ppm.

Table 6.3.2-1: Intergroup comparison of bodyweights - selected timepoints (g)

day	Dietary Concentration (ppm)									
	Males					Females				
	0	250	1500	8000	16000	0	250	1500	8000	16000
0	246	242	248	244	242	161	160	160	163	172
1	249	247	249	234	232*	165	163	161	156	161
4	263	261	265	250	246*	170	171	169	166	172
7	279	278	279	264	259*	181	182	177	176	181
14	304	300	301	286	280*	194	193	189	187	192
28	344	337	336	313	302**	207	206	203	199	207
56	393	380	386	359	347*	229	227	223	216	228
91	434	418	419	382*	368**	237	238	230	223	229
0-91	190±30	177±19	172±39	138±26**	126±30**	76±16	78±11	70±8	60±11*	57±12**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Food consumption, utilisation and achieved dose: The quantity of food consumed by animals dosed at 250 or 1500 ppm was similar to controls throughout the treatment period. Food consumption was reduced over the first 2 days of treatment for males and 3 days for females that received 8000 or 16000 ppm when compared with controls, achieving statistical significance. For the remainder of the treatment period, there were isolated incidences of statistical significance but generally food consumption was similar to controls.

Table 6.3.2-2: Intergroup comparison of food consumption - selected timepoints (g/animal/day)

day	Dietary Concentration (ppm)	
	Males	Females

	0	250	1500	8000	16000	0	250	1500	8000	16000
0	22.4	22.4	23.4	20.4	22.6	15.7	13.7	15.6	19.1	16.7
1	23.3	22.9	18.5*	11.0**	11.3**	16.2	14.2	15.8	9.9*	7.2**
2	20.8	19.9	20.5	13.1**	12.6**	14.6	14.8	11.9	15.3	9.0
3	22.9	23.0	23.4	22.7	22.1	17.7	15.5	14.5	10.5*	11.1*
91	23.6	23.9	23.0	21.3	22.0	17.7	16.9	16.9	18.1	17.3

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Food utilisation was statistically significantly lower over the duration of the study (Week 1-13) in animals receiving 8000 or 16000 ppm when compared with controls. This was consistent with the changes noted during the treatment period where food utilisation was statistically significantly lower in males during the Week 1-4 and 9-13 time periods at these dosages and in females during the Week 1-4 and 5-8 (8000 ppm only) time periods when compared with controls.

Table 6.3.2-3: Intergroup comparison of food utilisation - selected timepoints (g/animal/day)

weeks	Dietary Concentration (ppm)									
	Males					Females				
	0	250	1500	8000	16000	0	250	1500	8000	16000
1-4	14.2	14.1	12.8	10.4*	8.8**	9.1	9.2	9.0	6.8**	6.2**
5-8	7.4	6.9	7.7	7.2	7.3	4.4	4.3	4.1	3.1*	4.4
9-13	4.9	4.9	4.1	3.0*	2.7**	1.4	1.9	1.2	1.1	0.1
1-13	8.7	8.5	8.0	6.7**	6.0**	4.7	5.0	4.6	3.6**	3.5**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Estimated achieved dose: Dose rates (based on nominal dietary levels) were calculated in terms of mg SYN545974/kg body weight. Mean values are shown below:

Table 6.3.2-4: Mean dose received (mg/kg/day)

Dietary concentration (ppm)	250	1500	8000	16000
Males	18.6	111	587	1187
Females	21.6	127	727	1325

Water consumption: There were no treatment related effects on water consumption.

Ophthalmoscopic examinations: There were no treatment-related findings.

Functional observation battery parameters: There were no treatment-related effects on detailed clinical observations, quantitative functional observations or on locomotor activity.

Haematology and coagulation: There were no treatment related differences in haematology or coagulation parameters at dietary inclusion levels up to 16000 ppm in either sex.

For parameters where a statistically significant change from control was observed, the values and appropriate historical control data (HCD) from the conducting laboratory are presented in Table 6.3.2- 6.

Table 6.3.2-5: Intergroup comparison of selected haematology parameters

	Dietary Concentration (ppm)										Historical control data	
	Males					Females					Group Mean (min-max)	
	0	250	1500	8000	16000	0	250	1500	8000	16000	Male	Female
Red blood cell count (RBC)	8.65	8.67	8.70	8.92	9.48*	7.64	7.43	7.41	7.33	7.78	9.05 (7.94-9.91)	
Haemoglobin	14.6	14.5	14.4	14.9	15.8	13.6	13.3	13.3	13.4	13.7		
Mean corpuscular haemoglobin concentration (MCHC)	32.9	32.9	32.4	32.3	31.5*	32.9	32.9	32.7	32.7	32.1	33.1 (30.9-35.6)	
Mean corpuscular volume (MCV)	51.5	50.9	50.9	51.6	52.6	54.3	54.7	54.9	55.8*	54.8		54.0 (49.7-58.1)
Prothrombin time	24.1	22.9	22.4	23.4	22.1*	24.0	23.9	22.6	23.5	22.9	23.3 (19.4-28.0)	
White blood cell count	5.00	5.38	6.30*	7.14**	6.18	3.28	4.01	3.00	3.25	4.54	6.36 (3.11-12.23)	
Lymphocytes	3.86	4.36	4.88	5.77**	4.82	2.47	3.18	2.17	2.51	3.73*	5.04 (2.53-8.71)	3.18 (0.59-6.17)
Fibrinogen	301	286	293	282	301	178	194	193	232*	190		180 (114-306)

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Table 6.3.2-6: Historical control data (2010-2012) for selected haematology parameters

	Study Start	Jul-10	Oct-10	Dec-11	Apr-12	Jun-12	Overall ^a
	Route of Exposure	Rectal	Dietary	Dietary	Gavage	Dietary	
	Age at Termination (wks)	21/22	20/21	20/21	19-21	20/21	
Red blood cell count (RBC) ($\times 10^{12}/L$): Male	Number of animals	15	10	10	7	10	
	Mean	9.23	9	9.1	9.13	8.79	9.05
	SD	0.4	0.31	0.39	0.24	0.56	0.17
	Min	8.57	8.36	8.38	8.8	7.94	7.94
	Max	9.91	9.35	9.55	9.42	9.52	9.91
Mean corpuscular haemoglobin concentration (MCHC) (g/dL): Male	Number of animals	15	10	10	7	10	
	Mean	33.7	34.8	32.1	32.5	32.6	33.1
	SD	0.9	0.6	0.8	0.4	0.8	1.1
	Min	32.2	34.2	30.9	32.1	31.5	30.9
	Max	35.5	35.6	33.6	33.4	34	35.6
Mean corpuscular volume (MCV) (fL): Female	Number of animals	15	9	10	10	10	
	Mean	53.4	51.5	55.4	54.4	55.3	54
	SD	1.1	1	1.5	1.5	1.3	1.6
	Min	52.1	49.7	52.5	52.3	52.3	49.7
	Max	55.8	52.8	58.1	57.5	56.8	58.1
Prothrombin time (s): Male	Number of animals	15	9	10	n/a	9	
	Mean	22.4	23.4	22.5	n/a	24.9	23.3
	SD	1.2	2.1	2.2	n/a	2.7	1.2
	Min	20.2	20.1	19.4	-	19.9	19.4
	Max	24.5	27.5	25.3	-	28	28
White blood cell count ($\times 10^9/L$): Male	Number of animals	15	10	10	7	10	
	Mean	6.21	6.13	6.1	6.1	7.27	6.36
	SD	1.43	2.65	1	1.48	1.94	0.51
	Min	3.78	3.11	4.47	4.42	4.76	3.11
	Max	9.8	12.23	7.9	8.31	10.45	12.23
Lymphocytes ($\times 10^9/L$): Male	Number of animals	15	10	10	7	10	
	Mean	4.9	4.75	4.73	5.06	5.74	5.04
	SD	1.18	1.76	0.77	1.33	1.82	0.42
	Min	2.59	2.53	3.5	3.56	3.41	2.53
	Max	6.43	8.36	6.1	7.07	8.71	8.71
	Number of animals	15	9	10	10	10	

	Study Start	Jul-10	Oct-10	Dec-11	Apr-12	Jun-12	Overall ^a
	Route of Exposure	Rectal	Dietary	Dietary	Gavage	Dietary	
Lymphocytes (x10 ⁹ /L): Females	Age at Termination (wks)	21/22	20/21	20/21	19-21	20/21	
	Mean	3.37	3.54	2.86	2.69	3.42	
	SD	1.27	1.02	1.03	0.58	1.05	
	Min	0.59	2.11	1.29	1.94	2	
	Max	6.17	4.95	4.81	3.83	4.69	
Fibrinogen (mg/dL): Females	Number of animals	n/a	n/a	10	n/a	10	
	Mean	n/a	n/a	168	n/a	192	
	SD	n/a	n/a	31	n/a	43	
	Min			114		151	
	Max			221		306	

a) Overall means calculated from the mean of the study means presented; standard deviation displayed are for those calculations

Minor differences were observed in both sexes. In males that received 16000 ppm, statistically significant differences were observed in the following parameters, when compared with control: higher red blood cell count, lower mean cell haemoglobin concentration and a shorter prothrombin time. At 1500 or 8000 ppm, higher white blood cell counts, mainly as a consequence of higher lymphocytes, achieved statistical significance when compared with controls. In females, statistically significant differences were observed in lymphocyte counts (higher at 16000 ppm when compared with controls) and mean cell volume and fibrinogen levels (both higher at 8000 ppm when compared with controls). All values were within the historical control range for this performing laboratory. Due to the magnitude of these changes, a lack of dose-response, and that values were within the historical control range for this performing laboratory, the differences were considered to be of no toxicological significance.

Blood clinical chemistry: There were no treatment related differences in clinical chemistry parameters at dietary inclusion levels up to 16000 ppm in either sex. Alkaline phosphatase (ALP) was statistically lower both in males and females that received 1500, 8000 or 16000 ppm when compared with controls. These ALP values measured in males and females that received 1500, 8000 or 16000 ppm are within the historical control data from the conducting laboratories provided by the applicant (5 studies performed within a 5-year time frame (see Table 6.3.2-7)). However, as the decrease in this parameter observed in treated animals is relatively important compared to their corresponding controls (approximately from 30% to 40% decrease for male and female, respectively), HSE is of opinion that the toxicological relevance of this effect observed both in male and females at the 3 highest doses cannot be ruled out. Calcium levels were statistically higher in all treated groups in males, however, due to at least half of the individual values being within the control range and all values being within 10% biological variation this was considered not to be toxicologically significant. Higher cholesterol levels in females, which achieved statistical significance, were observed in animals that received 8000 or 16000 ppm. Other minor differences were observed in both sexes, however, due to the small magnitude of the changes and lack of a dose-response, all inter-group differences were attributed to normal biological variation. For parameters where a statistically significant change from control was observed, the values and appropriate historical control data (HCD) from the conducting laboratory are presented in Table 6.3.2-8.

Table 6.3.2-7: Intergroup comparison of selected clinical chemistry parameters

	Dietary Concentration (ppm)										Historical control data	
	Males					Group Mean (min-max)					Group Mean (min-max)	
	0	250	1500	8000	16000	0	250	1500	8000	16000	Male	Female
Alkaline phosphatase (ALP)	93	89	65**	64**	57**	59	45	35**	34**	36**	79.4 (47-138)	43.6 (21-81)
Total protein (TP)	60	60	61	63	63*	62	64	62	63	62	63 (55-105)	
Urea	5.7	5.7	6.2	5.5	6.2	6.0	5.7	5.6	5.7	5.1**		5.9 (4.0-9.5)
Albumin	40	39	41	44**	44**	46	47	45	45	45	42 (38-47)	
Calcium	2.57	2.61	2.65*	2.65*	2.69**	2.55	2.62	2.60	2.64*	2.58	2.46 (2.41-2.81)	2.65 (2.42-2.85)
Sodium	143	143	144	143	144*	143	144	144	144	144	143 (140-146)	
Potassium	4.2	4.3	4.3	4.6*	4.5	3.7	3.5	3.6	3.7	3.9	4.3 (3.5-5.9)	
Cholesterol	2.0	2.1	2.3	1.9	1.9	1.7	1.7	2.0	2.3**	2.3**		1.5 (0.7-2.6)

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Table 6.3.2-8: Historical control data (2010-2012) for selected clinical chemistry parameters

	Study Start	Jul-10	Oct-10	Dec-11	Apr-12	Jun-12	Overall ^a
	Route of Exposure	Rectal	Dietary	Dietary	Gavage	Dietary	
	Age at Termination (wks)	21/22	20/21	20/21	19-21	20/21	
Alkaline phosphatase (ALP) (U/L): Males	Number of animals	15	10	10	9	10	
	Mean	89	68	76	79	85	79.4
	SD	22	13	27	15	19	8
	Min	53	55	47	60	65	47
	Max	138	89	134	100	130	138
Alkaline phosphatase (ALP) (U/L): Females	Number of animals	15	10	10	10	10	
	Mean	51	36	35	50	46	43.6
	SD	11	5	9	14	14	8
	Min	31	28	21	34	27	21
	Max	68	45	44	81	72	81
Total protein (TP) (g/L): Males	Number of animals	15	10	10	9	10	
	Mean	61	61	60	70	61	63
	SD	3	3	2	14	2	4
	Min	55	57	56	59	57	55
	Max	65	65	65	105	64	105
Urea (mmol/L): Females	Number of animals	15	10	10	10	10	
	Mean	5.6	5	6.8	6.4	5.8	5.9
	SD	0.8	1	1.1	0.6	0.3	0.7
	Min	4.5	4	5.4	5.3	5.2	4
	Max	7.1	6.5	9.5	7.4	6.2	9.5
Albumin(g/L): Males	Number of animals	15	10	10	9	10	
	Mean	43	43	41	43	40	42
	SD	2	1	2	3	1	1
	Min	39	41	38	38	39	38
	Max	45	45	44	47	43	47
Calcium (mmol/L): Males	Number of animals	15	10	10	9	10	
	Mean	2.63	2.68	2.53	2.7	2.68	2.64
	SD	0.06	0.08	0.07	0.07	0.07	0.07
	Min	2.52	2.55	2.41	2.61	2.53	2.41
	Max	2.72	2.81	2.61	2.8	2.78	2.81
Calcium (mmol/L): Females	Number of animals	15	10	10	10	10	
	Mean	2.63	2.7	2.55	2.73	2.66	2.65
	SD	0.09	0.03	0.09	0.08	0.11	0.07
	Min	2.5	2.66	2.42	2.6	2.47	2.42
	Max	2.78	2.75	2.73	2.85	2.78	2.85
Sodium (mmol/L): Males	Number of animals	15	10	10	9	10	
	Mean	143	143	144	143	142	143
	SD	1	1	1	2	1	1

	Min	140	142	143	141	141	140
	Max	145	144	146	146	145	146
Potassium (mmol/L): Males	Number of animals	15	10	10	9	10	
	Mean	4.2	4.3	4	5	4.2	4.3
	SD	0.3	0.3	0.4	0.5	0.3	0.4
	Min	3.7	3.9	3.5	4.4	3.7	3.5
	Max	4.6	4.8	4.9	5.9	4.6	5.9
Cholesterol (mmol/L): Females	Study Start	Nov-12	Apr-13	Nov-13	Jul-14	Feb-15	Overall ^a
	Route of Exposure	Gavage	Gavage	Gavage	Gavage	Gavage	
	Age at Termination (wks)	19-21	20/21	20/21	21/22	20/21	
	Number of animals	10	10	9	10	15	
	Mean	1.3	1.6	1.1	1.6	1.8	1.5
	SD	0.2	0.2	0.3	0.3	0.3	0.3
	Min	1	1.3	0.7	1	1.5	0.7
	Max	1.5	2	1.5	2	2.6	2.6

a) Overall means calculated from the mean of the study means presented; standard deviation displayed are for those calculations

Urinalysis: There were no treatment related differences in urinalysis parameters at dietary inclusion levels up to 16000 ppm in either sex (Table 6.3.2-9).

Urinary pH was statistically lower when compared with controls in both males and females that received 8000 or 16000 ppm. The toxicological significance of this change is unclear.

Table 6.3.2-9: Intergroup comparison of selected urine parameters

	Dietary Concentration (ppm)										Historical control data Group Mean (min-max)	
	Males					Females					Male	Female
	0	250	1500	8000	16000	0	250	1500	8000	16000		
pH	8.2	8.2	8.4	6.7**	6.7**	7.7	7.1	7.1	6.5*	6.4*	7.4 (6.0-9.0)	6.6 (5.5-9.0)
Volume	0.6	0.7	1.9	0.7	0.9	0.5	0.6	0.3	0.4	1.1	8.7 (0.2-24.9)	5.7 (0.2-17.1)

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Bioanalysis evaluation: Bioanalytical evaluation showed that SYN545974 treated rats were generally continuously exposed to measurable concentrations of SYN545974 at all dietary inclusion levels.

In untreated animals, individual blood SYN545974 concentrations were generally found to be below the level of quantification (5.0 ng/mL) thus the absence of SYN545974 in the control animals was generally confirmed.

Sacrifice and pathology:

Macroscopic findings: An enlarged liver was observed in one male that received 16000 ppm; additionally a mass was recorded in the kidney of one male.

Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age or rat, and/or were similar incidence in control and treated animals and, therefore, were considered unrelated to administration of SYN545974.

Organ weights: Following adjustment for terminal body weight, a dose-related increase in covariant liver weight was observed in animals (males and females) that received 1500, 8000 or 16000 ppm (in males: 28%, 44% and 52% increases compared to control at 1500, 8000 or 16000 ppm, respectively; in females: 18%, 41% and 43% increases compared to control at 1500, 8000 or 16000 ppm, respectively). Absolute liver weights of both sexes were also increased at these dose levels: in males: 21%, 23% and 26% increases

compared to control at 1500, 8000 or 16000 ppm, respectively; in females: 16%, 36% and 40% increases compared to control at 1500, 8000 or 16000 ppm, respectively).

Relative liver weights (to body weight) were also increased in males and females receiving 1500, 8000 or 16000 ppm compared to their respective controls.

An increase in covariant kidney weight was also observed in females that received 8000 ppm (no dose-relationship evident) and in males and females that received 16000 ppm. Statistical significance was observed in females only.

A statistically significant increase in covariant kidney weight was observed in females receiving 8000 ppm or 16000 ppm, however no dose-relationship was evident. An increase in covariant kidney weight was also observed in males that received 16000 ppm. Based on no correlating histopathology the changes are considered not to be adverse.

Table 6.3.2-10: Intergroup comparison of selected organ weights

		Dietary Concentration (ppm)									
		Males					Females				
		0	250	1500	8000	16000	0	250	1500	8000	16000
Liver	Absolute (g)	14.51	14.39	17.57* (+21%)	17.80** (+23%)	18.30** (+26%)	7.63	8.19	8.85** (+16%)	10.38** (+36%)	10.71** (+40%)
	Covariate (g)	13.07	13.79	16.85** (+29%)	18.81** (+44%)	19.90** (+52%)	7.52	8.04	8.87** (+18%)	10.58** (+41%)	10.75** (+43%)
	Relative (% body weight)	3.342	3.473	4.195 (+26%)	4.695 (+40%)	5.005 (+50%)	3.297	3.513	3.903 (+18%)	4.714 (+43%)	4.736 (+44%)
Kidney	Absolute	2.64	2.55	2.62	2.56	2.64	1.59	1.63	1.63	1.74*	1.68
	Covariate (g)	2.52	2.51	2.56	2.66	2.78*	1.57	1.60	1.63	1.78**	1.69

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Microscopic findings: Hepatocyte hypertrophy was recorded in the liver at 1500 ppm or greater in males and 8000 ppm or greater in females. Follicular cell hypertrophy of the thyroid gland was observed at 1500 ppm or greater in males, this increased incidence achieved statistical significance at 8000 ppm or greater in males and females.

Any other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of SYN545974.

Table 6.3.2-11: Intergroup comparison of selected microscopic findings

		Dietary Concentration (ppm)									
		Males					Females				
		0	250	1500	8000	16000	0	250	1500	8000	16000
LIVER (number examined)		10	10	10	10	10	10	10	10	10	10
Hepatocyte hypertrophy	minimal	0	0	5*	7**	10**	0	0	0	6*	9**
Inflammatory cell foci		3	4	4	4	5	1	2	4	2	3
Infarct, total		1	0	0	0	0	0	0	0	0	0
THYROID (number examined)		10	10	10	10	10	10	10	10	10	10

Follicular cell hypertrophy										
minimal	0	0	4	5	4	0	0	0	3	2
mild	0	0	0	1	3	0	0	0	1	6
Total	0	0	4	6*	7**	0	0	0	4*	8**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

CONCLUSION:

In a 90-day repeated dose toxicity study, conducted according to GLP and OECD TG 408 (1998), Han Wistar rats (10/sex/dose) were administered dietary concentrations of 0, 250, 1500, 8000 or 16000 ppm, which equated to mean estimated doses of 0, 18.6, 111, 587 and 1187 mg/kg bw/d in males and 0, 21.6, 127, 727 and 1325 mg/kg bw/d in females. Blood samples were collected on Days 2, 9, 28 and 91 (i.e. after 1, 8, 27 and 90 full days dietary exposure) from designated animals for whole blood bioanalysis.

There were no treatment related mortalities or clinical signs of toxicity and there were no treatment-related findings upon ophthalmoscopic examinations. With regard to functional observational battery parameters (FOB), there was no effect on detailed clinical observations, quantitative functional observations, or locomotor activity.

Bioanalytical evaluation showed that treated rats were generally continuously exposed to measurable concentrations of pydiflumetofen at all dietary levels. In untreated animals, individual blood pydiflumetofen concentrations were generally found to be below the level of quantification (5.0 ng/mL); thus the absence of pydiflumetofen in the control animals was generally confirmed.

In males, group mean body weights at 8000 and 16000 ppm were lower than controls throughout treatment, reaching statistical significance at the end of the study at 8000 ppm and at all time points at 16000 ppm; thus, at the end of the treatment period mean body weights were 12% and 15% lower than controls at 8000 and 16000 ppm respectively. Overall mean body weight gain was also statistically significantly lower than controls at 8000 and 16000 ppm (27% and 34% respectively). Mean food consumption in males was statistically significantly lower than controls during the first 2 days of treatment at 8000 and 16000 ppm and food utilisation at these doses was statistically significantly lower over the duration of the study by 23% and 31% compared with controls (weeks 1-13), being particularly apparent at the week 1-4 and week 9-13 timepoints.

In females, group mean body weights were lower than controls throughout the treatment period, however, statistical significance was not reached. Overall mean body weight gain was statistically significantly lower than controls in females receiving 8000 and 16000 ppm pydiflumetofen (21% and 25% respectively). Mean food consumption in females was statistically significantly lower than controls during the first 3 days of treatment at 8000 and 16000 ppm and food utilisation at these doses was statistically significantly lower than controls at the week 1-4 and/or week 5-8 timepoints, resulting in an overall food utilisation efficiency (weeks 1-13) that was 23% and 26% lower than controls at these respective doses.

The main target organs in rats after 13-weeks' exposure were the liver and the thyroid.

Clinical chemistry analysis revealed that ALP was statistically significantly reduced in comparison with controls by 30%, 31% and 39% in males and 41%, 42% and 40% in females at 1500, 8000 and 16000 ppm respectively. The reported values fell within the range of the laboratory HCD (5-year time-period, same laboratory). Nonetheless, a clear dose response was evident, and the relatively large magnitude of the decrease means that the toxicological relevance of the effect cannot be ruled out. Therefore, the reduction in ALP is concluded to be related to treatment with pydiflumetofen. Further clinical chemistry findings comprised increased cholesterol in females from 8000 ppm (35%). Observed increases in serum calcium levels were mostly within normal biological variation and therefore were not related to treatment.

Liver weights were increased in both sexes at 1500, 8000, 16000 ppm in comparison with controls. In males, absolute liver weights were increased at these doses by 21%, 23% and 26% and relative liver weights

were increased by 26%, 40% and 50%. In females, liver weights were increased by 16%, 36% and 40% (absolute) and 18%, 43% and 44% (relative) at these doses when compared with controls.

Hepatocyte hypertrophy was noted in the livers of males from 1500 ppm and females from 8000 ppm. Follicular cell hypertrophy of the thyroid was also noted in males (from 1500 ppm) and females (from 8000 ppm), being statistically significant from 8000 ppm in both sexes (no incidences of follicular cell hypertrophy were recorded in the thyroid in the control or 250 ppm dose group in males and females or in the 1500 ppm dose group in females).

Overall, treatment of male and female rats with 250, 1500, 8000 and 16000 ppm pydiflumetofen for at least 90-days, resulted in impaired body weight development, reduced food consumption and reduced food utilisation from 8000 ppm, in addition to liver effects (increased weights, hepatocellular hypertrophy and reduced ALP) and thyroid effects (follicular cell hypertrophy in males only) from 1500 ppm.

Therefore, a **NOAEL of 250 ppm (equivalent to 18.6 and 21.6 mg/kg bw/d in males and females respectively)** was determined from this study. At the LOAEL of 1500 ppm (equivalent to 111 and 127 mg/kg bw/d in males and females respectively), liver weight increases, and reduced ALP were seen in both sexes, whilst hepatocellular hypertrophy and thyroid follicular cell hypertrophy (not statistically significant) were observed in males.

(██████████ and ██████████, 2015)

B.6.3.2.1 1 Mouse

Report:	K-CA 5.3.2/03 ██████████ (2015). SYN545974: A 13 Week Toxicity Study of SYN545974 by Oral (Dietary) Administration in Mice (Final Report Amendment 2). ██████████ ██████████. Laboratory Report No. 33011, issue date 28 July 2015. Unpublished. Syngenta File No. SYN545974_10211.
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Report:	K-CA 5.3.2/04 ██████████, ██████████, 2016 SYN545974 - Historical Control Data for SYN545974_10210 and SYN545974_10211, ██████████ ██████████, Syngenta File No. SYN545974_10448.
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Guidelines: Repeat dose oral toxicity study in rodents. OECD guideline reference 408 (1998): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.3100 (1998): EU Directive 96/52/EC, B.26 (2001).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

Additional Historical control Data for some clinical chemistry parameters are provided and the information incorporated into the study summary below.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rodent diet has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

The objective of this study was to determine the potential toxicity of SYN545974 in the mouse after oral administration by diet for a minimum of 91 days and to determine dose levels for a subsequent carcinogenicity study in mice. The study is part of animal toxicity experiments designed to provide information which will allow the test substance to be used safely.

Groups of 10 male and 10 female, 7-8 week old, CD-1 (■■■■:CD-1(ICR)) mice were fed diets containing 0, 100, 500, 4000 or 7000 ppm of SYN545974 (purity 99.5%) for a period of 91/92 consecutive days.

The following were assessed at pre-determined intervals from pre-trial until study completion from all animals: viability, clinical observations, body weight and food consumption. Blood samples were collected from all animals at termination for haematology and clinical chemistry investigations. Additional blood samples were collected on Days 2, 16, 30 and 91 (i.e. after 1, 15, 29 and 90 full day's dietary exposure) from designated animals for whole blood bioanalysis.

All animals were terminated after completion of 91/92 days of treatment and underwent a detailed necropsy examination with selected organs weighed. Tissues from all animals in the control and 7000 ppm dose groups were subjected to a comprehensive histological evaluation. Additionally, sections of liver were also evaluated from the 100, 500 and 4000 ppm dose animals.

The overall mean achieved dose levels were 17.5, 81.6, 630.1 and 1158.0 mg SYN545974/kg/day for males and 20.4, 105.9, 846.3 and 1482.6 mg/kg/day for females, corresponding to dietary inclusion levels of 100, 500, 4000 and 7000 ppm, respectively. Treated mice were generally continuously exposed to measurable concentrations of SYN545974 at all dietary inclusion levels.

There were no early deaths during the study. There were no clinical observations noted during treatment. Body weight change was lower in males throughout the treatment period and in females receiving 500 ppm and above from Day 63. There were no treatment related findings in food consumption or water consumption.

There were no differences in haematological parameters. Higher cholesterol and triglycerides levels were observed in males receiving ≥ 4000 ppm and in females receiving 7000 ppm, when compared with controls. Cholesterol was also higher in males receiving 500 ppm.

There were no macroscopic differences in treated animals noted at termination when compared with controls. An increased incidence of hepatocyte hypertrophy was observed in the livers of males treated with 500, 4000 or 7000 ppm and females treated with 4000 or 7000 ppm, with a corresponding increase in liver weight.

Under the conditions of this study the No Observed Adverse Effect Level (NOAEL) following at least 91 consecutive days of dietary exposure to SYN545974, was 100 ppm for male mice, equating to 17.5 mg/kg/day, based on the increased cholesterol, triglyceride concentrations, liver weights (>15%) and hepatocyte hypertrophy in the liver at ≥ 500 ppm (81.6 mg/kg/day). The No Observed Adverse Effect Level (NOAEL) for female mice was 500 ppm, equating to 105.9 mg/kg/day, based on the increased cholesterol, triglyceride concentrations, liver weight (+60%) and hepatocyte hypertrophy in the liver at ≥ 4000 ppm (846.3 mg/kg/day).

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Technical, white powder
Lot/Batch number:	2637-BA/110
Purity:	99.5 %
CAS#:	Not reported
Stability of test compound:	Stable until end July 2013 (stored at <30°C)

Vehicle and/or positive control: The test substance was administered via Certified Rodent Diet No. 5CR4 (14% protein) Ground, supplied by ■■■■■ (■■■■).

Test Animals:	
Species	mouse
Strain	CD-1, designation [REDACTED]:CD-1(ICR),
Age/weight at dosing	Approximately 7-8 weeks / 29-40 g (males), 22-30 g (females)
Source	[REDACTED]
Housing	Males were singly housed and females 2 per cage, in polypropylene cages (dimensions 48 x45 x 13 cm) with stainless steel grid tops, solid bottoms and containing a separate stainless steel food hopper. Sterilised wood shavings were provided as bedding (Datesand, Manchester, UK).
Acclimatisation period	14 days
Diet	Certified Rodent Diet No. 5CR4 (14% protein) supplied by [REDACTED] ([REDACTED]), <i>ad libitum</i>
Water	Water, in water bottles, from the public water supply <i>ad libitum</i>
Environmental conditions	Temperature: 19-21°C Humidity: 46-56% Air changes: 15 per hour Photoperiod: 12 hours light, 12 hours dark

Study Design and Methods:

In-life dates: Start: 18 October 2011, End: 05 May 2015

In a 13 week dietary toxicity study, groups of 10 male and 10 female, 7-8 week old, CD-1 ([REDACTED] CD-1(ICR)) mice were fed diets containing 0, 100, 500, 4000 or 7000 ppm of SYN545974 (purity 99.5%) for a period of 91/92 consecutive days. Control animals received blank diet only *i.e.* diet not containing the test substances.

Dose level selection: Dose levels were set after evaluation of data from a 28 day study ([REDACTED], 2012a, MCA Section 5.3.1/02) and took into account the maximum tolerated dose in the test model.

Animal assignment: On arrival from the suppliers, animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. Control animals were housed on a separate rack. Treated animals were housed on two racks, containing 2 groups per rack. During pre-trial, group mean body weights were checked to ensure all animals were within 20% of the mean weight of each sex. All groups were within $\pm 5\%$ indicating acceptable homogeneity of body weight.

Animals were allocated to 5 treatment groups and treated as follows:

Study design

Test group	Treatment	Dietary concentration (ppm)	# male	# female
1	control	0	10	10
2	low dose	100	10	10
3	intermediate dose I	500	10	10
4	intermediate dose II	4000	10	10
5	high dose	7000	10	10

Diet preparation and analysis: Diet formulations were prepared from the high dose level of 7000 ppm. This was prepared from a 200 g premix containing the total weight of test substance required and untreated [REDACTED] control diet. This was ground in an automated mortar and pestle for 5-6 min. The premix was then blended with the required amount of untreated [REDACTED] control diet and mixed for 20 minutes in a diet mixer (Winkworth change drum mixer). The diets at the lower concentrations (100, 500 and 4000 ppm) were prepared as a serial dilution from the higher concentration group (*i.e.* 4000 ppm prepared from 7000 ppm, 500 ppm prepared from 4000 ppm diet and so on) by adding an appropriate amount of higher concentration diet to the appropriate weight of blank control Certified Rodent Diet No. 5CR4. Diets were mixed for 20 minutes in a diet mixer (Winkworth change drum mixer).

Diet formulations were prepared for one week only on commencement of treatment, followed by once every two weeks, stored and used within the conditions established in a previous analytical method validation study (██████████ Study Number 427957). Diet formulations (without the test substance SYN545974) were dispensed for the control animals.

Analysis of diets was carried out with regard to achieved concentration and homogeneity. Duplicate 20 g samples were taken from the top, middle and bottom of each formulated diet (including control) immediately after preparation for feeding on Day 1 and during Weeks 4, 7 and 13. All samples were stored in the dark at ambient temperature until analysed.

Results: Analysed concentrations of the test substance in diets formulated for use on Day 1 and in Weeks 4, 7 and 13 for concentrations 100, 500, 4000 and 7000 ppm were found to be -7.6 to 1.0% of the theoretical concentrations indicating acceptable accuracy of formulations. The coefficient of variation for triplicate samples was 7.1% or lower indicating acceptable homogeneity of formulations, with the exception of the 7000 ppm formulation prepared for feeding in Week 4, which had a coefficient of variation of 27.3. This value was confirmed following analysis of the back-up samples and following an investigation it was thought the diet was not mixed correctly. However, as this was only one preparation fed over two weeks, the following preparation was prepared in the same way and was found to be within specification, the lowest concentration within the diet was still higher than the next dose level and the food consumed by the animals was considered not to have been affected, this was considered not to have affected the outcome or integrity of the study.

Observations: All animals were generally checked early morning and as late as possible each day for viability. All animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta, once pre-trial, daily for Days 1 to 8 and then weekly thereafter. At least once daily, all animals were examined in their cages for reaction to treatment. The onset, intensity and duration of any signs were recorded.

Bodyweight: Body weights were recorded twice during Pre-trial, daily during Week 1 of treatment and once each week thereafter until the completion of treatment.

Food consumption, utilisation and achieved dose: The quantity of food consumed by each cage of animals was measured and recorded once during pre-trial, daily during Week 1 of treatment and once each week thereafter until the completion of treatment.

Food utilisation was calculated for each cage as follows: (cage mean weight gain x 100)/cage total food consumption. Values were calculated for intervals of Weeks 1-4, 5-8, 9-13 and also an overall value for Weeks 1-13.

The amount of experimental diet ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{dose (ppm)} \times \text{cage mean food consumption (g/animal/day)}}{\text{cage mean body weight at the middle of the period}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Haematology: Blood was collected from all animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. They were not starved overnight. The following parameters were examined:

haemoglobin	mean cell haemoglobin concentration
haematocrit	platelet count
red blood cell count	total white cell count
mean cell volume	differential white cell count

mean cell haemoglobin	red cell distribution width
reticulocytes	reticulocyte count
blood smear (not examined)	

Clinical chemistry: Blood was collected from all animals prior to termination via a capillary tube from the orbital sinus under isoflurane anaesthesia. They were not starved overnight. The following parameters were examined:

urea	albumin
glucose	globulin
aspartate aminotransferase	albumin :globulin ratio
alanine aminotransferase	cholesterol
alkaline phosphatase	creatinine
lactate dehydrogenase	creatine phosphokinase
sodium	total bilirubin)
potassium	calcium
chloride	phosphate
total protein	triglycerides

In addition, some blood was collected from all animals for diagnostic serology sampling. The blood was transferred into a plain glass tube and left to clot for at least 1 h at room temperature. In order to ensure enough blood was collected to obtain a viable sample, blood samples from animals of the same sex were pooled.

Bioanalysis evaluation: Blood samples (approximately 0.1 mL) were obtained from all animals in each group for whole blood analysis of SYN545974 on Days 2, 16, 30 and 91 at the following timepoints: 0700 h, 1100 h, 1500 h and 1800 h. Samples were collected from the saphenous vein following warming of the animal in a heating cabinet (37-40°C) for an appropriate time. The samples were then stored at -80°C until analysed.

Concentrations of SYN545974 in each sample were determined using a suitable LC-MS/MS analytical method. The assay accuracy and precision was within 100±20% and ≤20% respectively.

Investigations *post mortem*:

Termination: Following a minimum of 91 days of treatment, all animals were killed in a random order by exposure to a rising concentration of carbon dioxide and had their terminal body weight recorded, followed by severance of major blood vessels.

Macroscopic examination: Each animal was subject to a detailed necropsy which consisted of a complete external and internal examination including body orifices (ears, nostrils, mouth, anus and vulva) and cranial, thoracic and abdominal organs and tissues.

Organ weights: The organs below were removed and weighed from all animals before sampling and preservation. Paired organs were weighed separately and the sum of the individual organs used for reporting purposes.

brain	ovaries
epididymides	spleen
adrenal glands	testes
liver	thymus
heart	uterus
kidneys	

Tissue submission: The following tissues from all animals were examined *in situ*, removed and examined and fixed in an appropriate fixative:

aorta (from thoracic segment)	lymph nodes (cervical and mandibular)
bone marrow smear	lymph nodes mesenteric
bone marrow (femur and sternum)	skeletal muscle
bone (femur and sternum)	nasal cavity
brain (forebrain, midbrain, cerebellum and medulla oblongata)	optic nerves
cervix	sciatic nerve
epididymis	oesophagus
eyes	ovaries
gallbladder	lung
adrenal glands	oviducts
Harderian glands	pancreas
lacrimal glands	pharynx
mammary glands (with inguinal skin (females only))	skin
parathyroid glands	duodenum
pituitary gland	ileum
prostate gland	jejunum
salivary glands (submandibular and submaxillary)	spinal cord (cervical, thoracic and lumbar)
seminal vesicles	spleen
thyroid gland	stomach (glandular and non-glandular region)
gut-associated lymphoid tissue	testes
heart	thymus
kidneys	tongue
caecum	trachea
colon	ureter
rectum	urinary bladder
larynx	uterus
liver	vagina

Microscopic examination: All processed tissues from all Control and high dose (7000 ppm) animals (with the exception of bone marrow smears and nasal cavity) were examined by light microscopy. Liver was processed from all animals in the low and intermediate dose groups (100, 500 and 4000 ppm) and examined by light microscopy.

Additional Samples: Following sampling for histopathology, two additional 5mm liver sections from the remaining tissue of the left lateral lobe were evenly divided (the number of samples taken per animal is recorded in the study data), and placed in separate RNA-ase free tubes. Samples were snap frozen in liquid nitrogen as quickly as possible after collection and stored at -80°C for possible future investigations to be performed at the discretion of the Sponsor. Additionally, a representative section of the left lateral lobe, the right median lobe and the caudate lobe were taken and fixed in formalin for 48 hours and processed to paraffin wax block. A small piece of duodenum was incorporated into each block. The remainder of the liver was snap frozen in liquid nitrogen for possible future analysis.

Statistics: The following statistical approaches were used in this study:

- All analyses were two-tailed for significance levels of 5% and 1%. Males and females were analysed separately.
- All means are presented with standard deviations.
- Body weights, cumulative body weight gain, food consumption, food utilisation, selected functional observation battery and motor activity data, absolute organ weights, haematology, coagulation, clinical chemistry and selected urinalysis parameters were analysed initially by a one-way analysis of variance (ANOVA).

- Organ weights were also analysed by analysis of covariance (ANCOVA) on final body weight. This statistical analysis provided Adjusted Organ Weight values.
- Summary values of organ to body weight ratios were not analysed statistically.
- For all parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant.
- Micropathology incidence data were analysed using Fisher's Exact Test.

RESULTS

Mortality: There were no treatment-related mortalities.

Clinical observations: There were no clinical observations that were thought to be related to treatment with SYN545974.

Bodyweight: Body weight change was lower in all male treated groups throughout the treatment period when compared with controls; isolated incidences of statistical significance were achieved. Body weight change was generally lower in females receiving 500 ppm and above from Day 63.

The body weight in animals receiving 7000 ppm was generally noted to be lower throughout the treatment period, when compared with control. However, due to the small magnitude and the fact that all animals were within the control range throughout the study, with the exception of 2 animals at Day 70 only, this was considered to be unrelated to treatment with SYN545974.

Table 6.3.2-12: Intergroup comparison of bodyweight change - selected timepoints (g)

Body weight change (days)	Dietary Concentration (ppm)									
	Males					Females				
	0	100	500	4000	7000	0	100	500	4000	7000
Initial weight day 0	33.7	34.6	36.1	34.1	34.1	26.2	26.0	25.8	26.1	25.2
0-1	-0.1	-0.4	-0.2	-0.7	-1.4*	-0.7	-0.5	-0.5	-1.1	-1.4
0-4	0.9	0.1	-0.5**	-0.3	0.0	0.8	0.2	0.2	0.3	0.7
0-7	0.5	0.2	-0.5	0.5	0.8	0.6	0.4	0.4	0.5	1.2
0-21	3.5	1.8*	1.8*	2.7	2.7	2.9	2.1	3.1	3.2	4.0
0-42	5.2	2.9*	3.8	4.0	4.2	4.9	4.6	4.8	5.8	5.0
0-63	8.9	5.3**	7.4	5.7**	6.6	8.3	7.8	7.7	7.3	7.0
0-91	10.1	8.2	9.6	7.3	7.5	8.3	8.5	7.1	8.2	6.9
Final weight day 91	43.9	42.7	45.7	41.1	41.6	34.5	34.5	33.0	34.3	32.1

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Food consumption, utilisation and achieved dose: There were no notable differences in quantity of food consumed or food utilisation in any treated group.

Food utilisation was generally lower throughout the treatment period in all male treated groups when compared with controls. However, due to the lack of a dose relationship and that individual values were generally within the control ranges, the differences were considered to be incidental to treatment with SYN545974.

Estimated achieved dose: Dose rates (based on nominal dietary levels) were calculated in terms of mg SYN545974/kg body weight. Mean values are shown below:

Table 6.3.2-13: Mean Dose Received (mg/kg/day)

Dietary concentration (ppm)	100	500	4000	7000
Males	17.5	81.6	630	1158
Females	20.4	106	846	1483

Water consumption: There were no treatment related effects on water consumption.

Haematology and coagulation: There were no treatment related differences in haematology parameters at dietary inclusion levels of up to 7000 ppm SYN545974 in either sex.

A number of statistically significant changes were observed in males only, however due to the small magnitude of the changes, a lack of a dose response and no correlating histological findings, the differences are considered to be spontaneous and not related to treatment. Following a request from EFSA, the applicant submitted historical control data (2010-2016) but only for some selected haematology parameters in dietary 13 week toxicity studies in mice. This additional information has been added in Table 6.3.2-14a and 6.3.2-14b.

Table 6.3.2-14a: Intergroup comparison of selected haematology parameters for males

Parameter	Dietary Concentration (ppm)					Historical control
	Males					
	0	100	500	4000	7000	
Haemoglobin g/dL	12.0	12.2	12.2	11.0*	11.6	-
RBC (x10 ¹² /L)	8.22	8.29	8.42	7.75	8.25	8.88 (6.09-9.91)
Red Cell Distribution Width (RDW) %	12.9	13.5	13.3	14.0*	13.4	-
Mean cell haemoglobin concentration (MCHC) g/dL	29.1	28.9	29.3	28.6	28.5	33.5 (30.9-36.6)
Mean cell volume (MCV) fL	50.2	50.8	49.6	49.7	49.5	-
White Blood Cells x10 ⁹ /L	3.92	5.04	5.81	5.64	5.11	6.63 (3.11-19.55)
Neutrophils x10 ⁹ /L	0.61	0.87	1.13**	0.89	0.75	-
Eosinophils x10 ⁹ /L	0.09	0.16	0.21*	0.15	0.12	-
Platelets x10 ⁹ /L	1296	1491	1395	1541	1616**	-
Lymphocytes x10 ⁹ /L	3.12	3.86	4.26	4.43	4.10	5.30 (2.53-15.25)

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Table 6.3.2-14b: Intergroup comparison of selected haematology parameters for females

Parameter	Dietary Concentration (ppm)					Historical control
	Females					
	0	100	500	4000	7000	
Haemoglobin g/dL	12.4	12.1	12.5	11.8	11.7	
RBC (x10 ¹² /L)	8.32	8.01	8.43	7.98	8.06	-
Red Cell Distribution Width (RDW) %	13.6	14.7	13.5	14.4	13.8	-
Mean cell haemoglobin concentration (MCHC) g/dL	29.5	29.1	29.3	29.2	29.0	-
Mean cell volume (MCV) fL	50.8	52.0	50.7	50.6	50.1	54.1 (48.4-58.1)
White Blood Cells x10 ⁹ /L	5.66	4.99	5.24	5.88	6.35	-
Neutrophils x10 ⁹ /L	0.75	0.80	0.68	0.82	0.89	-
Eosinophils x10 ⁹ /L	0.17	0.13	0.17	0.09	0.11	-
Platelets x10 ⁹ /L	1358	1269	1494	1380	1511	-
Lymphocytes x10 ⁹ /L	4.61	3.92	4.25	4.80	5.17	3.45 (0.75-9.77)

* Statistically significant difference from control group mean, $p < 0.05$ (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student's t-test, 2-sided)

Blood clinical chemistry: Cholesterol and triglyceride concentrations were higher in animals receiving 4000 or 7000 ppm when compared with controls, with statistical significance being achieved in both parameters at 7000 ppm and in cholesterol in males receiving 4000 ppm. Cholesterol was also higher in males receiving 500 ppm, however, no statistical significance was achieved.

A number of other statistically significant changes were observed in clinical chemistry parameters, however, due to the small magnitude of change, lack of a dose relationship, the individual values were mostly within the control range and in the absence of any histopathological findings, they were considered to be incidental to treatment. For parameters where a statistically significant change from control was observed, the values and appropriate historical control data (HCD) from the conducting laboratory are presented in Table 6.3.2-16.

Table 6.3.2-15: Intergroup comparison of selected clinical chemistry parameters

Parameter	Dietary Concentration (ppm)										Historical control data	
	Males					Females					Group Mean (min-max)	
	0	100	500	4000	7000	0	100	500	4000	7000	Male	Female
Cholesterol	3.9	4.1	4.8	4.9*	5.9**	2.8	2.6	2.7	3.6	3.8**	3.8 (2.7-6.3)	2.52 (1.3-4.6)
Triglycerides	1.50	1.26	1.46	1.70	2.79**	1.13	1.14	0.90	1.72	1.77*	1.60 (0.31-2.85)	1.23 (0.39-3.15)
Glucose	12.3	12.33	12.29	11.88	11.30	12.86	12.30	11.93	10.89*	10.88*	12.16 (8.06-18.11)	11.09 (5.56-15.01)
Total protein	51	53	52	52	56*	53	51	51	53	53	53 (46-61)	51 (43-60)
Albumin	32	32	31	32	34	36	35	34**	35	35	34 (29-40)	36 (31-40)
Globulin	19	21*	22**	21*	22**	17	17	17	18	18	19 (14-27)	16 (11-23)
AG-R	1.7	1.6*	1.4**	1.5*	1.6	2.2	2.	2.1	1.9**	1.9**	1.9 (1.3-2.8)	2.4 (1.5-3.2)
K	4.8	5.1	4.7	4.5	4.2*	4.3	4.2	4.1	3.8	3.9	4.2 (3.2-5.1)	3.9 (3.1-4.9)
Cl	118	116	117	116	115*	116	117	117	116	116	115 (108-120)	115 (102-120)
Phosphate	2.20	2.15	2.05	2.04	2.19	1.97	2.05	2.04	2.46*	2.31	2.04 (1.02-2.81)	2.21 (1.36-3.80)
Calcium	2.45	2.48	2.54	2.54	2.57	2.43	2.46	2.48	2.53	2.57*	2.43 (2.27-2.68)	2.40 (2.07-2.55)

* Statistically significant difference from control group mean, p<0.05 (Student's t-test, 2-sided)

** Statistically significant difference from control group mean, p<0.01 (Student's t-test, 2-sided)

Table 6.3.2-16: Historical data (2009-2014) for selected clinical chemistry parameters

	Study Start Route of Exposure Age at Termination (wks)	Apr-09 Dietary 20	Oct-10 Dietary 19	Sep-11 Dietary 20/21	Dec-11 Dietary 20	Nov-14 Dietary 19/20	Overall ^a
Cholesterol (mmol/L): Males	Number of animals	10	7	10	10	10	
	Mean	4.1	3.5	3.7	4.6	3.3	3.8
	SD	1.1	0.4	0.5	1	0.5	0.5
	Min	3	3	2.7	3.1	2.7	2.7
	Max	6.1	4.2	4.4	6.3	3.6	6.3
Cholesterol (mmol/L): Females	Number of animals	10	10	9	10	10	
	Mean	2.8	2.1	2.7	2.6	2.4	2.52
	SD	0.8	0.5	0.6	0.6	0.7	0.28
	Min	1.6	1.3	1.8	2	1.5	1.3
	Max	4.6	2.9	3.4	3.6	3.8	4.6
Triglycerides (mmol/L): Males	Number of animals	10	7	10	10	10	
	Mean	2.06	1.83	1.4	1.41	1.28	1.6
	SD	0.53	0.39	0.52	0.55	0.47	0.33
	Min	1.15	1.29	0.87	0.81	0.31	0.31
	Max	2.85	2.38	2.61	2.62	1.92	2.85
Triglycerides (mmol/L): Females	Number of animals	10	10	9	10	10	
	Mean	1.59	1.24	0.89	1.13	1.31	1.23
	SD	0.62	0.73	0.25	0.41	0.54	0.26
	Min	0.95	0.39	0.48	0.67	0.7	0.39
	Max	3.15	2.54	1.19	1.98	2.44	3.15
Glucose (mmol/L): Males	Number of animals	10	7	10	10	10	
	Mean	11.32	12.49	12.36	13.27	11.37	12.16
	SD	2.2	3.13	2.46	2.46	1.58	0.82
	Min	9.56	9.02	9.49	8.06	9.46	8.06
	Max	15.16	18.11	17.45	16.47	13.97	18.11
Glucose (mmol/L): Females	Number of animals	10	10	8	10	10	
	Mean	11.39	10.04	11.65	11.32	11.04	11.09
	SD	1.36	1.95	1.26	1.53	2.7	0.62
	Min	8.33	5.56	9.49	9.08	6.42	5.56
	Max	13	12.52	13.23	14.57	15.01	15.01
Total Protein (g/L): Males	Number of animals	10	7	10	10	10	
	Mean	56	54	51	52	50	53
	SD	3	2	2	3	1	2
	Min	51	52	49	46	49	46
	Max	61	58	54	54	53	61
Total Protein (g/L): Females	Number of animals	10	10	8	10	10	
	Mean	57	52	50	49	49	51
	SD	2	3	2	4	2	3
	Min	53	45	46	43	47	43
	Max	60	55	53	56	52	60

	Study Start Route of Exposure Age at Termination (wks)	Apr-09 Dietary 20	Oct-10 Dietary 19	Sep-11 Dietary 20/21	Dec-11 Dietary 20	Nov-14 Dietary 19/20	Overall ^a
Albumin (g/L): Males	Number of animals	10	7	10	10	10	
	Mean	35	37	33	33	34	34
	SD	2	2	2	2	1	2
	Min	31	34	31	29	33	29
	Max	37	40	36	35	35	40
Albumin (g/L): Females	Number of animals	10	10	8	10	10	
	Mean	37	38	34	35	36	36
	SD	1	2	2	3	1	2
	Min	35	34	31	31	34	31
	Max	39	40	36	39	38	40
Globulin (g/L): Males	Number of animals	10	7	10	10	10	
	Mean	22	17	19	19	17	19
	SD	2	2	1	2	1	2
	Min	20	14	17	15	15	14
	Max	27	21	21	21	19	27
Globulin (g/L): Females	Number of animals	10	10	8	10	10	
	Mean	20	14	16	15	13	16
	SD	1	2	2	2	1	3
	Min	18	11	13	12	12	11
	Max	23	17	18	19	15	23
AG-R: Males	Number of animals	10	7	10	10	10	
	Mean	1.6	2.2	1.8	1.8	2.1	1.9
	SD	0.2	0.4	0.1	0.2	0.2	0.2
	Min	1.3	1.7	1.5	1.5	1.7	1.3
	Max	1.8	2.8	2	2.3	2.4	2.8
AG-R: Females	Number of animals	10	10	8	10	10	
	Mean	1.9	2.6	2.1	2.4	2.8	2.4
	SD	0.2	0.3	0.3	0.4	0.3	0.4
	Min	1.5	2.3	1.8	1.6	2.3	1.5
	Max	2.1	3.1	2.6	3	3.2	3.2
Potassium (mmol/L): Males	Number of animals	8	4	10	10	10	
	Mean	4.1	4.2	4.2	4.3	4.1	4.2
	SD	0.3	0.3	0.4	0.5	0.2	0.08
	Min	3.8	3.8	3.2	3.6	3.9	3.2
	Max	4.8	4.4	4.7	5.1	4.5	5.1
Potassium (mmol/L): Females	Number of animals	6	4	8	10	10	
	Mean	4.1	4	3.8	4.1	3.7	3.9
	SD	0.6	0.5	0.5	0.6	0.4	0.2
	Min	3.5	3.4	3.1	3.4	3.4	3.1
	Max	4.9	4.5	4.3	4.9	4.3	4.9
	Number of animals	8	4	10	10	10	

	Study Start Route of Exposure Age at Termination (wks)	Apr-09 Dietary 20	Oct-10 Dietary 19	Sep-11 Dietary 20/21	Dec-11 Dietary 20	Nov-14 Dietary 19/20	Overall ^a
Chloride (mmol/L): Males	Mean	115	118	115	116	113	115
	SD	3	2	2	2	3	2
	Min	110	116	112	114	108	108
	Max	119	120	118	119	117	120
Chloride (mmol/L): Females	Number of animals	6	3	8	10	10	
	Mean	111	119	116	115	115	115
	SD	4	1	2	4	2	3
	Min	102	118	113	107	111	102
Phosphate (mmol/L): Males	Max	114	120	120	118	117	120
	Number of animals	10	7	7	9	10	
	Mean	2.12	2.26	2.14	2.03	1.63	2.04
	SD	0.36	0.26	0.38	0.41	0.23	0.24
Phosphate (mmol/L): Females	Min	1.54	1.94	1.57	1.02	1.17	1.02
	Max	2.59	2.63	2.81	2.45	1.93	2.81
	Number of animals	10	10	3	7	10	
	Mean	2.07	2.65	2.23	2.3	1.82	2.21
Calcium (mmol/L): Males	SD	0.35	0.76	0.35	0.36	0.3	0.31
	Min	1.65	1.73	1.9	1.67	1.36	1.36
	Max	2.71	3.8	2.59	2.71	2.33	3.8
	Number of animals	9	7	7	9	10	
Calcium (mmol/L): Females	Mean	2.4	2.53	2.48	2.42	2.34	2.43
	SD	0.08	0.07	0.09	0.03	0.06	0.07
	Min	2.29	2.45	2.34	2.37	2.27	2.27
	Max	2.57	2.68	2.59	2.47	2.42	2.68
	Number of animals	10	9	4	9	10	
	Mean	2.37	2.46	2.47	2.39	2.33	2.4
	SD	0.07	0.06	0.06	0.15	0.08	0.06
	Min	2.25	2.35	2.41	2.07	2.2	2.07
	Max	2.48	2.52	2.54	2.55	2.43	2.55

a) Overall means calculated from the mean of the study means presented; standard deviation displayed are for those calculations

Bioanalysis evaluation: Bioanalytical evaluation showed that SYN545974 treated mice were generally continuously exposed to measurable concentrations of SYN545974 at all dietary inclusion levels.

In untreated animals, individual blood SYN545974 concentrations were generally found to be below the level of qualification (<5.0 ng/mL) thus the absence of SYN545974 in the control animals was generally confirmed.

Sacrifice and pathology:

Macroscopic findings: No treatment-related gross findings were noted. The gross findings observed were considered incidental, of the nature commonly observed in this strain and age of mice, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of SYN545974.

Organ weights: Absolute and covariate liver weight was statistically significantly higher in animals that received 4000 or 7000 ppm when compared with controls. Absolute and covariate liver weight was also statistically significantly higher in males receiving 500 ppm when compared with controls.

Absolute and covariate spleen weight was higher in animals that received 4000 ppm when compared with controls. However, due to the small magnitude of change, lack of a dose relationship and in the absence of histopathological findings, they were considered to be incidental to treatment.

Table 6.3.2-17: Intergroup comparison of liver weights

		Dietary Concentration (ppm)									
		Males					Females				
		0	100	500	4000	7000	0	100	500	4000	7000
Liver	Absolute (g)	1.94	1.96	2.29* (+18%)	2.76** (+42%)	3.24** (+67%)	1.63	1.64	1.66	2.60** (+59%)	2.51** (+54%)
	Covariate (g)	1.90	1.99	2.18* (+15%)	2.82** (+48%)	3.32** (+75%)	1.58	1.61	1.71	2.56** (+62%)	2.59** (+64%)
	Relative (% body weight)	4.703	4.905	5.352 (+14%)	7.005 (+49%)	8.272 (+76%)	4.989	5.097	5.410	7.990 (+60%)	8.375 (+68%)

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Microscopic findings: Centrilobular hepatocyte hypertrophy was observed in the liver, at 500 ppm and above in males and 4000 ppm and above in females, which correlated with increased absolute and relative organ weights. There was no evidence of treatment related histopathological change in the thyroid (Table 6.3.2-18).

Any other microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rat, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of SYN545974.

Table 6.3.2-18: Intergroup comparison of selected microscopic findings

		Dietary Concentration (ppm)									
		Males					Females				
		0	100	500	4000	7000	0	100	500	4000	7000
LIVER (number examined)		10	10	10	10	10	10	10	10	10	10
Hepatocyte hypertrophy	mild centrilobular	0	0	2	4	5*	0	0	0	6*	7**
Focal necrosis		0	0	0	1	0	0	0	1	0	1
Infraction, lobar		0	0	0	0	0	0	0	0	0	1
Inflammatory cell infiltration, with or without necrosis		0	0	0	0	1	2	1	2	0	0
Hepatocyte vacuolisation		1	0	1	0	1	0	0	0	0	0
THYROID (number examined)		10	0	0	0	10	10	0	0	0	10
No abnormality detected		10	-	-	-	10	10	-	-	-	10
Ectopic thymus		0	-	-	-	0	2	-	-	-	1

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

CONCLUSION:

In a 90-day repeated dose toxicity study conducted according to GLP and OECD TG 408 (1998), CD-1 mice (10/sex/dose) were administered dietary concentrations of 0, 100, 500, 4000 or 7000 ppm, which equated to estimated achieved doses of 0, 17.5, 81.6, 630 and 1158 mg/kg bw/d in males and 0, 20.4, 106, 846 and 1483 mg/kg bw/d in females. Blood samples were collected on Days 2, 16, 30 and 91 (i.e. after 1, 15, 29 and 90 full day's dietary exposure) from designated animals for whole blood bioanalysis.

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

Body weight gain was lower in all treated males throughout the study, occasionally reaching statistical significance; however, final body weight gain was not statistically significantly lower at the end of the study. There was no treatment related effect on the mean body weights of males at any time point. In females, body weight gain was generally lower from 500 ppm and from day 63 onwards; however, statistical significance was not reached at any time-point. There was no treatment related effect on the mean body weights of females. There was no effect on food consumption in either sex and although food utilisation was generally lower, there was no dose response and all individual values were within the control ranges; therefore, the effect on food utilisation in males and females was not attributable to treatment with pydiflumetofen.

Bioanalytical evaluation showed that treated mice were generally continuously exposed to measurable concentrations of pydiflumetofen at all dietary levels. In untreated animals, individual blood pydiflumetofen concentrations were generally found to be below the level of qualification (<5.0 ng/mL); thus the absence of pydiflumetofen in the control animals was generally confirmed.

The main target organ in mice after 13 weeks' exposure was the liver.

Clinical chemistry analysis revealed higher cholesterol concentrations from 500 ppm in males (statistically significant from 4000 ppm) and at 7000 ppm in females. Triglyceride concentrations were also increased from 4000 ppm in both sexes (statistically significant at 7000 ppm). Other sporadic statistically significant changes in clinical chemistry parameters were incidental to treatment as they were small in magnitude and lacked a clear dose response relationship.

Liver weights were increased in males from 500 ppm; absolute liver weights were increased by 18%, 42% and 67% and relative liver weights by 14%, 49% and 76% at 500, 4000 and 7000 ppm respectively. Liver weights were increased in females from 4000 ppm; absolute weights were increased by 59% and 54% and relative weights by 60% and 68% at 4000 and 7000 ppm respectively. Histopathological correlates comprising hepatocellular hypertrophy were observed, being statistically significant at 7000 ppm in males and from 4000 ppm in females; incidences were 0, 0, 2, 4 and 5 in males and 0, 0, 0, 6 and 7 in females at 0 (control), 100, 500, 4000 and 7000 ppm respectively.

Overall, treatment of male and female mice with 100, 500, 4000 and 7000 ppm pydiflumetofen for at least 90-days, resulted in liver weight increases with clinical chemistry and histopathological findings indicative of liver impairment from 500 ppm in males and from 4000 ppm in females.

Therefore, a **NOAEL of 100 ppm (equivalent to 17.56 mg/kg bw/d) in males and 500 ppm (equivalent to 106 mg/kg bw/d) in females** was established from this study. At the LOAEL of 500 ppm in males (equivalent to 81.6 mg/kg bw/d), absolute and relative liver weights were increased by 18% and 14% respectively and increased cholesterol (not statistically significant) was noted. At the LOAEL of 4000 ppm in females (equivalent to 846 mg/kg bw/d), absolute and relative liver weights were increased by 59% and 60% respectively and histopathological correlates (hepatocellular hypertrophy) were noted. Triglyceride concentrations were also increased at the LOAEL in females (not statistically significant).

(██████████, 2015)

B.6.3.2.1 2 Dog

- 90 Day Oral study:

Report:	K-CA 5.3.2/05 ██████████ (2015). SYN545974 - 90 Day Oral (Capsule) Study in the Dog. ██ Laboratory Report No. ██████████, issue date 19 May 2015. Unpublished. Syngenta File No. SYN545974_10207.
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Guidelines: Subchronic Oral Toxicity (non-rodent) (Capsule) (Dog) OECD 409 (1998): OPPTS 870.3150 (1998): 87/302/EEC B.30 (1988): EC 440/2008 (2008): JMAFF, 12 NOUSAN, 8147; (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a subchronic toxicity study, thirty-two beagle dogs were divided into four groups, each of four males and four females and dosed orally, once daily, at 0 (empty gelatin capsules), 30, 300 or 1000 mg/kg/day SYN545974 for at least 90 days.

Animals were examined daily for clinical signs of toxicity. The amount of food consumed was recorded daily and animals were weighed weekly. Ophthalmoscopy examinations were conducted before the start of dosing and again in Week 12. Functional observations were made pen side and in a standard arena, on a weekly basis, from one week before the start of dosing to the end of the treatment period.

Clinical pathology blood and urine samples were taken before the start of treatment and again in Weeks 4, 8 and 13 (blood) or Weeks 6 and 13 (urine). Toxicokinetic blood samples were also taken on Days 1 and 28 and during Week 13.

All animals were necropsied the day after receiving their final dose, with weighing of selected organs and microscopic examination of a comprehensive list of tissues from all animals.

The toxicokinetics of SYN545974 were typically characterised by a supra-proportional increase in exposure relative to the dose increments. Animals that received 300 or 1000 mg/kg/day were continuously exposed over the 24 hour period after dosing, but at 30 mg/kg/day exposure to SYN545974 was limited to up to eight hours after dosing. Exposure tended to be higher in males, most notably at 300 mg/kg/day. There was no evidence of accumulation.

There were no deaths and no test item-related clinical signs during the study.

At 1000 mg/kg/day, two of the four males and all females lost weight during the initial weeks of dosing, with some taking several weeks to regain this lost weight and others, most notably females, continuing to show erratic weight change. Body weight gain was lower for the female group at this dose compared with controls and their final body weight was notably lower than controls. There was no significant

difference in overall weight gain or final body weight for the male group. There was a corresponding effect on food consumption in males and females at 1000 mg/kg/day.

At 300 mg/kg/day, one male and two females showed transient, slight, body weight losses during the early part of the treatment period. In the absence of a consistent and overall effect on body weight and body weight gain, this was considered not adverse.

There were no ocular abnormalities, detailed clinical observations, haematology, urinalysis or macroscopic pathology findings that were considered to be treatment-related.

Increases in alkaline phosphatase activity, plasma triglycerides and liver weight were observed at 300 mg/kg/day and 1000 mg/kg/day. In addition, minimal hepatocyte hypertrophy was observed in both sexes at 1000 mg/kg/day. There were no other changes in clinical chemistry, organ weights or histopathology findings which were considered to be related to treatment with SYN545974. Liver weights were higher than controls in males and/or females given 300 or 1000 mg/kg/day, as demonstrated by the statistical significance of group mean absolute and body weight covariate adjusted values.

Administration of SYN545974 once daily, by capsule, for at least 90 days to the beagle dog, was associated in males and females to reduced body weight gain, lower food consumption, increased liver weight (40%) and blood clinical chemistry (elevated ALP and Triglyceride) and liver histopathological changes (hepatocellular hypertrophy) at the highest dose of 1000 mg/kg bw/d.

At a dose level of 300 mg/kg bw/d in males, effects of treatment included increases in plasma alkaline phosphatase and increases in liver weight (but without corresponding liver histopathological changes). Due to the important liver weight increase (by approximatively 30%) associated with blood clinical chemistry changes observed in males at 300 mg/kg bw/day, the No Observed Adverse Effect Level (NOAEL) was considered to be 30 mg/kg bw/day in males.

At a dose level of 300 mg/kg bw/d in females, effects of treatment included reduction of bw gain (by 50% between day -1 to day 91), increase of liver weight (16-19% relative measures) associated with clinical parameters changes (increased ALP by > 200%); the No Observed Adverse Effect Level (NOAEL) was considered to be 30 mg/kg bw/day in females.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 % w/w
CAS#:	1228284-64-7
Stability of test compound:	30 June 2016

Vehicle and/or positive control: The test substance was administered in gelatin capsules.

Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	Approximately 7 to 8 months/9.8 kg to 13.2 kg (males) and 8.9 kg to 12.6 kg (females)
Source	[REDACTED]
Housing	Group housed in pens with access to at least 4.5 square metres, or individually whilst food intake was measured.
Acclimatisation period	Approximately three weeks
Diet	260 g pelleted diet per dog per day
Water	<i>ad libitum</i>
Environmental conditions	Temperature: between 17 °C and 28 °C* Humidity: 15% to 72% Photoperiod: Alternating 12 hour light and dark cycles.

* Morning temperature on Day 42 of dosing was found to be 28°C in the room housing the males. This reduced to 22°C in the afternoon and was an isolated incident.

Study Design and Methods:

In-life dates: Start: 15 March 2013 (animal arrival)
End: 16 August 2013 (pathology report)

Animal assignment: The study consisted of one control and three treatment groups each containing four male and four female dogs. The animals were distributed amongst experimental groups by means of a randomisation procedure based on body weight in such a way that littermates were, as far as possible, distributed across the groups and similar group mean body weights were obtained. The sexes were randomised separately.

The study design was as follows:

Study design

Group	Number of animals		Animal ID numbers		Dose level (mg/kg/day) SYN545974
	Males	Females	Males	Females	
1	4	4	325-328	341-344	0
2	4	4	329-332	345-348	30
3	4	4	333-336	349-352	300
4	4	4	337-340	353-356	1000

Observations: Animals were examined daily for mortality and clinical signs of toxicity or changes in behaviour and appearance, with a detailed examination completed once each week. In addition, the animals were examined by a veterinary surgeon towards the end of the treatment period. The temperament of the animals was also assessed weekly.

Body weight: The body weight of each animal was recorded weekly.

Food consumption: Food consumption for each animal was determined daily.

Ophthalmoscopic examination: The eyes of all animals were examined before the start of treatment and again during Week 12.

Standard arena observations: Before the start of treatment and then once weekly during the treatment period, a detailed assessment of the behaviour and clinical condition of the animals was made within the home pen and in a standard arena. Observations were made at approximately the same time of day on each occasion (afternoon).

Haematology and clinical chemistry: Blood was collected from all animals by jugular, saphenous or cephalic venepuncture during the acclimatisation period (pre-dose) and during Weeks 4, 8 and 13. The following parameters were analysed:

Haematology and coagulation:

haemoglobin concentration	total leucocyte count
red blood cell count	neutrophils
packed cell volume	lymphocytes
mean cell volume	monocytes
mean cell haemoglobin	eosinophils
mean cell haemoglobin concentration	basophils
reticulocytes	large unstained cells
platelet count	prothrombin time
cell morphology*	activated partial thromboplastin time

*Blood smears were prepared but in the absence of any haematological reasons for doing so were not examined.

Clinical chemistry:

urea	a/g ratio
creatinine	total bilirubin
glucose	gamma glutamyl transpeptidase
alkaline phosphatase	cholesterol
alanine aminotransferase	calcium
aspartate aminotransferase	sodium
total protein	potassium
albumin	chloride
globulin (calculated)	phosphorus
glutamate dehydrogenase	triglycerides
lactate dehydrogenase	creatinine kinase

Urinalysis: Urine was collected by catheterisation from all animals during the acclimatisation period (pre-dose) and during Weeks 6 and 13. The following parameters were examined:

volume (pre-dose and week 6 only)	protein
specific gravity	blood
glucose	colour and appearance
ph	

Toxicokinetics: Exposure to SYN545974 was assessed by the collection of blood on Days 1, 28 and during Week 13 at the following time-points: Pre-dose and 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours after dosing. The following parameters were derived from the blood concentrations of SYN545974:

C_{\max}	the maximum observed blood concentration
T_{\max}	the time of occurrence of C_{\max}
AUC_{0-24}	the area under the blood concentration versus time curve from time zero to 24hr
AUC_{INF}	the area under the blood concentration versus time curve from time zero to infinity, calculated from $AUC_{0-\text{tlast}} + C_t/\lambda_z$, where C_t is the last measurable concentration.
$AUC_{0-\text{tlast}}$	the area under the blood concentration versus time curve from time zero to the last time point with measurable concentrations of the test item
$AUC_{\% \text{EXTRAP}}$	% of AUC_{INF} due to extrapolation from the last time point to infinity
$t_{1/2}$	the apparent terminal half-life, calculated from $\ln 2/\lambda_z$
R_0	accumulation ratio calculated from the individual values of AUC_{0-24} (repeat dose) / AUC_{0-24} (single dose)

Post mortem investigations:

Macroscopic examination: All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	ovaries
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brain	spleen
epididymides	testes
heart	thymus
kidneys	thyroids (including parathyroids)
liver	uterus (with cervix and oviducts)

Paired organs were weighed together.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

adrenal glands	pancreas
aorta	<i>pharynx</i>
<i>bone marrow smear</i>	pituitary
brain (5 levels examined)	prostate
caecum	rectum
colon	salivary gland (submandibular)
duodenum	salivary gland (sublingual)
epididymides	salivary gland (parotid)
eyes (including optic nerves)	sciatic nerve
<i>femur (including marrow)</i>	site of mammary gland
gall bladder	skeletal muscle
heart	skin
<i>animal identification (skin tattoo)</i>	spinal cord (3 levels examined)
ileum (including Peyer's patch)	spleen
jejunum	sternum (including bone marrow)
kidneys	stomach
<i>larynx</i>	testes
liver	thymus
lungs (including mainstem bronchi)	thyroids (including parathyroids)
mesenteric lymph node	trachea
<i>nasal passages</i>	urinary bladder
oesophagus	uterus (including uterine cervix and oviducts)
ovaries	vagina
	all gross lesions

Microscopic examination: With the exception of the tissues in *italics* above, all processed tissues were examined by light microscopy.

Frozen liver samples: Samples from the liver of all animals were retained and frozen by snap-freezing in liquid nitrogen, with or without RNA-Later solution. Samples were not analysed as part of this study and are stored frozen pending possible future analysis.

Statistics: Data were processed to give group mean values and standard deviations, where appropriate. Where the data allowed, the following methods were used for statistical analysis.

General Approach: All statistical tests were two-sided. Probability values of less than 5 % were regarded as providing sufficient evidence to reject the null hypothesis and therefore statistical significance was identified at the $p < 0.05$ level. For illustrative purposes, significance levels of $p < 0.01$ was also noted.

Non-parametric statistics were not routinely conducted. When used, Dunnett's test was conducted regardless of the outcome of the analysis of variance (ANOVA) or analysis of covariance (ANCOVA). Data were examined for unusually high or low values which could influence the statistical analysis and interpretation (possible outliers). After examining for any outliers, if the variances are clearly heterogeneous, transformations (e.g. log, square root or, where applicable, double arcsine) were used in an attempt to stabilise the variances. If the transformations failed, the data set was examined for possible outliers.

Quantitative Data: Body weight, cumulative body weight gain from the start of dosing, food intake, haematology, coagulation, clinical chemistry, quantitative urinalysis values (e.g. specific gravity) and absolute organ weights were analysed using ANOVA and pairwise analysis by Dunnett's test.

Organ weights were also analysed by analysis of covariance (ANCOVA) with final body weight as the covariate. Organ to body weight ratios are presented but not be analysed statistically.

Macroscopic and microscopic pathology: The incidence of macroscopic and microscopic findings were analysed using Fisher's Exact Test.

Outliers: Exclusion of individual outlier values was considered unnecessary as it was decided that the data reflected variation expected for non-rodent study data. The decision not to exclude these values was also considered not to have affected the scientific interpretation of the study findings.

Dunnett's test: For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the final report.

RESULTS

Mortality: There were no deaths in the study.

Clinical observations: There were no treatment-related clinical signs, with those that were observed representing background signs commonly seen in laboratory maintained beagles. Furthermore, veterinary examination at the end of the treatment period did not identify an effect of treatment on the clinical condition of the animals.

Bodyweight and weight gain: Two of the four males and all of the females given 1000 mg/kg/day lost weight over the first week of dosing. Some of these animals regained their lost weight after several weeks, although others, most notably females, continued to show erratic body weight change. This resulted in a lower mean body weight gain for the female group given 1000 mg/kg/day compared with controls ($p < 0.05$ or $p < 0.01$) and a notably lower final body weight than all other groups, including controls, but the difference did not achieve statistical significance. Although two of the four males lost weight initially, there was no significant difference in overall group mean body weight gain or final body weight for the males (Table 6.3.2-19).

One male and two females given 300 mg/kg/day showed slight body weight losses during the early part of the treatment period. The effect on group mean body gain for the females achieved statistical significance when compared to controls (Week 1 to 2: $p < 0.05$). Although the affected females gained weight during the latter part of the treatment period, the overall body weight gain for the group was slightly lower than the controls. There were no effects on overall group mean body weight or body weight gain for males. There was no effect on body weight at 30 mg/kg/day.

Table 6.3.2-19: Intergroup comparison of bodyweight and weight change - selected timepoints (kg)

Body weight (kg)	Group/sex							
	Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 300 mg/kg/day		Group: 4 SYN545974 1000 mg/kg/day	
	M	F	M	F	M	F	M	F

-1	11.45	10.48	11.73	10.08	11.55	10.53	11.78	10.85
1	11.50	10.55	11.93	10.20	11.55	10.45	11.68	10.48
3	11.65	10.75	12.15	10.45	11.55	10.40	11.78	10.53
5	11.53	10.95	12.05	10.58	11.63	10.50	11.73	10.35
7	11.63	11.28	12.35	10.93	11.93	10.70	11.90	10.58
9	11.78	11.43	12.43	11.18	11.85	11.10	12.03	10.60
11	11.78	11.53	12.45	11.20	11.90	11.13	12.03	10.30
13	11.68	11.63	12.58	11.43	12.03	11.10	11.90	10.83
Weight gain (kg)								
-1 to 7	0.05	0.08	0.20	0.13	0.00	-0.08	-0.10	-0.38*
-1 to 14	0.15	0.18	0.20	0.23	-0.05	-0.15**	-0.10	-0.38*
-1 to 21	0.20	0.28	0.43	0.38	0.00	-0.13	0.00	-0.33*
-1 to 28	0.18	0.40	0.28	0.45	0.00	-0.03	-0.05	-0.45*
-1 to 35	0.08	0.48	0.33	0.50	0.08	-0.03	-0.05	-0.50*
-1 to 42	0.15	0.68	0.48	0.68	0.15	0.13	0.03	-0.28*
-1 to 49	0.18	0.80	0.63	0.85	0.38	0.18	0.13	-0.28*
-1 to 56	0.23	0.83	0.38	0.85	0.05	0.30	-0.20	-0.33*
-1 to 63	0.33	0.95	0.70	1.08	0.30	0.45	0.25	-0.13*
-1 to 70	0.33	0.95	0.75	1.10	0.35	0.58	0.30	-0.25*
-1 to 77	0.33	1.05	0.73	1.13	0.35	0.60	0.25	-0.55*
-1 to 84	0.35	1.23	0.83	1.30	0.43	0.63	0.25	-0.10**
-1 to 91	0.23	1.15	0.85	1.35	0.48	0.58	0.13	-0.03**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Food consumption: Males and females given 1000 mg/kg/day typically consumed less food than the controls over the dosing period. This was most apparent for two of the four females, that did not completely consume their ration (approximately 70 %) over the majority of the treatment period (Week 1 to 13). The group mean total intake for males and females given 1000 mg/kg/day was also statistically significantly lower than controls for the overall dosing period.

There was no effect on the food intake of animals given 30 or 300 mg/kg/day, with differences in the amount of food consumed reflecting normal variation for the beagle dog.

The group mean values for overall food intake are shown in Table 6.3.2-20 below:

Table 6.3.2- 20: Overall Food Intake - Group Mean Values

Food Total Intake (Week Number)		Group/sex							
		Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 300 mg/kg/day		Group: 4 SYN545974 1000 mg/kg/day	
		M	F	M	F	M	F	M	F
1 to 13	Mean	23720.0	23324.0	23689.5	22985.3	23647.0	22263.5	22363.0^{d1}	19003.3^{d1}
	SD	0.0	672.0	61.0	1018.5	146.0	1569.3	1133.1	2948.4
	N	4	4	4	4	4	4	4	4

1 [d - Test: Dunnett 2 Sided $p < 0.05$]

Standard Arena Observations (Functional Observations): There was no indication of neurotoxicity from observation of the animals at the cage side, on removal from the home pen or within a standard arena.

Ophthalmoscopic examination: There were no ocular changes following SYN545974 administration for at least 12 weeks.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: Alkaline phosphatase activity was markedly increased in males and females that received 300 or 1000 mg/kg/day SYN545974, when compared with controls and individual pre-dose levels. The effect was most pronounced in the high dose group, with individual values up to four-fold higher than the normal range (ALP: 39 to 161 U/l for males, 21 to 169 U/l for females).

There was no effect of treatment on alkaline phosphatase activity in animals given 30 mg/kg/day and all individual values were within the ranges seen in the concurrent control group.

Triglyceride concentrations were higher for males at 300 and 1000 mg/kg/day and females given 1000 mg/kg/day compared to controls and pre-dose values, and on occasion achieved statistical significance. The effect persisted throughout the study, but showed variability between individual animals.

Group mean alkaline phosphatase activities are shown in Table 6.3.2-21 with group mean triglyceride concentrations detailed in Table 6.3.2-22.

Table 6.3.2- 21: Alkaline Phosphatase - Group Mean Values

ALP U/l (Week Number)		Group/sex							
		Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 300 mg/kg/day		Group: 4 SYN545974 1000 mg/kg/day	
		M	F	M	F	M	F	M	F
-1	Mean	73.5	100.5	81.5	83.8	89.3	94.5	71.5	88.5
	SD	19.8	32.3	10.4	12.8	16.2	35.9	23.9	23.0
	N	4	4	4	4	4	4	4	4
4	Mean	71.5	94.0	85.8	91.0	251.8	214.3	349.0 ^{dd2}	328.3 ^{dd1}
	SD	25.6	25.9	5.2	23.1	86.6	79.8	167.8	149.3
	N	4	4	4	4	4	4	4	4
8	Mean	74.0	86.0	86.0	87.3	286.8	247.5	379.0 ^{d1}	374.3 ^{dd1}
	SD	28.1	31.4	8.7	20.0	134.7	89.0	198.1	195.7
	N	4	4	4	4	4	4	4	4
13	Mean	68.5	77.8	70.0	76.8	252.3 ^{dd2}	236.5	384.3 ^{dd2}	327.8 ^{d2}
	SD	24.0	26.7	6.3	17.0	132.9	99.7	203.8	193.9
	N	4	4	4	4	4	4	4	4

1 [d - Test: Dunnett 2 Sided p < 0.05]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

Table 6.3.2-22: Triglycerides - Group Mean Values

Trigs mg/dl (Week Number)		Group/sex			
		Group: 1 Control	Group: 2 SYN545974	Group: 3 SYN545974	Group: 4 SYN545974

		0 mg/kg/day		30 mg/kg/day		300 mg/kg/day		1000 mg/kg/day	
		M	F	M	F	M	F	M	F
-1	Mean	28.5	38.8	34.8	42.0	36.5	38.8	33.5	32.3
	SD	3.1	7.1	8.6	1.4	9.5	5.0	8.8	2.9
	N	4	4	4	4	4	4	4	4
4	Mean	28.3	50.3	35.8	54.8	68.3^{dd1}	51.8	96.8^{dd1}	79.3
	SD	1.7	12.3	5.7	14.9	20.0	7.7	23.5	28.3
	N	4	4	4	4	4	4	4	4
8	Mean	26.3	44.5	31.0	58.0	41.3	54.3	67.3^{dd1}	71.0
	SD	2.1	6.1	4.8	13.3	9.9	9.8	29.7	29.8
	N	4	4	4	4	4	4	4	4
13	Mean	30.3	44.8	29.0	52.8	51.0	47.5	74.0^{d2}	61.5
	SD	6.2	9.4	5.2	21.9	10.9	8.1	37.4	3.1
	N	4	4	4	4	4	4	4	4

1 [dd - Test: Dunnett 2 Sided p < 0.01]

2 [d - Test: Dunnett 2 Sided p < 0.05]

Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

Toxicokinetics: Animals given 300 or 1000 mg/kg/day SYN545974 were continuously exposed to SYN545974 over the 24 hours following dosing, whereas SYN545974 was typically detected up to eight hours after dosing for animals given 30 mg/kg/day. Group mean elimination half-lives were found to vary from 2.4 to 4.9 hours. T_{max} typically occurred 1 to 2, 2 to 4 or 4 to 8 hours after dosing for animals receiving 30, 300 or 1000 mg/kg/day, respectively; however, for some animals T_{max} occurred as late as 12 hours.

Following a single dose, increases in peak (C_{max}) and total (AUC_{0-24}) systemic exposure were supra-proportional in relation to dose. With the exception of females given 300 mg/kg/day, a supra-proportional increase in SYN545974 exposure continued with repeat administration, but was less prominent than on Day 1.

Exposure to SYN545974 tended to be higher in males, most notably at 300 mg/kg/day. There was no consistent evidence of accumulation over the treatment period.

No SYN545974 was detected in the blood of control animals.

Sacrifice and pathology:

Organ weights: Liver weights were higher than controls in males and females given 300 or 1000 mg/kg/day, as demonstrated by the statistical significance of group mean absolute and body weight covariate adjusted values. This was most pronounced in the high dose group, with group mean adjusted liver weights approximately 40 % higher than the concurrent control group. At 300 mg/kg bw/d, liver weights were also statistically higher than the concurrent control group (34% and 31% for mean absolute and body weight adjusted values, respectively) but only for the males. There was no effect of treatment on liver weight at 30 mg/kg/day (Table 6.3.2-23).

The adjusted group mean epididymides weight was significantly lower than controls for the males given 30 mg/kg/day. In the absence of a decrease in adjusted epididymides weight at higher dose levels, the outcome of the statistical analysis was considered to be incidental and unrelated to treatment (Table 6.3.2-23).

There were no statistical significant changes observed in mean thyroid weights (absolute and body weight covariate adjusted values) both in males and females. An increase by 22% compared to control was observed for mean relative thyroid weight in females receiving the dose of 1000 mg/kg/day (no statistical analysis performed). However, it should be noted that no histological findings were seen in thyroids irrespective of the administered dose of SYN545974.

Table 6.3.2-23: Organ Weights - Absolute, Adjusted and % Body Weight - Group Mean Values

Organ weights (kg/g) (Week Number)		Group/sex							
		Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 300 mg/kg/day		Group: 4 SYN545974 1000 mg/kg/day	
		M	F	M	F	M	F	M	F
Terminal bodyweight (kg)	Mean	11.8	11.7	12.6	11.4	12.2	11.1	12.1	11.0
	SD	1.0	1.3	0.1	0.8	1.4	1.5	1.0	1.7
	N	4	4	4	4	4	4	4	4
Liver (g) (absolute)	Mean	367.5	369.9	393.2 (+7%)	358.9	492.9^{d1} (+34%)	408.4 (+10%)	527.6^{dd2} (+44%)	511.6^{d1} (+38%)
	SD	50.5	49.2	63.2	64.5	32.8	20.5	78.6	95.5
	N	4	4	4	4	4	4	4	4
Liver (g) (covariate)	Mean	375.7	356.6	382.7 (+2%)	355.9	492.9^{d1} (+31%)	414.3 (+16%)	529.9^{dd2} (+41%)	522.0^{dd2} (+46%)
	N	4	4	4	4	4	4	4	4
Liver (%) (relative to body weight)†	Mean	3.099	3.143	3.114	3.139	4.088 (+32%)	3.748 (+19%)	4.372 (+41%)	4.660 (+48%)
	SD	0.234	0.091	0.496	0.438	0.539	0.678	0.586	0.279
	N	4	4	4	4	4	4	4	4
Thyroid (g) (absolute)	Mean	0.87	0.91	0.99	0.91	1.02	0.90	0.99 (+14%)	1.04 (+14%)
	SD	0.14	0.13	0.13	0.16	0.22	0.04	0.16	0.24
	N	4	4	4	4	4	4	4	4
Thyroid (g) (covariate)	Mean	0.89	0.90	0.97	0.90	1.02	0.91	1.0 (+12%)	1.04 (+16%)
	N	4	4	4	4	4	4	4	4
Thyroid (%) (relative to body weight)†	Mean	0.0074	0.0078	0.0078	0.0079	0.0084	0.0083	0.0082 (+11%)	0.0095 (+22%)
	SD	0.0013	0.0018	0.0010	0.0010	0.0017	0.0013	0.0013	0.0021
	N	4	4	4	4	4	4	4	4
Epididymides (g) (absolute)	Mean	4.52		3.99		4.21		4.37	
	SD	0.42		0.46		0.53		0.33	
	N	4		4		4		4	
Epididymides (g) (covariate)	Mean	4.59		3.89 ^{d1}		4.21		4.39	
	N	4		4		4		4	
Epididymides (%) (relative to body weight)	Mean	0.0384		0.0316		0.0346		0.0362	
	SD	0.0048		0.0038		0.0029		0.0004	
	N	4		4		4		4	

1 [d - Test: Dunnett 2 Sided p < 0.05]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

† No statistical analysis performed

Macroscopic findings: There were no treatment-related macroscopic findings in dogs given SYN545974, with all macroscopic findings consistent with changes typically seen in laboratory beagles of this age.

Microscopic findings: The only treatment-related finding was minimal hepatocyte hypertrophy in the liver of all males and females given SYN545974 at 1000 mg/kg/day.

There were no treatment-related microscopic changes in dogs given 30 or 300 mg/kg/day.

Table 6.3.2-24: Intergroup comparison of selected microscopic findings

		Dietary Concentration (mg/kg/day)							
		Males				Females			
		0	30	300	1000	0	30	300	1000
LIVER (number examined)		4	4	4	4	4	4	4	4
Hepatocyte hypertrophy centrilobular	minimal	0	0	0	4*	0	0	0	4*

* Statistically significant difference from control group mean, $p < 0.05$

CONCLUSION:

In a 90-day repeated dose toxicity study conducted according to GLP and OECD TG 409 (1998), four male and four female Beagle dogs were administered doses of 0, 30, 300 or 1000 mg/kg bw/d pydiflumetofen *via* oral capsule. Systemic exposure to pydiflumetofen was assessed by the collection of blood on Days 1, 28 and during Week 13 at the following time-points: pre-dose and 0.5, 1, 1.5, 2, 4, 8, 12 and 24 hours after dosing.

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

In the kinetic investigations, animals given 300 or 1000 mg/kg bw/d pydiflumetofen were continuously exposed to the test substance over the 24 hours following dosing, whereas pydiflumetofen was typically detected up to 8 hours after dosing for animals given 30 mg/kg bw/d. Group mean elimination half-lives were found to vary from 2.4 to 4.9 hours. T_{max} typically occurred 1 to 2, 2 to 4 or 4 to 8 hours after dosing for animals receiving 30, 300 or 1000 mg/kg bw/d, respectively; however, for some animals T_{max} occurred as late as 12 hours. Following a single dose, increases in peak (C_{max}) and total (AUC_{0-24}) systemic exposure were supra-proportional in relation to dose. With the exception of females given 300 mg/kg bw/d, a supra-proportional increase in pydiflumetofen systemic exposure continued with repeat administration, but was less prominent than on Day 1. Systemic exposure to pydiflumetofen tended to be higher in males, most notably at 300 mg/kg bw/d. There was no consistent evidence of accumulation over the treatment period. No test substance was detected in the blood of control animals.

Two males at 1000 mg/kg bw/d lost weight during week one of dosing. Overall weight gain was sporadic thereafter, resulting in the initial weight losses not being fully recovered. The overall body weight gain for high dose males at the end of the study (days 1-91) was 43% lower than controls (not statistically significant). There was no effect on overall mean body weights in males at the high dose and body weight development was not affected in males at the lower doses (one male at 300 mg/kg bw/d lost weight during the initial part of the study but the overall group means for body weight and body weight gain were not affected). Mean food consumption was statistically significantly lower than controls for males at 1000 mg/kg bw/d (-6%).

Four females lost weight during week one and body weight losses were recorded for high dose females at all time points thereafter. This resulted in a final overall body weight gain for high-dose females that

was statistically significantly lower than controls (females at 1000 mg/kg bw/d lost 0.03 kg during weeks 1-91 compared with gains of 1.15, 1.35 and 0.58 kg at 0, 30 and 300 mg/kg bw/d). Final mean body weights of high dose females were also lower than controls (7% at week 13); however, statistical significance was not achieved. At 300 mg/kg bw/d two females lost weight during the initial part of the study. (statistically significant during weeks 1-2), resulting in lower overall lower body weight gains for females at this dose (-50%), although the change was not statistically significant and overall group mean body weights were not affected. Food consumption was statistically significantly lower than controls for females at 1000 mg/kg bw/d (-19%), largely driven by two females that consumed approximately 70% of the allocated food during weeks 1-13.

The liver was the main target organ in males and females.

With regard to clinical chemistry parameters, alkaline phosphatase (ALP) was increased in males and females at 300 and 1000 mg/kg bw/d, in comparison with both the controls and the pre-dosing measurements. ALP measurements at the end of the study were approximately 4-fold and 3-fold higher than controls in males and females respectively at 1000 mg/kg bw/d and approximately 2.5-fold greater in both sexes at 300 mg/kg bw/d (not statistically significant in females). Triglyceride concentrations were also higher than controls in males from 300 mg/kg bw/d and in females at 1000 mg/kg bw/d.

At 1000 mg/kg bw/d absolute and relative liver weights were statistically significantly increased in comparison with controls by 44% and 48% in males and 38% and 48% in females. At 300 mg/kg bw/d absolute and relative liver weights were increased by 34% and 32% in males and by 10% and 19% in females, although statistical significance was not reached in females. Other organ weight changes (epididymides, thyroid) were not statistically significant, showed no dose response, were related to body weight reductions and/or had no histopathological correlates and were therefore incidental and not related to treatment.

Minimal hepatocyte hypertrophy was seen in the livers of all males and females at 1000 mg/kg bw/d (there were no incidences in the controls or lower dose groups).

Overall, administration of pydiflumetofen to beagle dogs at doses of 0, 30, 300 and 1000 mg/kg bw/d resulted in impaired body weight development from 300 mg/kg bw/d in females and at 1000 mg/kg bw/d in males. Additionally, increased liver weights from 300 mg/kg bw/d in both sexes with histopathological correlates at 1000 mg/kg bw/d and clinical chemistry changes (increased ALP and triglycerides) from 300 mg/kg bw/d were noted.

Therefore, a **NOAEL of 30 mg/kg bw/d in males and females** is proposed from this study. At the LOAEL of 300 mg/kg bw/d in females, body weight gain was decreased (-50%, days 1-91, not statistically significant) and liver weights were increased (19% relative and 10% absolute, not statistically significant) with associated clinical chemistry changes (approximate 2-fold increase in ALP, not statistically significant). At the LOAEL of 300 mg/kg bw/d in males, liver weights were increased (34% absolute and 32% relative) with associated clinical chemistry changes (approximate 2-fold increase in ALP and transient increases in triglyceride concentrations).

HSE notes that the effects at the LOAEL in females did not reach statistical significance; however, owing to the low number of animals tested (4/sex/dose), the statistical power is somewhat reduced, and the lack of statistical significance does not exclude a relation to treatment.

(██████, 2015)

- 1-year Oral study:

There is no longer a requirement in the EU for the 52 week toxicity study in the dog, however, as the study is required in other regions, the study was conducted and included here for completeness.

Report:	K-CA 5.3.2/06. [REDACTED] (2015a). SYN545974 - 52 Week Oral (Capsule) Toxicity Study in the Dog. [REDACTED] [REDACTED]. Laboratory Report No. [REDACTED], issue date 19 May 2015. Unpublished. Syngenta File No. SYN545974_10205.
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Guidelines: Chronic Oral Toxicity (non-rodent) (Capsule) (Dog) OECD 452 (2009): OPPTS 870.4100 (1998): JMAFF, 12 – NohSan No. 8147 (2000)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a chronic toxicity study, thirty-two beagle dogs were divided into four groups, each of four males and four females, and dosed orally once daily at 0 (empty gelatin capsules), 30, 100 or 300 mg/kg/day SYN545974 for 52 weeks.

Animals were observed for mortality and morbidity daily, weighed weekly and food intake was recorded daily from arrival. More detailed clinical observations were made during the treatment period. Blood and urine samples were collected for clinical pathology once pre-dose and then again in Weeks 13 (blood only), 26 and 52. Further blood samples were taken in Weeks 26 and 52, at 24 hours after dosing, for possible proof of absorption analysis. The eyes of all animals were examined pre-dose and again in Week 52.

At the end of the treatment period all animals were necropsied, selected organs were weighed and a comprehensive list of tissues was examined microscopically from all animals. Liver samples were also taken and frozen for possible exploratory analysis by the Sponsor.

There were no deaths and no treatment-related clinical signs or ocular changes were observed during the study. There was no evidence of an adverse effect of treatment on either food intake or body weight.

Plasma alkaline phosphatase activity at 300 mg/kg/day was significantly higher than controls at all time-points after dose initiation. There were no other blood chemistry changes nor were there any test item-related haematological or urine composition changes.

At necropsy, there were no treatment-related macroscopic abnormalities. Group mean liver weights were significantly higher than controls at 300 mg/kg/day (+35%). Other organ weights were significantly higher than controls, however in the absence of histopathological changes in all organs, these increases were considered not adverse.

There were no histopathological changes, in particular any evidence of hepatic hypertrophy, in the animals given 300 mg/kg /day.

When administered orally for 52 weeks to the beagle dog at doses of 30, 100 or 300 mg/kg/day, SYN545794 elicits in males increases in liver weight (34-35% relative measures), in thyroid weight (52-54% relative measures) and blood clinical changes (3.8 fold increased in plasma alkaline phosphatase) at 300 mg/kg/day, but without histopathological changes. In females, SYN545794 elicits increase in liver weight (28-31% relative measures) associated with a strong increase of plasma alkaline phosphatase (3 fold). Thus, the No Observed Adverse Effect Level (NOAEL) is 100 mg/kg bw/day for males and females.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545794
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 % w/w
CAS#:	Not reported
Stability of test compound:	30 June 2016

Vehicle and/or positive control: The test substance was administered in gelatine capsules.

Test Animals:	
Species	Dog
Strain	Beagle
Age/weight at dosing	Approximately 6 months/7.8 kg to 11.2 kg (males) and 6.4 kg to 8.8 kg (females)
Source	[REDACTED]
Housing	Group housed in pens with access to at least 4.5 square metres, or individually whilst food intake was measured.
Acclimatisation period	Approximately three weeks
Diet	260 g pelleted diet per dog per day
Water	<i>ad libitum</i>
Environmental conditions	Temperature: between 14 °C and 26 °C Humidity: 20 % to 83 % Photoperiod: Alternating 12 hour light and dark cycles.

Study Design and Methods:

In-life dates: Start: 11 October 2013 (animal arrival)
End: 25 February 2015 (pathology report)

Animal assignment: The study consisted of one control and three treatment groups each containing four male and four female dogs. The animals were distributed amongst experimental groups by means of a randomisation procedure based on body weight in such a way that littermates were, as far as possible, distributed across the groups and similar group mean body weights were obtained. The sexes were randomised separately.

The study design was as shown below:

Study design

Group	Number of animals		Animal ID numbers		Dose level (mg/kg/day) SYN545974
	Males	Females	Males	Females	
1	4	4	202 - 205	218 - 221	0
2	4	4	206 - 209	222 - 225	30
3	4	4	210 - 213	227 - 229, 234	100
4	4	4	214 - 217	230 - 233	300

Observations: Animals were examined twice daily for mortality and morbidity and were given a detailed clinical examination once each week from arrival. Where the clinical condition of an animal gave cause for concern, monitoring was adjusted accordingly and, if necessary, the animal was isolated to prevent further deterioration. In addition, the animals were examined by a veterinary surgeon towards the end of the treatment period and found to be unremarkable.

Body weight: Body weights were recorded on arrival and then at weekly intervals throughout the study. Where animals lost weight, additional body weights were recorded.

Food consumption: The amount of food consumed by each animal was recorded daily throughout the study and presented weekly.

Ophthalmoscopic examination: The eyes of all animals were examined before the start of treatment and again during Week 52.

Haematology and clinical chemistry: Blood samples were collected from all animals once during the acclimatisation period and again during Weeks 13, 26 and 52. Samples were taken by jugular venepuncture, or from the cephalic veins if necessary. The following parameters were analysed:

Haematology and coagulation:

haemoglobin concentration	total leucocyte count
red blood cell count	neutrophils
packed cell volume	lymphocytes
mean cell volume	monocytes
mean cell haemoglobin	eosinophils
mean cell haemoglobin concentration	basophils
reticulocytes	large unstained cells
platelet count	prothrombin time
cell morphology	activated partial thromboplastin time
red cell distribution width	

Clinical chemistry:

urea	A/G ratio
creatinine	total bilirubin
glucose	gamma glutamyl transpeptidase
alkaline phosphatase	cholesterol

alanine aminotransferase	calcium
aspartate aminotransferase	sodium
total protein	potassium
albumin	chloride
globulin (calculated)	phosphorus
glutamate dehydrogenase	triglycerides
lactate dehydrogenase	creatinine kinase
bile acids (weeks 26 and 52 only)	

Urinalysis: Urine samples were collected from all animals by catheterisation during the acclimatisation period (pre-dose) and again during Weeks 26 and 52. The following parameters were examined:

appearance	colour
specific gravity	leucocytes*
glucose	erythrocytes*
bilirubin	crystals*
ketones	debris*
ph	casts*
protein	epithelial cells*
blood	urobilinogen*

* Weeks 26 and 52 only.

Proof of absorption: Blood samples were collected from all animals in Weeks 26 and 52 at 24 hours after dosing, for possible proof of absorption analysis. These samples were not analysed as part of this study and are stored frozen pending possible future analysis.

Investigations *post mortem*:

Macroscopic examination: All animals were examined *post mortem*. This involved an external observation and an internal examination of all organs and structures.

Organ weights: From all animals surviving to scheduled termination, the following organs were removed, trimmed free of extraneous tissue and weighed:

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	thymus
kidneys	thyroids (including parathyroids)
liver	uterus
pituitary	prostate

Paired organs were weighed together.

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in an appropriate fixative:

adrenal glands	pharynx
aorta	pituitary
brain (5 levels examined)	prostate
caecum	rectum
colon	sciatic nerve

duodenum	site of mammary gland
epididymides	skeletal muscle
eyes (including optic nerves)	skin
femur (including marrow)	spinal cord (3 levels examined as transverse and longitudinal sections)
gall bladder	
heart	spleen
ileum (including peyer's patch)	sternum (including bone marrow)
jejunum	stomach
kidneys	submandibular lymph node
lacrimal glands	testes
larynx	thymus
liver	thyroids (including parathyroids)
lungs (including mainstem bronchi)	trachea
mesenteric lymph nodes	tongue
<i>nasal passages</i>	urinary bladder
oesophagus	uterus (including uterine cervix and oviducts)
ovaries	vagina
pancreas	all gross lesions
salivary gland (submandibular)	

Microscopic examination: With the exception of the tissues in *italics* above, all processed tissues were examined by light microscopy.

Frozen liver samples: Samples from the liver of all animals were retained and frozen by snap-freezing in liquid nitrogen, with or without RNA-Later solution. Samples were not analysed as part of this study and are stored frozen pending possible future analysis.

Statistics: Data were processed to give group mean values and standard deviations, where appropriate. Where the data allowed, the following methods were used for statistical analysis:

General Approach: All statistical tests were two-sided. Probability values of less than 5 % were regarded as providing sufficient evidence to reject the null hypothesis and therefore statistical significance was identified at the $p < 0.05$ level. For illustrative purposes, significance levels of $p < 0.01$ was also noted.

Non-parametric statistics were not routinely conducted. When used, Dunnett's test was conducted regardless of the outcome of the analysis of variance (ANOVA) or analysis of covariance (ANCOVA).

Data were examined for unusually high or low values which could influence the statistical analysis and interpretation (possible outliers). After examining for any outliers, if the variances are clearly heterogeneous, transformations (e.g. log, square root or, where applicable, double arcsine) were used in an attempt to stabilise the variances. If the transformations failed, the data set was examined.

Quantitative Data: Body weight, cumulative body weight gain from the start of dosing, food intake, haematology, coagulation, clinical chemistry, quantitative urinalysis values (e.g. specific gravity) and absolute organ weights were analysed using ANOVA and pairwise analysis by Dunnett's test.

Organ weights were also analysed by analysis of covariance (ANCOVA) with final body weight as the covariate. Organ to body weight ratios are presented but not be analysed statistically.

Macroscopic and microscopic pathology: The incidence of macroscopic and microscopic findings were analysed using Fisher's Exact Test.

Outliers: Exclusion of individual outlier values was considered unnecessary as it was decided that the data reflected variation expected for non-rodent study data. The decision not to exclude these values was also considered not to have affected the scientific interpretation of the study findings.

Dunnett's test: For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the final report.

RESULTS

Mortality: There were no deaths in the study.

Clinical observations: There were no treatment related clinical signs, with most (occasional emesis or passage of loose faeces and excessive salivation), representing background signs in orally dosed, laboratory maintained, beagles.

A notable incidental finding was an episode of tonic convulsions, of longer than 5 minutes duration, on Day 112 in one female given 100 mg/kg/day. Given the lack of convulsions in other animals from this group or the higher (300 mg/kg/day), dose group, this episode is considered to have been an idiopathic response. There were no subsequent convulsions in this animal, although she did show tremors and unsteady gait on Day 329.

Bodyweight and weight gain: There were no adverse effects of treatment on body weight. Although group mean body weights and body weight gains for the SYN545974 treated groups were generally lower than the concurrent controls, there was no dose response, with the difference most apparent for low dose group (30 mg/kg/day). These inter-group differences were considered to reflect a high degree of body weight variation in individual animals; for example, the body weight of the control males at the end of the study ranged from 11.4 kg to 16.0 kg. Consequently, when group mean differences from control values achieved statistical significance it was considered to reflect this large variation and not represent an adverse effect of SYN545974 administration.

Table 6.3.2-25: Intergroup comparison of bodyweight and weight change - selected timepoints (kg)

Body weight (kg)	Group/sex							
	Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 100 mg/kg/day		Group: 4 SYN545974 300 mg/kg/day	
	M	F	M	F	M	F	M	F
1	9.63	7.65	9.33	7.18	9.65	7.85	9.95	7.83
11	11.23	8.88	10.18	7.45	10.65	9.08	11.18	9.03
18	11.80	9.30	10.35	7.70*	10.58	9.48	11.55	9.15
25	12.30	9.38	10.53	7.98	10.98	9.23	11.68	9.43
32	12.55	9.65	10.63	8.13	11.18	9.48	11.83	9.33
39	12.75	9.98	10.73	8.40	11.48	10.18	11.85	9.55
46	13.08	10.30	10.88	8.43	11.83	10.18	12.05	9.65
53	13.30	10.40	10.90	8.60	11.85	10.00	12.25	9.48
Weight gain (kg)								
-1 to 7	1.25	0.98	0.30*	0.38	0.68	1.13	0.75	1.20
-1 to 14	2.05	1.65	0.58**	0.55*	1.03*	1.65	1.30	1.50
-1 to 21	2.43	1.75	0.95*	0.63	1.10**	1.53	1.58	1.53
-1 to 28	2.95	2.13	1.00*	0.95	1.55*	1.63	1.78**	1.73
-1 to 35	3.48	2.23	1.43*	1.20	1.98**	2.03	2.13**	1.70

-1 to 42	3.58	2.63	1.35*	1.28	2.23	2.55	1.98**	1.98
-1 to 49	3.65	2.85	1.50*	1.25	2.30	2.38	2.18	1.83
-1 to 52	3.68	2.75	1.30*	1.45	2.15	2.43	2.15	1.85

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Food consumption and utilisation: Food intake was unaffected by SYN545974 treatment.

Ophthalmoscopic examination: There were no treatment related ocular abnormalities or changes, the only new finding in the treatment period being a unilateral lens opacity in a control male.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: Group mean plasma alkaline phosphatase (ALP) activities in males and females given 300 mg/kg/day were significantly higher than controls at Week 13, 26 and 52. One male and one female consistently had the highest ALP activities at all time-points. Increases in plasma ALP have been associated with induction of a dog-specific ALP isoenzyme in response to some compounds, which may account for the increase seen in some animals. Such a response was considered to be adaptive and non-adverse by the applicant.

ALP activities were not increased at 100 mg/kg/day, with the exception of one male whose alkaline phosphatase activity was increased throughout the treatment period when compared with pre-dose values.

There were no effects on ALP activity in males or females that received 30 mg/kg/day, with individual values within the normal range during the treatment period (0 to 180 U/l for males and 9 to 158 U/l for females).

Group mean ALP activities are shown below:

Table 6.3.2-26: Alkaline Phosphatase - Group Mean Values

ALP U/l (Week Number)		Group/sex							
		Group: 1 Control 0 mg/kg/day		Group: 2 SYN545974 30 mg/kg/day		Group: 3 SYN545974 100 mg/kg/day		Group: 4 SYN545974 300 mg/kg/day	
		M	F	M	F	M	F	M	F
-1	Mean	103.8	81.8	114.0	101.5	105.0	78.8	100.0	98.3
	SD	17.0	4.6	40.9	40.8	15.4	23.6	12.8	18.9
	N	4	4	4	4	4	4	4	4
13	Mean	56.8	52.8	98.8	71.8	104.8	58.5	200.5 ^{dd1}	144.3 ^{d1}
	SD	8.8	16.7	23.5	11.5	51.8	16.0	77.2	69.0
	N	4	4	4	4	4	4	4	4
26	Mean	45.3	43.8	83.5	63.8	109.0	55.5	173.5 ^{dd1}	133.5 ^{d1}
	SD	11.3	18.2	19.9	15.4	67.8	22.9	64.5	70.7
	N	4	4	4	4	4	4	4	4
52	Mean	38.3	40.3	66.5	65.0	92.3	61.3	146.5 ^{d2}	125.5 ^{d1}
	SD	11.2	27.2	20.7	9.6	54.6	26.6	64.5	75.2
	N	4	4	4	4	4	4	4	4

1 [dd - Test: Dunnett 2 Sided $p < 0.01$] 2 [d - Test: Dunnett 2 Sided $p < 0.05$]

Urinalysis: There were no differences in urine clinical chemistry parameters which were considered to be related to treatment.

Sacrifice and pathology:

Organ weights: At 300 mg/kg/day, group mean liver weights were higher than controls (absolute, adjusted and relative to body weight), with a statistically significant difference for the male adjusted values (35%) (Table 6.3.2-27). These increases were attributed to particularly high liver weights for one male and two females in this group (only 4 animals/group). Following an EFSA request, the applicant submitted the historical control values for liver weight in 52-year dog studies performed between 2003 and 2017 by the conducting laboratory (Table 6.3.2-27). With these additional data (HCD 2003-2017), the values in males and females are within the background range at 300 mg/kg/day. Therefore, the applicant concluded that in the absence of histopathology changes, the statistical increase in liver weight should be considered adaptive and not adverse. However, it should be noted that these HCD come from 84 one-year dog studies performed in a time frame of 14 years (2003 to 2017) and HSE points out that the ranges of these HCD are relatively large (i.e. 2.24 to 4.17 % and 1.99 to 4.26% for relative liver weight in males and females, respectively). When limiting the number of studies to the time frame of 5-year from the [REDACTED] (2015a) study (HCD 2015-2017), the liver weight increases (absolute and relative) observed at 300 mg/kg/day exceed the HCD ranges both in males and females (Table 6.3.2-27).

Thyroid weights were slightly higher than controls for the male and female groups given 100 or 300 mg/kg/day. The differences were significantly higher for the male adjusted thyroid weights at both dose levels, and for male absolute thyroid weight at 300 mg/kg/day. This difference was considered to be slight, with group mean thyroid weights relative to body weight only marginally higher than the normal background data range (male relative thyroid weight: 0.004 % to 0.012+ %, female relative thyroid weight: 0.005 % to 0.012+ %).

Following an EFSA request, the applicant submitted the historical control values for thyroid weight in 52-year dog studies performed between 2003 and 2017 by the conducting laboratory (Table 6.3.2-27). With the additional HCD (HCD 2003-2017), the values in males and females at 100 and 300 mg/kg for absolute and relative thyroid weight are within the background range. Therefore, the applicant considered the marginal increase in thyroid weight relative to bodyweight in the absence of histopathology changes is not adverse. By limiting the number of studies to the time frame of 5-year from the [REDACTED] (2015a) study (HCD 2015-2017), the values in males and females at 100 and 300 mg/kg for absolute and relative thyroid weights are still within the background range except for absolute weight in males at 300 mg/kg/day (Table 6.3.2-27). Thus, as no treatment related microscopy pathology findings were observed in thyroids, the effect on thyroid weight observed at 100 mg/kg/day in males and females and 300 mg/kg/day in females has not been considered adverse. At 300 mg/kg in males, the increase by 50% of the relative thyroid weight is considered as treatment related and adverse even without histopathological associated findings.

Other differences from controls that attained statistical significance, particularly higher adjusted mean testes weights at 300 mg/kg/day in males and pituitary and adrenal weights for females given 100 mg/kg/day, were considered to reflect typical variability in beagle dogs. In the absence of corroborative histopathological changes these effects on organ weights in males and females given SYN545974 were considered not adverse.

Table 6.3.2-27: Organ Weights - Absolute, Adjusted and % Body Weight - Group Mean Values

Organ weights (kg/g)		Males				Historical control data Group Mean (min-max)	
		Group: 1 Control 0 mg/kg/day	Group: 2 SYN545974 30 mg/kg/day	Group: 3 SYN545974 100 mg/kg/day	Group: 4 SYN545974 300 mg/kg/day	2003-2017	2015-2017
Terminal bodyweight (kg)	Mean	13.3	10.9	11.8	12.2	-	-
	SD	2.0	0.5	2.3	1.1	-	-
	N	4	4	4	4	-	-
Liver (g) (absolute)	Mean	376.75	369.85	371.68	468.33 (+24%)	386.93 (214.6 – 607.0)	300.85 (219.1-361.8)
	SD	70.40	37.83	53.97	98.69	84.89	49.9
	N	4	4	4	4	84 (studies)	5 (studies)
Liver (g) (covariate)	Mean	346.07	398.31 (+15%)	377.24 (+9%)	464.99^{d1} (+34%)	-	-
	N	4	1	4	4	-	-
Liver (%) (relative to body weight)†	Mean	2.848	3.380 (+19%)	3.174 (+11%)	3.843 (+35%)	3.01 (2.24 – 4.17)	2.87 (2.55-3.16)
	SD	0.433	0.202	0.280	0.689	0.34	0.06
	N	4	4	4	4	84 (studies)	5 (studies)
Thyroid (g) (absolute)	Mean	0.988	1.063 (+8%)	1.215 (+23%)	1.385^{d1} (+40%)	1.00 (0.49 – 1.82)	0.88 (0.49-1.22)
	SD	0.158	0.154	0.231	0.174	0.29	0.15
	N	4	4	4	4	84 (studies)	5 (studies)
Thyroid (g) (covariate)	Mean	0.897	1.146 (+28%)	1.231^{d1} (+37%)	1.375^{dd2} (+53%)	-	-
	N	4	4	4	4	-	-
Thyroid (%) (relative to body weight)†	Mean	0.0075	0.0097 (+29%)	0.0103 (+37%)	0.0114 (+52%)	0.0078 (0.0041 -0.0126)	0.0085 (0.00645- 0.0115)
	SD	0.0009	0.0015	0.0012	0.0008	0.0018	0.0008
	N	4	4	4	4	84 (studies)	5 (studies)
Organ weights (kg/g)		Females				Historical control data Group Mean (min-max)	
		Group: 1 Control 0 mg/kg/day	Group: 2 SYN545974 30 mg/kg/day	Group: 3 SYN545974 100 mg/kg/day	Group: 4 SYN545974 300 mg/kg/day	2003-2017	2015-2017
Terminal bodyweight (kg)	Mean	10.4	8.6	10.0	9.4	-	-
	SD	1.2	0.8	1.2	0.7	-	-
	N	4	4	4	4	-	-
Liver (g) (absolute)	Mean	313.53	324.20 (+3%)	339.63 (+8%)	376.68 (+20%)	331.07 (220.4– 507.1)	275.70 (224.0-393.7)
	SD	29.90	92.78	47.03	48.63	65.18	35.84
	N	4	4	4	4	84 (studies)	3 (studies)
Liver (g) (covariate)	Mean	297.78	345.21 (+16%)	330.70 (+11%)	380.35 (+28%)	-	-
	N	4	4	4	4	-	-
Liver (%) (relative to body weight)†	Mean	3.061	3.732 (+22%)	3.421 (+12%)	4.001 (+31%)	3.03 (1.99 – 4.26)	3.00 (2.49-3.71)
	SD	0.456	0.763	0.559	0.471	0.41	0.18
	N	4	4	4	4	84 (studies)	3 (studies)

Thyroid (g) (absolute)	Mean	0.795	0.660	1.038 (+31%)	1.138 (+43%)	0.87 (0.47 – 1.65)	0.81 (0.57-1.23)
	SD	0.061	0.139	0.471	0.349	0.22	0.087
	N	4	4	4	4	84 (studies)	3 (studies)
Thyroid (g) (covariate)	Mean	0.863	0.570	1.076 (+25%)	1.122 (+30%)	-	-
	N	4	4	4	4	-	-
Thyroid (%) (relative to body weight)†	Mean	0.0078	0.0077	0.0109 (+40%)	0.0120 (+54%)	0.0079 (0.0045 – 0.0121)	0.0087 (0.006-0.012)
	SD	0.0012	0.0015	0.0066	0.0034	0.0015	0.0005
	N	4	4	4	4	84 (studies)	3 (studies)

1 [d - Test: Dunnett 2 Sided p < 0.05]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

† No statistical analysis performed

Macroscopic findings: There were no treatment related macroscopic necropsy findings.

Microscopic findings: There were no treatment-related microscopic pathology findings and, in particular, no findings to account for the raised liver weights observed at 300 mg/kg/day. Findings that were observed reflected typical background pathology in laboratory maintained beagle dogs of this age.

CONCLUSION:

In a repeated dose toxicity study conducted according to GLP and OECD TG 452 (2009), four male and four female Beagle dogs were administered doses of 0, 30, 100 or 300 mg/kg bw/d pydiflumetofen *via* oral capsule for 12 months.

There were no deaths or treatment-related clinical signs of toxicity and there was no effect on body weight development or food consumption. Group mean body weights and body weight gains were lower than concurrent controls occasionally reaching statistical significance; however, there was no evidence of a dose response (the most marked reductions were noted in the 30 mg/kg bw/d dose group) and the changes reflected a large degree of weight variation amongst the individual animals (e.g., 11.4-16 kg in the control group). Therefore, a treatment related effect on body weight development could not be determined.

The liver was identified as the main target organ in males and females.

There was no effect on haematological parameters. Clinical chemistry analysis revealed large increases in alkaline phosphatase (ALP) in males and females at 300 mg/kg bw/d, being statistically significantly greater than controls during weeks 13, 26 and 52, where ALP was increased by up to approximately 4-fold in males and 3-fold in females. The applicant proposed that the increase in ALP was secondary to the induction of dog specific ALP isoenzymes. This proposal, which is assessed in the report [REDACTED] (2018), is not supported by HSE.

Liver weights were increased at 300 mg/kg bw/d in both sexes. In males at this dose absolute and relative liver weights were statistically significantly increased by 24% and 35% respectively when compared with controls, whilst in females absolute and relative liver weights were 20% and 31% greater than controls, although the changes were not statistically significant (it is noted that one male and two females in this group presented with unusually large liver weights and contributed significantly to these values). The absolute and relative liver weights at this dose exceeded historical control values when compared with HCD covering a five-year period from the present study. There were no histopathological correlates; however, the extent of the liver weight increases and the fact that they are above the HCD

confirms that the liver weight increases at the high dose are treatment related and adverse. There was no statistically significant increase in liver weights at the lower doses in either sex.

Thyroid weights were increased from 100 mg/kg bw/d in males and females, being statistically significant in males at 300 mg/kg bw/d (40% absolute and 52% relative). When compared with historical control data (same laboratory, 5-year time period), the thyroid weight changes in both males and females were mostly within the expected background variation in both sexes at 100 mg/kg bw/d and in females at 300 mg/kg bw/d. Furthermore, there were no histopathological correlates in either sex at any dose. Therefore, only the increase in thyroid weights in males at 300 mg/kg bw/d is related to treatment with pydiflumetofen.

Therefore, a **NOAEL of 100 mg/kg bw/d** is determined in male and female dogs from this study. At the LOAEL of 300 mg/kg bw/d in males, liver weights were statistically significantly increased by 24% (absolute) and 35% (relative) and a large increase in ALP (up to 4-fold compared with controls) were seen. Thyroid weights were also increased at the LOAEL in males (40% absolute and 52% relative). In females, liver weights were increased by 20% (absolute) and 31% (relative), with a large statistically significant increase in ALP (up to 3-fold).

HSE notes that the liver weight increases at the LOAEL in females did not reach statistical significance; however, owing to the low number of animals tested the statistical power is somewhat reduced and the lack of statistical significance does not exclude a relation to treatment.

(██████████, 2015a)

During the commenting period, EFSA requested to the applicant the following additional information regarding the dog studies:

- Applicant to provide individual ALP values tabulated and a statement to support that the increase in plasma ALP has been associated with induction of dog-specific ALP isoenzyme (52-week study in the dog).

A further assessment to support the increase of ALP has been provided in document presented below.

Report:	K-CA 5.3.2/07 ██████████, (2018) SYN545974 - Rebuttal for Increase in ALP in SYN545974 52 Week Dog Study, ██████████. Syngenta File No. SYN545974_10622.
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Guidelines: Not applicable.

GLP: This report contains an assessment of data from completed studies and additional scientific information and is not a study per se, a Good Laboratory Practice (GLP) statement as defined by OECD is not appropriate.

EXECUTIVE SUMMARY

A 52 week oral (capsule) toxicity study in the dog was conducted on SYN545974 at ██████████ in 2013-2014, using beagle dogs (██████████, 2015).

This document outlines that the variability within the individual plasma alkaline phosphatase levels is not treatment related. It is concluded that the effects on clinical chemistry parameters and liver weight should be considered adaptive and not adverse in the absence of histopathology effects.

RESULTS

In the 52 week SYN545974 dog study, plasma alkaline phosphatase (ALP) levels at 300mg/kg were significantly higher than controls at all time points after dose initiation. The ALP activities were not increased at 100 mg/kg bw per day, with the exception of one male dog whose alkaline phosphatase activity was increased throughout the treatment period when compared to pre-dose values. The ALP values of these individual animals are presented in Table 6.3.2-28.

Table 6.3.2-28. Individual dog alkaline phosphatase (ALP) values in 52 week, SYN545974 dog study.

Group	Animal ID Males	ALP U/I (Week Number)				Animal ID Females	ALP U/I (Week Number)			
		-2	13	26	52		-1	13	26	52
Group: 1 Control 0 mg/kg/day	202	123	59 ^a	42 ^c	33	218	77	49	35	26
	203	90	52b	38	32	219	84	46	33	25
	204	89	48	39	33a	220	79	39	36	29
	205	113a	68b	62	55	221	87	77	71	81
Group: 2 SYN545974 30 mg/kg/day	206	95	71	56	42d	222	84	61	43	51
	207	175	124	102	7	223	73	63	68	68d
	208	88a	89	83	58	224	162	79	64	68
	209	98	111b	93a	89	225	87	84i	80a	73
Group: 3 SYN545974 100 mg/kg/day	210	112	103	120	128d	227	67	64	53	63
	211	115	175d	199	149	228	114	75	81	97
	212	82	51	43	37d	229	70	58	62	51j
	213	111d	90	74	55	234	64	37	26	34
Group: 4 SYN545974 300 mg/kg/day	214	83	116e	134	127	230	101	112	97	69
	215	101	155	146a	131	231	96	147	114	91
	216	102	165	144g	89	232	121	239c	238	236j
	217	114	316a	270	239d	233	75	79	85a	106

a [SC:Plasma sample slightly haemolysed]

b [SC:Plasma sample moderately haemolysed/partially clotted]

c [SC:Partially clotted sample]

d [SC:Plasma sample moderately haemolysed]

e [SC:Plasma sample slightly haemolysed/partially clotted]

f [RC:Actual GGT result less than 3 U/L, SC:Plasma sample slightly haemolysed]

g [SC:Sample clotted]

h [RC:Actual GGT result less than 3 U/L, SC:Plasma sample moderately haemolysed]

i [SC:Plasma sample grossly haemolysed/clotted sample]

j [SC:Plasma sample grossly haemolysed]

At necropsy, there were no treatment related, macroscopic abnormalities in the liver and, despite liver weight increases, there were no treatment related histopathological changes in the liver. Histopathological damage to the liver is generally correlated with increases in three liver enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase. The lack of increase in AST and ALT levels as well as the lack of treatment related histopathology findings in the livers of 300 mg/kg/day treated animals, strongly suggests that the increase in ALP is spurious and is not related to treatment.

Furthermore, the main serum alkaline phosphatase (ALP) isoenzymes in beagle dogs are bone, liver and small intestine derived (██████ et al. 2011). Thus, although individual ALP isoenzymes were not measured in this study, the ALP increases noted in the SYN545974-treated dogs could originate from the bone (particularly in young, growing animals) or small intestine and are unlikely to be derived from the liver, particularly in view of the absence of histopathological lesions observed in the livers of treated dogs.

In addition, there were no other blood chemistry changes, nor were there any test item hematological changes in this study and there was no dose-related increase in ALP in the 30 and 100 mg/kg/day dose groups, all suggesting that the increase in ALP in treated dogs was spontaneous and not linked to treatment.

ALP activity, particularly in dogs, can be induced due to increased cortisol levels noted in stress reactions (██████ et al. 2013; ██████ et al. 2002). Thus the increase in ALP in the 300mg/kg/day treated dogs could be related to stress and is unlikely to treatment related.

CONCLUSION:

In a repeated dose toxicity study conducted according to GLP and OECD TG 452 (2009) (██████, 2015a), four male and four female Beagle dogs were administered doses of 0, 30, 100 or 300 mg/kg bw/d pydiflumetofen via oral capsule for 12 months.

Clinical chemistry analysis revealed large increases in alkaline phosphatase (ALP) in males and females at 300 mg/kg bw/d, being statistically significantly greater than controls during weeks 13, 26 and 52, where ALP was increased by up to approximately 4-fold in males and 3-fold in females. The applicant proposed that the increase in ALP was secondary to the induction of dog specific ALP isoenzymes.

ALP (alkaline phosphatase) levels were greater than controls at all time points following dosing; however, there were no effects on the levels of AST (aspartate aminotransferase) or ALT (alanine amino transferase). There were liver weight increases but no unusual histopathological findings in the liver at necropsy.

The applicant proposed that the lack of histopathological findings in the liver and the lack of an effect on AST or ALT indicates that the observed increases in ALP were not related to treatment with pydiflumetofen. It was further proposed by the applicant that the observed increases in ALP enzymes were not indicative of liver function impairment, but instead could have originated in the bone (secondary to bone growth in young animals) or intestines, or that increases in ALP in dogs could be secondary to increased cortisol levels in the stress response. However, no particular mechanism for the increase in ALP was proposed by the applicant.

Furthermore, no evidence was provided to substantiate the origin of the ALP (no individual ALP isoenzymes were measured) and no further data was provided to support the proposed mode of action.

Therefore, HSE considers that in light of the increased liver weights and the large and statistically significant increase in ALP (up to 3-fold in females and up to 4-fold in males), and with no evidence offered to the contrary, the increase in ALP observed in the 1-year dog study is treatment related and adverse.

The proposed NOAEL of 100 mg/kg bw/d in males and females is therefore unaffected by this additional information.

REFERENCES:

Hatayama K, Nishihara Y, Kimura S, Goto K, Nakamura D, Wakita A, Urasoko Y. 2011 Serum alkaline phosphatase isoenzymes in laboratory beagle dogs detected by polyacrylamide-gel disk electrophoresis. *J Toxicol Sci.* 36:653-60.

Wiedmeyer CEI, Solter PE, Hoffmann WE. 2002 Alkaline phosphatase expression in tissues from glucocorticoid-treated dogs. *Am J Vet Res.* Aug;63:1083-8.

(██████, 2018)

B.6.3.3. Other routes

The applicant submitted a 28-day dermal toxicity study in rat. However, no circumstances which required this type of study have been met according to Regulation 283/2013. Therefore, a justification for conducting the study should have been provided.

Report: K-CA 5.3.3/01. [REDACTED] (2013). SYN545974: 28-Day Dermal Toxicity Study in the Wistar Rat (Final Report Amendment 1). [REDACTED]
[REDACTED]. Laboratory Report No. D62072, issue date 14 January 2013. Unpublished. Syngenta File No. SYN545974_10047.

Guidelines: Repeated Dose Dermal Toxicity. OECD Guidelines No. 410 (1981); EPA OPPTS 870.3200 (1998); EC No. 440/2008, B.9 (2008); MAFF, 12 NohSan No. 8147 (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a 28 day dermal toxicity study, four groups of male and female Han-Wistar rats were exposed to SYN545974 at dose levels of 100, 300 and 1000 mg/kg body weight by dermal semi-occlusive application for six hours per day, five days per week for 28 days.

Parameters determined in this study included mortality, clinical and local dermal signs, functional observational battery, grip strength, locomotor activity, food consumption, body weights, haematology, clinical biochemistry and urinalysis parameters and organ weights, macroscopic findings and histopathology.

No test item-related effects on mortality, functional observational battery parameters, grip strength, locomotor activity, ophthalmoscopic findings, food consumption, body weight development, urinalysis parameters, organ weights and no clinical or local dermal signs, or macroscopic findings were observed.

Small changes in clinical biochemistry parameters, such as higher calcium, phospholipid and total cholesterol levels in females exposed to 1000 mg/kg body weight as well as higher globulin and total protein level in males exposed to 1000 mg/kg body weight are considered to be of minor toxicological relevance and not adverse as all values are within the range of historical control data.

Microscopically, a minor increase of alveolitis in high dose animals is deemed to be due to accidental inhalation of dust and/or test item that is not unusual in a dermal study. Similar lesions, although lower in incidence were recorded in control animals.

The no observed adverse effect level (NOAEL) for dermal treatment with SYN545974 in the Wistar rat for 28 days is 1000 mg/kg body weight /day.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974 technical
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Stability of test compound:	30 June 2016 (stored at room temperature (20 ± 5 °C), away from direct sunlight)

Vehicle: None, used as supplied.

Test Animals:	
Species	Rat
Strain	Rat, RccHanTM: WIST(SPF)
Age/weight at dosing	Approximately 8 weeks / 239.1-265.5 g (males), 160.0-191.0 g (females)
Source	
Housing	Individually in Makrolon type-3 cages with wire mesh tops
Acclimatisation period	6 days
Diet	Pelleted standard 2914C rodent maintenance diet; ad libitum except for overnight prior to blood sampling.
Water	Community tap-water ad libitum
Environmental conditions	Temperature: 22 ± 3 °C Humidity: 30-70% Air changes: 10-15 per hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

In-life dates: Start: 20 August 2012, End: 18 September 2012

Animal assignment: The rats were allocated to groups using a computer-generated random algorithm.

Study design

Test group	Dose level (mg/kg bw/day)	Number of males	Number of females
1 (Control)	0	10	10
2	100	10	10
3	300	10	10
4	1000	10	10

Dose preparation and analysis: No dose formulations were prepared, the test item was used as supplied and was moistened with approximately 0.5 mL purified water before application.

The dose for each group was calculated using the group mean (per sex) of the most recent weekly body weight measurement.

Preparation and treatment of animal skin: Approximately 24 hours before the test item was applied, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10% of the total body surface. For groups 2 - 4, the test item was weighed on a surgical gauze pad (ca. 5 x 5 cm) and moistened with approximately 0.5 mL of purified water. For group 1, a gauze pad was moistened with purified water only. The gauze pad was applied to the clipped skin area and held in contact with the skin by an adhesive hypoallergenic aerated semi-occlusive dressing and an elastic adhesive restrainer bandage wrapped around the abdomen. After at least six hours, the dressing was removed and the skin

was washed with lukewarm water and dried with disposable paper towels. The fur was clipped as needed but at least once weekly.

The dermal applications were made five days per week for at least six hours, at approximately 24 hour intervals, for four weeks.

Observations: Animals were observed twice daily for viability/mortality. Clinical signs (cage-side) were recorded once daily during acclimatization and during the treatment period, at approximately the same time each day. Local dermal signs were recorded twice daily during treatment on the days of test item application (5 days per week), prior to test item application and after removal of the bandage; once daily on days without test item application (2 days per week). Skin irritation was scored according to Draize. Detailed clinical observations were recorded weekly.

Functional observational battery:

Detailed observations: Once weekly the following detailed observations were made: appearance (piloerection, salivation, hunched posture), motor (ataxia, tremor/twitching, prostration, circling, spasm), behaviour (hyperactivity, somnolence, increased exploration, reduced grooming, vocalisation), respiration (dyspnea, tachypnea, bradypnea), reflexes (blink, pinna, iridic light reflex, push off – hind leg, pain response, startle/hearing, righting reflex), miscellaneous (lachrymation, limbs cyanotic, mydriasis, miosis, exophthalmos, reduced muscle tone).

Grip strength: Once in week 4 of treatment; forelimb and hindlimb grip strength measurements were made using a push-pull strain gauge.

Locomotor activity: Once in week 4 of treatment, locomotor activity (decreased or increased) was measured quantitatively with an AMS Föhr Medical Instruments GmbH (FMI) and DeMeTec GmbH Activity Monitor System. Animals were monitored for a 60-minute period and the total activity of this time period recorded. Low beams count was reported in 10-minute intervals as well as the total activity of the measuring period.

Body weight: Animals were weighed once during acclimatization and weekly during treatment.

Food consumption: Measured weekly.

Ophthalmoscopy: Eyes were examined once during acclimatisation (all animals). Once in week 4 of treatment (Groups 1 and 4 only; since there were no treatment-related findings, groups 2 and 3 were not examined in week 4).

Haematology and coagulation: Blood was collected from the retro-orbital plexus from all animals under light isoflurane anaesthesia on Day 28. The animals were fasted for approximately 18 hours before blood sampling but allowed access to water *ad libitum*. The following parameters were determined:

haemoglobin	mean corpuscular haemoglobin concentration
haematocrit	platelet count
erythrocyte count	total leukocyte count
mean corpuscular volume	differential leukocyte count
mean corpuscular haemoglobin	haemoglobin concentration distribution width
red cell volume distribution width	reticulocyte maturity index
reticulocyte count	prothrombin time
	activated partial thromboplastin time

Clinical chemistry: Blood was collected from the retro-orbital plexus from all animals under light isoflurane anaesthesia on Day 28. The animals were fasted for approximately 18 hours before blood sampling but allowed access to water ad libitum. The following parameters were determined:

urea	alkaline phosphatase activity
creatinine	alanine aminotransferase activity
glucose	gamma-glutamyl transferase activity
total bilirubin	calcium
total protein	phosphorus
total cholesterol	sodium
triglycerides	potassium
phospholipids	chloride
creatine kinase activity	A/G ratio
lactate dehydrogenase activity	globulin
aspartate aminotransferase activity	albumin

Urinalysis: Urine was collected on Day 28 from fasting animals into a specimen vial. The following parameters were determined:

volume (18 hour)	glucose
colour	ketones
appearance	protein
specific gravity	bilirubin
pH	erythrocytes
nitrite	leukocytes
urobilinogen	

Investigations post mortem:

Macroscopic examination: All animals were anaesthetised by intraperitoneal injection of pentobarbitone and killed by exsanguination on Day 29/30 and examined post mortem. This involved an external observation and an internal examination of all organs and structures.

Organ weights: The following organs were weighed:

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	thymus
kidneys	uterus (with cervix and vagina)
liver	

Tissue submission: The following tissues were examined in situ, removed and examined and placed in appropriate fixative:

adrenal gland	ovary
aorta	pancreas
bone (sternum and femur including joint)	pharynx
bone marrow (femur)	pituitary gland
brain (including section of medulla/pons, cerebral and cerebellar cortex)	prostate gland, seminal vesicles, coagulating gland
caecum	rectum
colon	salivary glands (mandibular and sublingual)
duodenum	sciatic nerve
epididymis	skeletal muscle (thigh)
eyes (with optic nerve)	skin (treated and untreated)
heart	spleen
ileum (with Peyer's patches)	spinal cord (cervical, mid thoracic, lumbar)
jejunum (with Peyer's patches)	stomach
kidney	testis
larynx	thymus
liver	thyroid gland (including parathyroid)
lung	tongue
lymph node (mesenteric, mandibular and inguinal)	trachea
mammary gland area	ureter
nasal cavity	urinary bladder
oesophagus	uterus (with cervix and vagina)
	gross lesions

Microscopic examination: All processed tissues from all the animals from the control and high dose group (1000 mg/kg bw/day) animals were examined by light microscopy.

Statistics: The following statistical approaches were used in this study:

- All analyses are two-tailed for significance levels of 5% and 1%.
- All means are presented with standard deviations.
- Body weights, cumulative body weight gain, food consumption, quantitative FOB measurements (grip strength, locomotor activity data at each measurement interval and overall activity) haematology, clinical chemistry, urinalysis and absolute organ weights were analysed initially by a one-way analysis of variance (ANOVA).
- Organ weights were also analyzed by analysis of covariance (ANCOVA) on final body weight. This statistical analysis provides an adjusted organ weight value, which is displayed in the results table in the final report along with flags for statistical significance.
- Summary values of organ to body weight ratios are presented but not analysed statistically.
- For all parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and test item-treated groups. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the final report.

- Macropathology and micropathology incidence data was analysed using Fisher's Exact Test.
- Any other parameters not specifically mentioned above that yield qualitative data were presented as summary data, but were not analysed statistically.

RESULTS

Mortality: All animals survived until scheduled sacrifice.

Clinical observations (cage-side): There were no treatment-related effects.

Local dermal signs: There were no treatment-related effects.

Functional observational battery:

Detailed observations: There were no treatment-related effects.

Grip strength: There were no treatment-related effects.

Locomotor activity: There were no treatment-related effects.

Body weight and weight gain: There were no treatment-related effects.

Food consumption: There were no treatment-related effects.

Ophthalmoscopy: There were no treatment-related effects.

Haematology: There were no differences in haematological parameters which were considered to be related to treatment.

Blood clinical chemistry: Statistically significantly higher calcium, phospholipids and total cholesterol levels in females exposed to 1000 mg/kg body weight and statistically significantly higher globulin and total protein level in males exposed to 1000 mg/kg body weight were seen. These may be test item-related but are considered to be of minor toxicological relevance and not adverse. All values were within the range of historical control data.

Table 6.3.3-1: Intergroup comparison of selected clinical chemistry parameters

Parameter	Dose level (mg/kg bw/day)							
	Males				Females			
	0	100	300	1000	0	100	300	1000
Calcium (mmol/l)	2.78	2.77	2.80	2.78	2.74	2.78	2.79	2.84**
Phospholipids (mmol/l)	1.80	1.73	1.69	1.71	1.65	1.91	1.88	2.06*
Total cholesterol (mmol/l)	2.11	2.02	1.95	1.97	1.56	1.85	1.83	1.99*
Globulin (g/l)	21.91	22.58	22.56	23.36*	20.74	21.60	21.44	20.84
Total protein (g/l)	66.76	67.64	67.16	69.01*	70.01	73.05	72.36	72.84
* Statistically significant difference from control group mean, p<0.05								
** Statistically significant difference from control group mean, p<0.01								

Urinalysis: There were no treatment-related effects.

Sacrifice and pathology:

Macroscopic findings: There were no treatment-related effects.

Organ weights: There were no treatment-related effects.

Microscopic findings: There were no treatment-related effects. A minor increase of alveolitis in high dose animals is deemed to be due to accidental inhalation of dust and/or test item that is not unusual in a dermal study. Similar lesions, although less in incidence were recorded in control animals.

CONCLUSION:

In a dermal toxicity study, conducted according to GLP and OECD TG 410 (1981), pydiflumetofen was administered to Han Wistar rats (10/sex/dose) at dose levels of 100, 300 and 1000 mg/kg bw/d for five days/week for 28 days.

There were no deaths or treatment-related clinical signs of toxicity and there were no local dermal effects observed.

There was no effect on body weight development or food consumption and the functional observational battery and ophthalmoscopy observations were unremarkable.

There were no unusual haematological findings; with regard to clinical chemistry, calcium, phospholipids and total cholesterol were increased in females at 1000 mg/kg bw/d and globulin and total protein levels were increased in males at the same dose. All changes were statistically significant but were within the range of the laboratory historical control data. Therefore, the clinical chemistry changes, although potentially treatment related were of minor toxicological significance and not adverse.

There was no effect on organ weights and there were no unusual macroscopic or microscopic findings at necropsy.

Therefore the **NOAEL was determined to be 1000 mg/kg bw/d** in males and females (the highest dose tested).

(████████, 2013)

B.6.3.4. Overall summary of short-term toxicity

The short-term toxicity of pydiflumetofen has been investigated via the oral route in GLP and guideline studies for 28 and 90 days in rats and mice and for 90 days and 1 year in dogs; an investigation via the dermal route in rats (28-days) was also provided.

There was no effect in rats when pydiflumetofen was administered dermally.

Rats

When pydiflumetofen was administered orally to rats for 28 days, the liver was identified as the main target organ. Dietary concentrations of 0, 500, 4000, 8000 and 16000 ppm equated to estimated mean achieved doses of 0, 43, 343, and 1322 mg/kg bw/d in males and 0, 40, 322, 619 and 1174 mg/kg bw/d in females. There were no deaths or clinical signs of toxicity and body weight development was affected in males only at the high dose of 1600 ppm (1322 mg/kg bw/d). Liver weights were increased from 4000 ppm by 18% and 20% (absolute) and 21% and 24% (relative) in males and females respectively, associated with centrilobular hypertrophy in both sexes and statistically significantly lower ALT from 8000 ppm in females only. A **NOAEL of 500 ppm (equivalent to 43/40 mg/kg bw/d in males/females)**

was therefore identified in rats following 28 days' exposure, based on increased liver weights and hepatocellular hypertrophy at the LOAEL of 4000 ppm (343/322 mg/kg bw/d in males and females).

When pydiflumetofen was administered to rats orally for 90 days, the liver and thyroid were identified as target organs. Dietary concentrations of 0, 250, 1500, 8000 and 16000 ppm equated to mean estimated achieved doses 0, 18.6, 111, 587 and 1187 mg/kg bw/d in males and 0, 21.6, 127, 727 and 1325 mg/kg bw/d in females. There were no deaths or clinical signs of toxicity and body weight development and food consumption were affected in both sexes from 8000 ppm. Liver weights were increased from 1500 ppm by 21% (absolute) and 26% (relative) in males and 16% (absolute) and 18% (relative) in females. Liver weight increases were associated with hepatocellular hypertrophy in males from 1500 ppm (only apparent in females from 8000 ppm) and with reduced ALP in both sexes from 1500 ppm. Follicular cell hypertrophy of the thyroid was noted in males from 1500 ppm (not statistically significant at this dose) and in females from 8000 ppm. **A NOAEL of 250 ppm (equivalent to 18.6 and 21.6 mg/kg bw/d in males and females respectively)** was identified in rats following 90-days' exposure, based on liver weight increases, hepatocellular hypertrophy, reduced ALP, and thyroid follicular cell hypertrophy at the LOAEL of 1500 ppm (111/127 mg/kg bw/d in males and females).

In rats the most sensitive NOAEL was 18.6 mg/kg bw/d, derived from the 90-day study.

Mice

Consistent with the findings in rats, when pydiflumetofen was administered to mice for 28 days, the liver was identified as the main target organ. Dietary concentrations of 0, 500, 1500, 4000 and 7000 ppm equated to estimated achieved doses of 0, 76, 213, 612 and 1115 mg/kg bw/d in males and 0, 96, 266, 701 and 1312 mg/kg bw/d in females. There were no deaths or clinical signs of toxicity. Body weight development was affected in males at all doses, resulting in final body weight gains that were 55% lower than controls at 500 ppm (the lowest tested dose); females were not affected. Liver weights were increased from 500 ppm by 9% (absolute) and 16% (relative) in males and 14% (absolute) and 21% (relative) in females. **A NOAEL could not be identified for mice following 28 days' exposure** as it was less than the lowest dose tested of 500 ppm (equivalent to 76/96 mg/kg bw/d in males/females).

When pydiflumetofen was administered to mice for 90-days, the liver was again identified as the main target organ. Dietary concentrations of 0, 100, 500, 4000 and 7000 ppm equated to estimated achieved doses of 0, 17.5, 81.6, 630 and 1158 mg/kg bw/d in males and 0, 20.4, 106, 846 and 1483 mg/kg bw/d in females. There were no deaths or clinical signs of toxicity. Liver weights were increased from 500 ppm in males by 18% (absolute) and 14% (relative); in females liver weights increases became apparent at 4000 ppm with an increase in absolute weight of 59% and relative weight of 60%. Hepatocellular hypertrophy was noted, reaching statistical significance from 7000 ppm in males and 4000 ppm in females. Clinical chemistry changes, indicative of liver impairment, were noted in both sexes; cholesterol was increased from 500 ppm in males (reaching statistical significance from 4000 ppm) and at 7000 ppm in females, whilst triglyceride concentrations were increased from 4000 ppm in both sexes (reaching statistical significance at 7000 ppm). **A NOAEL of 100 ppm (17.56 mg/kg bw/d) in males and 500 ppm (106 mg/kg bw/d) in females was determined in mice following 90 days' dietary exposure** based on increased liver weights and increased cholesterol at the LOAEL of 500 ppm (106 mg/kg bw/d) in males and increased liver weights, hepatocellular hypertrophy, and increased triglycerides at the LOAEL of 4000 ppm (846 mg/kg bw/d) in females.

In mice the most sensitive NOAEL is 17.56 mg/kg bw/d, derived from males in the 90-day study

Dogs

Consistent with the findings in rodents, the liver was identified as a target organ in dogs.

Following 90 days' exposure to pydiflumetofen at doses of 0, 30, 300 and 1000 mg/kg bw/d, body weight gain was reduced in female dogs from 300 mg/kg bw/d, along with liver weight increases and clinical chemistry changes (increased ALP) at the same dose. In males body weight gain was reduced

at 1000 mg/kg bw/d, whilst liver weight increases and clinical chemistry changes comprising increases in ALP and triglyceride concentrations were noted from 300 mg/kg bw/d. **A NOAEL of 30 mg/kg bw/d** was therefore identified in male and female dogs following 90 days' exposure, based on reduced body weight gain in females and increased liver weights with associated clinical chemistry changes in both sexes at the LOAEL of 300 mg/kg bw/d.

When the length of exposure was increased to one year in dogs (at doses of 0, 100, 300 & 1000 mg/kg bw/d), there were no treatment related effects on body weight development or food consumption and there were no deaths or clinical signs of toxicity. However, the liver was again identified as a target organ in both sexes. Liver weights were increased from 300 mg/kg bw/d; absolute and relative weights were increased by 24% and 35% in males and 20% and 31% in females. There were no unusual histopathological findings in the liver; however, clinical chemistry changes, indicative of liver impairment, were noted comprising large increases in ALP (up to 4-fold in males and 3-fold in females). **A NOAEL of 100 mg/kg bw/d** was therefore identified in male and female dogs following 1 year' exposure of pydiflumetofen, based on increased liver weights and concomitant increases in ALP at the LOAEL of 300 mg/kg bw/d.

In dogs, the most sensitive NOAEL is 30 mg/kg bw/d, derived from the 90-day study.

Overall, administration of pydiflumetofen to rats, mice or dogs, results in impaired body weight development. The liver was identified as a target organ in all species, whilst the thyroid was additionally identified as a target organ in rats only. HSE agreed with RAC that no classification for STOT RE was warranted as no effects were observed at doses relevant for classification (see [GB MCL Technical Report](#) for further details).

The most sensitive NOAEL was 17.56 mg/kg bw/d, identified in mice following 90-days' exposure.

The table below summarises the main findings from the repeated-dose toxicity studies:

Study & Acceptability	Test material & Dose levels	NOAEL	LOAEL	Effects at LOAEL
28-day rat dietary study (██████, 2012) <i>Acceptable GLP and guideline study</i>	Pydiflumetofen 0, 500, 4000, 8000 & 16000 ppm Equivalent to: Males: 0, 43, 343, 677 & 1322 mg/kg bw/d Females: 0, 40, 322, 619 & 1174 mg/kg bw/d in females	500 ppm (43/40 mg/kg bw/d in M/F)	4000 ppm (343/322 mg/kg bw/d in M/F)	↑ Liver weights in M & F ↑ centrilobular hepatocellular hypertrophy in M & F
28-day mouse dietary study (██████, 2012a) <i>Acceptable GLP and guideline study</i>	Pydiflumetofen 0, 500, 1500, 4000 & 7000 ppm Equivalent to:	< 500 ppm (76/96 mg/kg bw/d in M/F)	500 ppm (76/96 mg/kg bw/d in M/F)	↓ Body weight & Body weight gain in M ↑ Liver weights in M & F

	<p>Males: 0, 76, 213, 612 & 1115 mg/kg bw/d</p> <p>Females: 0, 96, 266, 701 & 1312 mg/kg bw/d</p>			
<p>90-day rat dietary study (█ & █, 2015)</p> <p><i>Acceptable GLP and guideline study</i></p>	<p>Pydiflumetofen</p> <p>0, 250, 1500, 8000 & 16000 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 18.6, 111, 587 & 1187 mg/kg bw/d</p> <p>Females: 0, 21.6, 127, 727 & 1325 mg/kg bw/d</p>	<p>250 ppm (18.6/21.6 mg/kg bw/d in M/F)</p>	<p>1500 ppm (111/127 mg/kg bw/d in M/F)</p>	<p>↑ Liver weights in M & F</p> <p>↓ ALP in M & F</p> <p>↑ hepatocellular hypertrophy in M</p> <p>↑ Follicular cell hypertrophy in M</p>
<p>90-day mouse dietary study (█, 2015)</p> <p><i>Acceptable GLP and guideline study</i></p>	<p>Pydiflumetofen</p> <p>0, 100, 500, 4000 & 7000 ppm</p> <p>Equivalent to:</p> <p>Males: 0, 17.5, 81.6, 630 & 1158 mg/kg bw/d</p> <p>Females: 0, 20.4, 106, 846 & 1483 mg/kg bw/d</p>	<p>Males: 100 ppm (17.56 mg/kg bw/d)</p> <p>Females: 500 ppm (106 mg/kg bw/d)</p>	<p>Males: 500 ppm (81.6 mg/kg bw/d)</p> <p>Females: 4000 ppm (846 mg/kg bw/d)</p>	<p>Males: ↑ Liver weights</p> <p>↑ Cholesterol</p> <p>Females: ↑ Liver weights</p> <p>↑ Hepatocellular hypertrophy</p> <p>↑ Triglyceride</p>
<p>90-day dog oral (capsule) study (█, 2015)</p> <p><i>Acceptable GLP and guideline study</i></p>	<p>Pydiflumetofen</p> <p>0, 30, 300 & 1000 mg/kg bw/d</p>	<p>30 mg/kg bw/d</p>	<p>300 mg/kg bw/d</p>	<p>↓ Body weight gain in F</p> <p>↑ Liver weights in M & F</p> <p>↑ ALP in M & F</p> <p>↑ Triglyceride in M</p>
<p>1-year dog oral (capsule) study (█, 2015a)</p> <p><i>Acceptable GLP and guideline study</i></p>	<p>Pydiflumetofen</p> <p>0, 30, 100 & 300 mg/kg bw/d</p>	<p>100 mg/kg bw/d</p>	<p>300 mg/kg bw/d</p>	<p>↑ Liver weights in M & F</p> <p>↑ ALP in M & F</p> <p>↑ Thyroid weights in M</p>

28-day rat dermal study (██████, 2013) <i>Acceptable GLP and guideline study</i>	Pydiflumetofen 0, 100, 300 & 1000 mg/kg bw/d	1000 mg/kg bw/d	>1000 mg/kg bw/d	No effects up to and including the highest dose tested of 1000 mg/kg bw/d
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B.6.4. GENOTOXICITY

B.6.4.1. In vitro studies

Pydiflumetofen was investigated for genotoxicity in vitro in two Ames test (one using a standard batch of material and one using a batch spiked with impurities to support the technical specification), a mouse lymphoma assay and a chromosome aberration test.

Report:	K-CA 5.4.1/01 ████████ (2012). SYN545974: Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay. Harlan, Cytotest Cell Research GmbH (Harlan CCR), In den Leppsteinswiesen 19, 64380 Rossdorf, Germany. Laboratory Report No. 1498901, 22 October 2012. Unpublished. Syngenta File No. SYN545974_10018.
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Guidelines: Bacterial Reverse Mutation Test. OECD 471 (1997); US EPA OPPTS 870.5100 (1998); Commission Regulation (EC) 440/2008 B13/14 (2008).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

In a reverse gene mutation assay in bacteria, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100, and Escherichia coli strains WP2uvrApKM101 and WP2pKM101, were exposed to SYN545974 (purity 98.5%) in DMSO at concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation in the plate incorporation test (experiment 1) and the pre-incubation test (experiment 2).

The plates incubated with the test substance showed normal background growth up to 5000 µg/plate with and without metabolic activation.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), were observed in strains TA1535 and TA1537 with metabolic activation in experiment 1. In experiment 2, cytotoxic effects were observed in strains TA1537 and TA98 without metabolic activation and in strain TA1535 with metabolic activation.

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545974 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations

in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test substance did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, SYN545974 is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 % w/w
CAS#:	1228284-64-7
Stability of test compound:	Expiry date end June 2016 (stored at room temperature < 30°C, light protected, moisture protected)

Control Materials:	
Negative:	Yes (untreated)
Solvent control (final concentration):	DMSO (10 µL/plate)
Positive controls:	No metabolic activation: Sodium azide 10 µg/plate TA100, TA1535 4-nitro-o-phenylene-diamine 10 µg/plate TA98 50 µg/plate TA1537 methyl methane sulfonate 3.0 µg/plate WP2 uvrA (pKM101), WP2 (pKM101)
	Metabolic activation: 2-Aminoanthracene 2.5 µg/plate TA1535, TA1537, TA98, TA100 10 µg/plate WP2 uvrA (pKM101), WP2 (pKM101)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbital		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from 8-12 weeks old male Wistar rats (Hsd Cpb:WU) induced by intraperitoneal administration of 80 mg/kg bw phenobarbital and by oral administrations of β-naphthoflavone each, on 3 consecutive days. The livers were prepared 24 hours after the last treatment. The S9 fractions were produced by dilution of the liver homogenate with a KCl solution (1+3 parts) followed by centrifugation at 9000 g. Aliquots of the supernatant were frozen and stored in ampoules at –80 °C. Small numbers of the ampoules can be kept at –20 °C for up to one week. Each batch of S9 mix was routinely tested with 2-aminoanthracene as well as benzo[a]pyrene.

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors were added to the S9 mix to reach the following concentrations in the S9 mix: 8mM MgCl₂, 33 mM KCl, 5 mM Glucose-6-phosphate, 4 mM NADP in 100 mM sodium-ortho-phosphate-buffer, pH 7.4. During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames *et al.*(1977).

Test organisms:

S. typhimurium strains									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
E. coli strains									
X	WP2 uvrA pKM101	X	WP2 pKM101						
Properly maintained?							X	Yes	No
Checked for appropriate genetic markers (rfa mutation, R factor)?							X	Yes	No

Test compound concentrations used

First test (pre-experiment for toxicity):

Nonactivated conditions: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Activated conditions: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

For all strains triplicate plates were used for all test substance, positive control, negative control and solvent control treatments.

Second test:

Nonactivated conditions: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Activated conditions: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

For all strains triplicate plates were used for all test substance, positive control and solvent control treatments.

Study Design and Methods:

Experimental dates: Start: 09 August 2012, End: 27 August 2012.

Test Performance:

Preliminary Cytotoxicity Assay: Not performed.

Type of Bacterial assay:

- X standard plate test (first experiment –S9, +S9)
- X pre-incubation (60 minutes) (second experiment –S9, +S9)
- ___ “Prival” modification (i.e. azo-reduction method)
- ___ spot test
- ___ other

Protocol:

Standard plate test: Bacterial cultures were prepared from frozen stocks by incubating for 4 hours at 37°C in a shaking incubator. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8 - 10^9 cells/mL).

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent and positive controls;

500 µL S9 mix or phosphate buffer;

100 µL Bacteria suspension;

2 mL Overlay agar (for Salmonella strains contains 7.0 g Agar, 6.0 g NaCl, 10.5 mg L-Histidine HCl H₂O and 12.2 mg Biotin per litre; for Escherichia coli contains 7.0 g Agar, 6.0 g NaCl and 10.2 mg tryptophan)

Plates were also prepared without the addition of bacteria.

Pre-incubation test: For the pre-incubation method 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacteria suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After preincubation 2.0 mL overlay agar (45°C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for 48-72 hours at 37°C in the dark.

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: The Salmonella typhimurium and Escherichia coli reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of the historical data
- the positive control substances should produce a significant increase in mutant colony frequencies
- a minimum of five analysable dose levels should be present with at least four dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

A test substance is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

RESULTS

Preliminary cytotoxicity assay: Not performed.

Mutagenicity assay:

First test (standard plate test): The plates incubated (72 hours) with the test substance showed normal background growth up to 5000 µg/plate with and without metabolic activation. Cytotoxic effects were observed at 2500 and 5000 µg/plate +S9 for TA1535 and at 2500 µg/plate +S9 for TA1537. Precipitation of the test substance was observed in the overlay agar in the test tubes from 1000 to 5000 µg/plate +S9 mix. Precipitation was also observed on the incubated agar plates from 1000 - 5000 µg/plate with and without S9 mix (Table 6.4.1-1).

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545974 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Second test (pre-incubation test): The plates incubated (72 hours) with the test substance showed normal background growth up to 5000 µg/plate with and without metabolic activation. Cytotoxic effects were observed at 1000 to 5000 µg/plate +S9 for TA1535 and at 5000 µg/plate without S9 for TA1537 and at 2500 and 5000 µg/plate without S9 for TA98. Precipitation of the test substance was observed in the overlay agar in the test tubes from 1000 to 5000 µg/plate +S9 mix. Precipitation was also observed on the incubated agar plates from 333 – 5000 µg/plate with S9 mix and from 1000 - 5000 µg/plate without S9 (Table 6.4.1-2).

No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545974 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

Table 6.4.1-1: Summary of results Pre-experiment/ Experiment I

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 <i>uvrA</i> pKM101
Without Activation	DMSO Untreated SYN545974		12 ± 4	21 ± 4	22 ± 4	147 ± 9	224 ± 8	363 ± 25
			15 ± 5	27 ± 1	22 ± 2	178 ± 12	248 ± 1	387 ± 26
		3 µg	13 ± 1	19 ± 4	28 ± 8	162 ± 18	222 ± 6	358 ± 16
		10 µg	14 ± 1	20 ± 3	26 ± 8	151 ± 15	224 ± 12	369 ± 14
		33 µg	16 ± 3	20 ± 1	25 ± 4	171 ± 20	238 ± 21	354 ± 18
		100 µg	12 ± 3	24 ± 5	24 ± 5	166 ± 3	224 ± 9	380 ± 32
		333 µg	16 ± 6	17 ± 4	25 ± 8	145 ± 10	221 ± 20	354 ± 17
		1000 µg	12 ± 3 ^{PM}	20 ± 3 ^{PM}	24 ± 5 ^{PM}	139 ± 13 ^P	226 ± 22 ^{PM}	355 ± 14 ^{PM}
		2500 µg	11 ± 2 ^{PM}	17 ± 2 ^{PM}	21 ± 5 ^{PM}	152 ± 10 ^P	240 ± 20 ^{PM}	349 ± 17 ^{PM}
		5000 µg	12 ± 3 ^{PM}	21 ± 4 ^{PM}	21 ± 4 ^{PM}	154 ± 10 ^P	211 ± 17 ^{PM}	377 ± 35 ^{PM}
	NaN3 4-NOPD 4-NOPD MMS	10 µg	1771 ± 49		296 ± 23	1936 ± 66		
		10 µg 50 µg 3.0 µL		80 ± 4			4139 ± 59	4166 ± 186
With Activation	DMSO Untreated SYN545974		22 ± 5	28 ± 1	38 ± 4	157 ± 15	277 ± 18	434 ± 21
			18 ± 4	29 ± 2	39 ± 16	165 ± 15	288 ± 17	431 ± 10
		3 µg	20 ± 6	31 ± 7	44 ± 5	149 ± 2	281 ± 16	436 ± 11
		10 µg	20 ± 2	27 ± 10	39 ± 7	141 ± 11	256 ± 11	431 ± 4
		33 µg	17 ± 1	32 ± 4	34 ± 6	141 ± 5	281 ± 20	440 ± 15
		100 µg	20 ± 5	33 ± 4	35 ± 2	147 ± 9	271 ± 12	420 ± 20
		333 µg	15 ± 4	28 ± 7	38 ± 4	122 ± 8	254 ± 12	430 ± 10
		1000 µg	12 ± 2 ^{PM}	15 ± 4 ^{PM}	35 ± 3 ^{PM}	94 ± 14 ^P	215 ± 11 ^{PM}	412 ± 31 ^{PM}
		2500 µg	9 ± 3 ^{PM}	12 ± 2 ^{PM}	29 ± 4 ^{PM}	101 ± 4 ^P	223 ± 12 ^{PM}	390 ± 28 ^{PM}
		5000 µg	9 ± 1 ^{PM}	15 ± 3 ^{PM}	27 ± 3 ^{PM}	86 ± 8 ^{PM}	155 ± 8 ^{PM}	312 ± 18 ^{PM}
	2-AA	2.5 µg	295 ± 23	258 ± 21	1491 ± 51	2144 ± 20		
	2-AA	10.0 µg					1985 ± 9	2566 ± 54
Key to Positive Controls			Key to Plate Postfix Codes					
NaN3	sodium azide				P	Precipitate		
2-AA	2-aminoanthracene				M	Manual count		
4-NOPD	4-nitro-o-phenylene-diamine							
MMS	methyl methane sulfonate							

Table 6.4.1-2: Summary of results Experiment II

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 <i>uvrA</i> pKM101
Without Activation	DMSO Untreated SYN545974		12 ± 2	11 ± 3	31 ± 3	137 ± 17	188 ± 10	347 ± 10
			12 ± 2	15 ± 5	29 ± 3	152 ± 15	201 ± 15	330 ± 5
		3 µg	14 ± 2	8 ± 2	41 ± 3	118 ± 3	186 ± 8	287 ± 18
		10 µg	14 ± 2	13 ± 3	34 ± 3	141 ± 17	193 ± 7	327 ± 12
		33 µg	16 ± 2	11 ± 1	34 ± 7	127 ± 8	183 ± 10	329 ± 13
		100 µg	15 ± 3	12 ± 2	37 ± 2	128 ± 5	213 ± 6	332 ± 3
		333 µg	13 ± 3	12 ± 2	35 ± 7	119 ± 1	181 ± 3	325 ± 13
		1000 µg	12 ± 3 ^{PM}	6 ± 2 ^{PM}	17 ± 4 ^{PM}	95 ± 5 ^{PM}	182 ± 6 ^{PM}	314 ± 15 ^{PM}
		2500 µg	7 ± 2 ^{PM}	7 ± 2 ^{PM}	14 ± 5 ^{PM}	102 ± 4 ^P	178 ± 8 ^{PM}	333 ± 15 ^{PM}
		5000 µg	9 ± 3 ^{PM}	4 ± 1 ^{PM}	11 ± 2 ^{PM}	95 ± 11 ^P	167 ± 9 ^{PM}	316 ± 18 ^{PM}
	NaN3 4-NOPD 4-NOPD MMS	10 µg	1782 ± 99		302 ± 22	1802 ± 71		
		10 µg 50 µg 3.0 µL		69 ± 6			3043 ± 21	2082 ± 245
With Activation	DMSO Untreated SYN545974		29 ± 6	12 ± 4	48 ± 13	151 ± 7	230 ± 10	378 ± 10
			27 ± 5	18 ± 2	57 ± 2	191 ± 8	282 ± 9	412 ± 10
		3 µg	32 ± 5	13 ± 8	55 ± 5	163 ± 20	234 ± 21	372 ± 16
		10 µg	41 ± 6	13 ± 4	59 ± 10	144 ± 6	226 ± 15	360 ± 5
		33 µg	32 ± 2	14 ± 5	55 ± 16	147 ± 5	232 ± 19	355 ± 14
		100 µg	29 ± 1	17 ± 5	51 ± 6	138 ± 8	212 ± 14	361 ± 19
		333 µg	36 ± 1 ^P	15 ± 2 ^{PM}	58 ± 3 ^P	145 ± 4 ^P	242 ± 25 ^P	367 ± 7 ^P
		1000 µg	10 ± 1 ^{PM}	8 ± 4 ^{PM}	29 ± 4 ^{PM}	89 ± 8 ^{PM}	167 ± 17 ^{PM}	356 ± 11 ^{PM}
		2500 µg	8 ± 3 ^{PM}	9 ± 4 ^{PM}	26 ± 5 ^{PM}	88 ± 12 ^P	163 ± 6 ^{PM}	308 ± 22 ^{PM}
		5000 µg	7 ± 2 ^{PM}	10 ± 1 ^{PM}	23 ± 1 ^{PM}	92 ± 9 ^{PM}	155 ± 8 ^{PM}	313 ± 17 ^{PM}
	2-AA	2.5 µg	287 ± 25	170 ± 15	1541 ± 206	2172 ± 129		
	2-AA	10.0 µg					2507 ± 26	2393 ± 228
Key to Positive Controls			Key to Plate Postfix Codes					
NaN3	sodium azide				P	Precipitate		
2-AA	2-aminoanthracene				M	Manual count		
4-NOPD	4-nitro-o-phenylene-diamine							
MMS	methyl methane sulfonate							

CONCLUSION:

In this GLP and OECD test guideline compliant Ames test ([REDACTED], 2012), *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strains WP2 uvrA pKM101 and WP2 pKM101 were exposed to concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate pydiflumetofen in the presence and absence of mammalian metabolic activation in the plate incorporation test (experiment 1) and the pre-incubation test (experiment 2).

The plates incubated with the test substance displayed normal background growth up to 5000 µg/plate with and without metabolic activation. Cytotoxic effects (evident as a reduction in the number of revertants, below the indication factor of 0.5) were observed in experiment 1 in strains TA1535 and TA1537 with metabolic activation. Cytotoxic effects were also observed in experiment 2 in strains TA1537 and TA98 without metabolic activation and in strain TA1535 with metabolic activation.

After exposure to pydiflumetofen, there was no substantial increase in revertant colony numbers of any strain tested at any concentration, either with or without metabolic activation. There was also no tendency of higher mutation rates with increasing test item concentrations in the range below the border of biological relevance. The reference mutagens used as positive controls showed a distinct increase in revertant colony numbers, demonstrating the sensitivity and validity of the test system.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, pydiflumetofen is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

HSE notes that cytotoxic effects and precipitation were observed in the study, therefore the test item was tested at high enough concentrations to be adequately investigated for genotoxic potential in this in vitro bacterial mutation assay.

([REDACTED], 2012)

Report:	K-CA 5.4.1/02 [REDACTED] (2014). SYN545974 - Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay. Harlan Cytotest Cell Research GmbH, In den Leppsteinswiesen 19, 64380 Rossdorf Germany. Laboratory Report No. 1648701, issue date: 03 December 2014. Unpublished. Syngenta File No. SYN545974_10127.
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Guidelines: Reverse Mutation Test Using Bacteria. OECD 471 (1997): OPPTS 870.5100 (1998): EC 440/2008 B.13/14 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

This study was performed to investigate the potential of SYN545974 to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *Escherichia coli* strains WP2 uvrA pKM101 and WP2 pKM101.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), were observed in strain TA 1537 in experiment I with and without S9 mix and in strain TA 98 in experiment II with metabolic activation.

No increase in revertant colony numbers of any of the six tested strains was observed following treatment with SYN545974 at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance. Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test substance did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, SYN545974 is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	White powder
Lot/Batch number:	SMU4FL762
Purity	96.7 % w/w
CAS#:	1228284-64-7
Stability of test compound:	Not indicated by the sponsor

Control Materials:	
Negative:	Concurrent untreated and solvent controls were performed
Solvent control (final concentration):	100 µl/plate
Positive control:	Nonactivation: Sodium azide 10 µg/plate TA100, TA1535 4-nitro-o-phenylene-diamine, 50 µg/plate TA 1537, 10 µg/plate TA98 methyl methane sulfonate 2 µL/plate WP2 (pKM101), WP2 uvrA (pKM101)
	Activation: 2-Aminoanthracene 2.5 µg/plate TA1535, TA1537, TA100, TA98 10 µg/plate WP2 (pKM101), WP2 uvrA (pKM101)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbital		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 10% v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8	mM	MgCl ₂
33	mM	KCl
5	mM	Glucose-6-phosphate
4	mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4. During the experiment the S9 mix was stored in an ice bath.

Test organisms:

S. typhimurium strains									
	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		list any others		
E. coli strains									
X	WP2 (pKM101)	X	WP2 uvrA (pKM101)						
Properly maintained?						X	Yes		No
Checked for appropriate genetic markers (rfa mutation, R factor)?						X	Yes		No

Test compound concentrations used

The test substance was tested at the following concentrations in both experiments:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Study Design and Methods:

In-life dates: Start: 27 August 2014

End: 10 September 2014

Test Performance

Preliminary Cytotoxicity Assay: Not performed.

Type of Bacterial assay:

X standard plate test (pre-experiment/experiment I; –S9, +S9)

X pre-incubation (60 minutes) (second experiment ; –S9, +S9)

___ “Prival” modification (i.e. azo-reduction method)

___ spot test

___ other

Protocol:

For each strain and dose level including the controls, three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µL Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),

500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),

100 µL Bacteria suspension (cf. test system, pre-culture of the strains; OD = 0.8 - 1.2, wavelength = 500 nm; approx. 8×10^8 cells/mL), 50 µL of bacteria suspension of strain TA1535 (experiment I) and 100 µL in experiment Ia (reported as part of experiment I)

2000 µL Overlay agar

For the pre-incubation method 100 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer* and 100 µL bacteria suspension were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45°C) was added to each tube. The mixture was poured on selective agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

* Substitution buffer: 7 parts of the 100 mM sodium-ortho-phosphate-buffer pH 7.4 with 3 parts of KCl solution 0.15 M

Statistical analysis: None – see Evaluation Criteria below.

Evaluation criteria: A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

RESULTS

Preliminary cytotoxicity assay: Not performed.

Mutagenicity assay: The test substance SYN545974 was assessed for its potential to induce gene mutations in the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using *S. typhimurium* strains TA1535, TA1537, TA98, and TA100, and the *E. coli* strains WP2 uvrA pKM101 and WP2 pKM101.

The assay was performed with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test substance was tested at the following concentrations in both experiments:

3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

The test item precipitated in the overlay agar in the test tubes from 1000 to 5000 µg/plate. Precipitation of the test item in the overlay agar on the incubated agar plates was observed from 1000 to 5000 µg/plate. The undissolved particles had no influence on the data recording.

The plates incubated with the test item showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

Cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), was observed at the following concentrations (µg/plate):

Strain	Experiment I		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	/	/	/	/
TA 1537	1000 - 5000	2500 - 5000	/	/
TA 98	/	/	/	5000
TA 100	/	/	/	/
WP2 pKM101	/	/	/	/
WP2 uvrA pKM101	/	/	/	/

/ = no cytotoxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5)

No increase in revertant colony numbers of any of the six tester strains was observed following treatment with SYN545974 at any concentration, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in the number of induced revertant colonies.

In experiment I with metabolic activation (untreated control 222 vs 245 colonies) and experiment II, without metabolic activation (untreated and solvent control; 185 vs 189 and 211 vs 213 colonies respectively), the number of colonies did not quite reach the lower limit of the historical control data in strain WP2 pKM101. Since this deviation is small, this effect is judged to be based upon statistical fluctuations and has no detrimental impact on the outcome of the study (Tables 6.4.1-3 and 6.4.1-4).

Table 6.4.1-3: Summary of results Pre-Experiment/Experiment I

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	DMSO Untreated SYN545974	3 µg	10 ± 3	18 ± 6	25 ± 5	148 ± 18	206 ± 7	380 ± 26
			8 ± 1	12 ± 3	33 ± 5	157 ± 8	216 ± 23	375 ± 25
			10 ± 4	16 ± 6	25 ± 8	155 ± 11	213 ± 25	388 ± 10
			10 ± 2	14 ± 6	28 ± 4	137 ± 2	192 ± 14	331 ± 9
			11 ± 1	14 ± 5	27 ± 4	147 ± 12	218 ± 14	340 ± 9
			10 ± 4	10 ± 3	28 ± 3	144 ± 15	201 ± 11	340 ± 23
		333 µg	11 ± 3	11 ± 6	27 ± 1	152 ± 7	225 ± 23	365 ± 30
		1000 µg	10 ± 2 ^P	6 ± 2 ^{MP}	31 ± 4 ^P	160 ± 13 ^P	201 ± 12 ^P	365 ± 30 ^P
		2500 µg	10 ± 1 ^P	7 ± 1 ^{MP}	19 ± 3 ^M	159 ± 10 ^P	197 ± 11 ^P	369 ± 10 ^P
		5000 µg	11 ± 1 ^P	4 ± 1 ^{MP}	22 ± 1 ^M	180 ± 23 ^P	209 ± 23 ^P	360 ± 25 ^P
		NaN3	10 µg	846 ± 26	372 ± 32	2026 ± 93		
With Activation	DMSO Untreated SYN545974	3 µg	15 ± 1	20 ± 5	43 ± 9	146 ± 15	204 ± 36	415 ± 7
			14 ± 2	19 ± 6	39 ± 4	151 ± 5	222 ± 31	426 ± 36
			19 ± 6	18 ± 9	40 ± 4	149 ± 13	202 ± 3	387 ± 40
			18 ± 7	20 ± 4	41 ± 7	149 ± 15	178 ± 2	336 ± 11
			18 ± 4	17 ± 4	37 ± 8	139 ± 6	196 ± 22	346 ± 17
			17 ± 4	19 ± 6	40 ± 6	143 ± 20	201 ± 6	396 ± 15
		333 µg	11 ± 2	17 ± 8	38 ± 4	130 ± 11	225 ± 20	392 ± 19
		1000 µg	14 ± 3 ^P	10 ± 2 ^M	29 ± 6 ^M	132 ± 8 ^P	195 ± 43 ^P	359 ± 9 ^P
		2500 µg	12 ± 1 ^M	6 ± 1 ^{MP}	22 ± 2 ^M	140 ± 4 ^M	133 ± 8 ^{MP}	324 ± 8 ^{MP}
		5000 µg	8 ± 3 ^{MP}	5 ± 2 ^{MP}	20 ± 1 ^M	132 ± 4 ^M	131 ± 10 ^{MP}	281 ± 19 ^{MP}
		2-AA	2.5 µg	499 ± 18	275 ± 43	2767 ± 116	3189 ± 133	1747 ± 156
		2-AA	10.0 µg					1800 ± 118

Key to Positive Controls

Key to Plate Postfix Codes

NaN3 sodium azide
 2-AA 2-aminoanthracene
 4-NOPD 4-nitro-o-phenylene-diamine
 MMS methyl methane sulfonate

P Precipitate
 M Manual count

* date of the repeated experiment with strain TA 1535 without S9 mix

Table 6.4.1-3: Summary of results Experiment II

Metabolic Activation	Test Group	Concentration (per plate)	Revertant Colony Counts (Mean ±SD)					
			TA 1535	TA 1537	TA 98	TA 100	WP2 pKM101	WP2 uvrA pKM101
Without Activation	DMSO Untreated SYN545974	3 µg	11 ± 2	12 ± 3	20 ± 3	97 ± 12	185 ± 14	305 ± 24
			12 ± 2	9 ± 1	21 ± 1	115 ± 10	211 ± 8	325 ± 21
			11 ± 3	12 ± 3	21 ± 7	89 ± 5	190 ± 22	285 ± 22
			13 ± 1	10 ± 3	16 ± 1	88 ± 8	180 ± 12	284 ± 28
			10 ± 4	10 ± 3	22 ± 5	82 ± 16	170 ± 15	277 ± 8
			11 ± 3	10 ± 2	21 ± 1	89 ± 21	182 ± 23	292 ± 18
		333 µg	13 ± 4	8 ± 2	20 ± 4	84 ± 23	177 ± 14	302 ± 16
		1000 µg	12 ± 2 ^P	6 ± 3 ^{PM}	17 ± 2 ^P	109 ± 2 ^P	178 ± 25 ^P	278 ± 14 ^P
		2500 µg	12 ± 3 ^P	8 ± 2 ^{PM}	17 ± 5 ^P	107 ± 7 ^P	178 ± 10 ^P	293 ± 19 ^P
		5000 µg	11 ± 2 ^P	6 ± 2 ^{PM}	13 ± 5 ^P	91 ± 8 ^P	202 ± 28 ^P	276 ± 31 ^P
		NaN3	10 µg	996 ± 19	453 ± 32	2174 ± 175		
With Activation	DMSO Untreated SYN545974	3 µg	14 ± 1	14 ± 2	40 ± 4	102 ± 13	205 ± 11	375 ± 19
			14 ± 2	13 ± 3	31 ± 6	104 ± 8	264 ± 28	422 ± 17
			11 ± 1	15 ± 2	33 ± 3	84 ± 17	222 ± 7	388 ± 44
			14 ± 3	15 ± 0	33 ± 7	93 ± 6	221 ± 8	352 ± 49
			12 ± 3	12 ± 3	34 ± 8	79 ± 15	218 ± 19	366 ± 30
			13 ± 3	15 ± 1	40 ± 2	88 ± 12	213 ± 13	374 ± 34
		333 µg	14 ± 5	13 ± 2	43 ± 1	72 ± 12	200 ± 38	394 ± 27
		1000 µg	10 ± 2 ^P	12 ± 1 ^P	30 ± 2 ^P	75 ± 7 ^P	222 ± 22 ^P	339 ± 48 ^P
		2500 µg	12 ± 1 ^P	11 ± 1 ^P	26 ± 2 ^P	67 ± 4 ^P	200 ± 4 ^{PM}	307 ± 27 ^{PM}
		5000 µg	9 ± 3 ^{PM}	10 ± 2 ^P	18 ± 3 ^P	64 ± 9 ^P	196 ± 4 ^{PM}	231 ± 19 ^{PM}
		2-AA	2.5 µg	353 ± 37	135 ± 9	3138 ± 407	2897 ± 313	1774 ± 166
		2-AA	10.0 µg					1845 ± 143

Key to Positive Controls

Key to Plate Postfix Codes

NaN3 sodium azide
 2-AA 2-aminoanthracene
 4-NOPD 4-nitro-o-phenylene-diamine
 MMS methyl methane sulfonate

P Precipitate
 M Manual count

CONCLUSION:

In this GLP and OECD test guideline compliant Ames test ([REDACTED], 2014), *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 and *Escherichia coli* strains WP2 uvrA pKM101 and WP2 pKM101 were exposed to concentrations of 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate pydiflumetofen in the presence and absence of mammalian metabolic activation in the plate incorporation test (experiment 1) and the pre-incubation test (experiment 2). The batch tested in this assay (SMU4FL762) was spiked with potential impurities to support the technical specification of pydiflumetofen.

The plates incubated with the test substance displayed normal background growth up to 5000 µg/plate with and without metabolic activation. Cytotoxic effects (evident as a reduction in the number of revertants, below the indication factor of 0.5) were observed in experiment 1 in strain TA1537 with and without metabolic activation. Cytotoxic effects were also observed in experiment 2 in strain TA98 with metabolic activation.

After exposure to pydiflumetofen, there was no substantial increase in revertant colony numbers of any strain tested at any concentration, either with or without metabolic activation. There was also no tendency of higher mutation rates with increasing test item concentrations in the range below the border of biological relevance. The reference mutagens used as positive controls showed a distinct increase in revertant colony numbers, demonstrating the sensitivity and validity of the test system.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, pydiflumetofen is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

HSE notes that cytotoxic effects and precipitation were observed in the study, therefore the test item was tested at high enough concentrations to be adequately investigated for genotoxic potential in this in vitro bacterial mutation assay.

([REDACTED], 2014)

Report:	K-CA 5.4.1/03 [REDACTED] (2013). SYN545974 - Chromosome Aberration Test in Human Lymphocytes In Vitro. Harlan Cytotest Cell Research GmbH (Harlan CCR), In den Leppsteinswiesen 19, 64380 Rossdorf, Germany. Laboratory Report No. 1498902, issue date: 21 January 2013. Unpublished. Syngenta File No. SYN545974_10048.
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Guidelines: Chromosome Aberration Test in Human Lymphocytes In Vitro. OECD 473 (1997); OPPTS 870.5375 (1998); EC 440/2008 B10 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

This *in vitro* assay was performed to assess the potential of SYN545974 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 control in Experiment IIA without metabolic activation where only 50 metaphases were evaluated.

The highest applied concentration in this study (4332.0 $\mu\text{g/mL}$ of the test substance, approx. 10 mM) was chosen with regard to the molecular weight and the purity (98.5 %) of the test substance and with respect to the current OECD Guideline 473.

Dose selection of the cytogenetic experiments was performed considering the toxicity data and test substance precipitation and in accordance with OECD Guideline 473.

In Experiment I and IIA in the absence of S9 mix, concentrations showing clear cytotoxicity were not scorable for cytogenetic damage. In Experiment I and IIA in the presence of S9 mix and in the confirmatory Experiment IIB in the absence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

In Experiment I in the absence and presence of S9 mix and in Experiment IIA in the presence of S9 mix, no clastogenicity was observed at the concentrations evaluated. 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 $\mu\text{g/mL}$. No dose-dependency was observed. In the confirmatory Experiment IIB statistically significant increases occurred after treatment with 20.0 and 40.0 $\mu\text{g/mL}$ (7.5 and 9.5 % aberrant cells, excluding gaps) clearly exceeding the laboratory historical solvent control range of 0.0 - 3.0 %.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test substance as compared to the control cultures.

Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p < 0.05$) in cells with structural chromosome aberrations.

In conclusion, it can be stated that under the experimental conditions reported, the test substance induced structural chromosomal aberrations in human lymphocytes *in vitro*. Therefore, SYN545974 is considered to be clastogenic in this chromosome aberration test in the absence of metabolic activation, when tested up to the highest required and/or precipitating concentrations.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 % w/w, dose calculation adjusted to purity
CAS#:	1228284-64-7
Stability of test compound:	Not indicated by the sponsor

Control Materials:	
Negative:	-

Control Materials:	
Solvent control (final concentration):	DMSO (1.0 %)
Positive control:	Absence of S9 mix: Ethyl methanesulfonate, 770.0 µg/mL (Experiment I, IIA and IIB)
	Presence of S9 mix: Cyclophosphamide, 20.0 µg/mL (Experiment I), 15.0 µg/mL (Experiment IIA)

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		
X indicates those that apply							

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM MgCl₂
 33 mM KCl
 5 mM glucose-6-phosphate
 4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4. During the experiment, the S9 mix was stored in an ice bath.

Test cells: mammalian cells in culture

	V79 cells (Chinese hamster lung fibroblasts)
X	Human lymphocytes. Obtained on the days of culture initiation from healthy, non-smoking donors. All donors had a previously established low incidence of chromosomal aberrations in their peripheral blood lymphocytes.
	Chinese hamster ovary (CHO) cells
X indicates those that apply	

Media: DMEM/Ham's F12 (1:1)				
Properly maintained?	X	Yes		No
Periodically checked for Mycoplasma contamination?		Yes		No
Periodically checked for karyotype stability?		Yes		No
X indicates those that apply				

Test compound concentrations used:

Absence of S9 mix	Experiment I Experiment IIA Experiment IIB	16.1, 28.1, 150.8 µg/mL 5.3, 9.2, 16.1 µg/mL 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 15.0, 20.0, 40.0 µg/mL
Presence of S9 mix	Experiment I Experiment IIA	16.1, 28.1, 49.2 µg/mL 9.2, 16.1, 2475.4, 4332.0 µg/mL

Study Design and Methods

In-life dates: Start 30 July 2012 End: 29 November 2012

Test Performance

Preliminary Cytotoxicity Assay: Not performed.

Cytogenetic Assay:

Cell exposure time:		Test Material	Solvent Control	Positive Control
- S9 mix	Experiment I	4 h	4 h	4 h
+ S9 mix	Experiment II	4 h	4 h	4 h
-S9 mix		22 h	22 h	22 h
+ S9 mix		4 h	4 h	4 h

Spindle inhibition:	
Inhibitor used/ concentration:	Colcemid 0.2 µg/mL
Administration time:	3 hours (before cell harvest)

Cell harvest time after termination of treatment:	Test Material	Solvent Control	Positive Control
- S9 mix (4 hour treatment)	18 h	18 h	18 h
+ S9 mix (4 hour treatment)	18 h	18 h	18 h
- S9 mix (22 hour treatment)	0 h	0 h	0 h

Exposure time 4 hours: About 72 h after seeding for each test group 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks. The culture medium was replaced with serum-free medium containing the test substance. For the treatment with metabolic activation 50 µL S9 mix per mL medium were used. Concurrent solvent and positive controls were performed. After 4 h the cells were spun down by gentle centrifugation for 5 minutes (approx. 900 x g). The supernatant with the dissolved test substance was discarded and the cells were re-suspended in "saline G". The washing procedure was repeated once as described.

The "saline G" solution was composed as follows (per litre):

NaCl 8000 mg
 KCl 400 mg
 glucose•H₂O 1100 mg
 Na₂HPO₄•2H₂O 192 mg
 KH₂PO₄ 150 mg
 pH was adjusted to 7.2

After washing the cells were re-suspended in complete culture medium and cultured until preparation.

Exposure time 22 hours (without S9 mix): About 72 h after seeding for each test group 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks. The culture medium was replaced with complete medium (with 10 % FBS) containing the test substance without S9 mix. The culture medium at continuous treatment was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

All cultures were incubated at 37 °C in a humidified atmosphere with 5.5 % CO₂ (94.5 % air).

Details of slide preparation: Three hours before harvesting, Colcemid was added to the cultures (final concentration 0.2 µg/mL). The cultures were harvested by centrifugation for 5 minutes (approx. 900 x g) 22 h after beginning of treatment. The supernatant was discarded and the cells were re-suspended in

approximately 5 mL hypotonic solution (0.0375 M KCl). The cell suspension was then allowed to stand at 37 °C for 20 minutes. After removal of the hypotonic solution by centrifugation for 5 minutes (approx. 900 x g) the cells were fixed with a mixture of methanol and glacial acetic acid (3 parts plus 1 part). At least two slides per experimental group were prepared by dropping the cell suspension onto a clean microscope slide. The cells for evaluation of cytogenetic damage were stained with Giemsa.

Metaphase analysis

No. of cells examined per dose: At least 100, except for the positive control in Experiment IIA without metabolic activation where only 50 metaphases were evaluated.				
Scored for structural?	X	Yes		No
Scored for numerical?		Yes (polyploidy noted if observed)	X	No
Coded prior to analysis?	X	Yes		No
X indicates those that apply				

Evaluation criteria: A test substance is classified as non-mutagenic if:

The number of induced structural chromosome aberrations in all evaluated dose groups is in the range of the laboratory historical control data (see Appendix I).

No statistically significant increase of the number of structural chromosome aberrations is observed.

A test substance is classified as mutagenic if:

The number of induced structural chromosome aberrations is not in the range of the laboratory historical control data (see Appendix I)

and

Either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

A test substance not meeting the criteria for classification as non-mutagenic or mutagenic may be considered equivocal in this assay and may be subject to further investigation.

Statistical analysis: Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the above mentioned criteria for the test substance are not clearly met, the classification with regard to the historical data and the biological relevance is discussed and/or a confirmatory experiment is performed.

RESULTS

Preliminary cytotoxicity assay: Not performed.

Cytogenetic assay: In Experiment I and IIA in the absence of S9 mix, concentrations showing clear cytotoxicity were not scorable for cytogenetic damage. In Experiment I and IIA in the presence of S9 mix and in Experiment IIB in the absence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

In Experiment I in the absence and presence of S9 mix and in Experiment IIA in the presence of S9 mix, no clastogenicity was observed at the concentrations evaluated. The aberration rates of the cells after treatment with the test substance (0.5 – 3.0 % aberrant cells, excluding gaps) were slightly above the solvent control values (0.5 – 1.0 % aberrant cells, excluding gaps) but within the range of the laboratory historical solvent control data. 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. No dose-dependency was observed. In the confirmatory Experiment IIB statistically significant increases occurred after treatment with 20.0 and 40.0 µg/mL (7.5 and 9.5 % aberrant cells, excluding gaps) clearly exceeding the laboratory historical solvent control range of 0.0-3.0 % (Table 6.4.1-1).

No evidence of an increase in polyploid metaphases was noticed after treatment with the test substance as compared to the control cultures. Either EMS (770 µg/mL) or CPA (15.0 or 20.0 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

Table 6.4.1-1: summary of results of the chromosomal aberration study with SYN545974

Exp.	Preparation interval	Test substance concentration in µg/mL	Mitotic indices in % of control	Aberrant cells		
				incl. gaps*	excl. gaps*	carrying exchanges
Exposure period 4 hrs without S9 mix						
I	22 hrs	Solvent control ¹	100.0	0.5	0.5	0.0
		Positive control ²	86.5	10.0	9.5 ^S	1.0
		16.1	84.5	1.0	1.0	0.0
		28.1 ^P	88.1	2.0	2.0	0.0
		150.8 ^P	94.4	1.0	1.0	0.0
Exposure period 22 hrs without S9 mix						
IIA	22 hrs	Solvent control ¹	100.0	0.5	0.5	0.0
		Positive control ^{2#}	68.9	43.0	42.0 ^S	17.0
		5.3 ^{##}	89.4	6.5	6.5 ^S	0.0
		9.2	104.4	2.0	1.5	0.0
		16.1 ^P	105.6	1.0	1.0	0.0
IIB	22 hrs	Solvent control ¹	100.0	1.5	1.0	0.0
		Positive control ²	37.6	21.5	21.5 ^S	6.5
		3.0	117.4	0.5	0.5	0.0
		4.0	110.3	2.0	2.0	0.0
		5.0	111.6	1.0	1.0	0.0
		6.0	106.6	0.5	0.5	0.0
		7.0	83.5	0.5	0.5	0.0
		10.0	95.5	0.0	0.0	0.0
		15.0 ^{##}	76.9	3.5	3.0	0.3
		20.0 ^{P##}	76.0	7.8	7.5 ^S	0.3
		40.0 ^P	73.1	10.5	9.5 ^S	0.0

Exp.	Preparation	Test substance	Mitotic indices	Aberrant cells		
	interval	concentration	in %	in %		carrying exchanges
		in µg/mL	of control	incl. gaps*	excl. gaps*	
Exposure period 4 hrs with S9 mix						
I	22 hrs	Solvent control ¹	100.0	0.5	0.5	0.0
		Positive control ²	75.7	19.5	18.0^S	4.5
		16.1	110.5	1.0	1.0	0.0
		28.1	123.8	0.5	0.5	0.0
		49.2 ^P	123.4	2.5	2.0	0.0
IIA	22 hrs	Solvent control ¹	100.0	1.0	1.0	0.0
		Positive control ³	54.8	13.5	13.5^S	4.0
		9.2	95.0	1.5	1.0	0.0
		16.1 ^P	110.0	2.0	2.0	0.0
		2475.4 ^P	91.3	3.0	3.0	0.0
		4332.0 ^P	63.0	2.0	2.0	0.0

* Including cells carrying exchanges

^P Precipitation occurred at the end of treatment

^S Aberration frequency statistically significant higher than corresponding control values

¹ DMSO 1.0% (v/v)² CPA 20.0 µg/mL³ CPA 15.0 µg/mL**CONCLUSION:**

In this GLP and OECD test guideline compliant in vitro chromosome aberration test in human lymphocytes (██████████, 2013), human lymphocytes were exposed to pydiflumetofen in the presence and absence of mammalian metabolic activation at the following concentrations:

Metabolic activation	Experiment	Concentration of test compound (µg/mL)
Absence of S9 mix	Experiment I (4hr)	16.1, 28.1, 150.8
	Experiment IIA (22hr)	5.3, 9.2, 16.1
	Experiment IIB (22hr)	3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 15.0, 20.0, 40.0
Presence of S9 mix	Experiment I (4hr)	16.1, 28.1, 49.2
	Experiment IIA (22hr)	9.2, 16.1, 2475.4, 4332.0

In experiment I and IIA without metabolic activation, concentrations showing clear cytotoxicity were not scorable for cytogenetic damage. In experiment I and IIA with metabolic activation and experiment IIB without metabolic activation, no cytotoxicity was observed up to the top concentration applied.

In Experiment I with and without metabolic activation and in Experiment IIA with metabolic activation, no clastogenicity was observed at the concentrations evaluated. The aberration rates of the cells after treatment with the test substance (0.5-3.0 % aberrant cells, excluding gaps) were slightly above the solvent control values (0.5-1.0 % aberrant cells, excluding gaps) but within the range of the laboratory historical solvent control data. In Experiment IIA without metabolic activation, there was 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) observed after treatment with 5.3 µg/mL test substance. There was no dose-dependency observed. In the confirmatory Experiment IIB, statistically significant increases occurred after treatment with 20.0 and 40.0 µg/mL test substance (7.5 and 9.5 % aberrant cells, excluding gaps), clearly exceeding the laboratory historical solvent control range of 0.0-3.0 %. Overall, a weakly positive/equivocal response was seen in the absence of metabolic activation.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test substance as compared to the control cultures. The positive controls showed distinct increases in cells with structural chromosome aberrations.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen induced a small increase in structural chromosomal aberrations in human lymphocytes in vitro in the absence of metabolic activation. No significant cytotoxicity was evident, although precipitation occurred at the highest concentrations tested. Precipitation did not impede scoring. Therefore, pydiflumetofen gave a weakly positive/equivocal response in the absence of metabolic activation in this in vitro chromosome aberration study.

(██████████, 2013)

Report:	K-CA 5.4.1/04 ██████████ (2013). SYN545974 - Cell Mutation Assay at the Thymidine Kinase Locus (TK ^{+/+}) in Mouse Lymphoma L5178Y Cells. Harlan Cytotest Cell Research GmbH (Harlan CCR), In den Leppsteinswiesen 19, 64380 Rossdorf, Germany. Laboratory Report No. 1498903, issue date: 24 January 2013. Unpublished. Syngenta File No. SYN545974_10049.
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Guidelines: *In Vitro* Mammalian Cell Gene Mutation Test OECD 476 (1997): OPPTS 870.5300 (1998): EC 440/2008 B17 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

The study was performed to investigate the potential of SYN545974 to induce mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y.

The assay was performed in three independent experiments, using two parallel cultures each. Experiments I and II were performed with and without liver microsomal activation and a treatment period of 4 hours at concentrations of :

In the absence of S9 mix

Experiment I: 7.5; 15.0; 22.5; 30.0; 60.0

Experiment II: 7.5; 15.0; 30.0; 45.0; 60.0 µg/mL

In the presence of S9 mix

Experiment I: 7.5; 15.0; 30.0; 45.0; 60.0

Experiment II 7.5; 15.0; 30.0; 60.0; 90.0

Experiment III 40.0; 80.0; 90.0; 100.0; 110.0.

Experiment III was solely performed in the presence of metabolic activation with a treatment period of 4 hours. The concentration range of the main experiments was limited by cytotoxic effects. No precipitation of the test item occurred up to the maximum concentration with and without metabolic activation.

Relevant cytotoxic effects indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50% in both cultures occurred in experiment I at 60 µg/mL without metabolic activation. In experiment II toxic effects as described above were noted at 45.0 µg/mL and above in the absence of metabolic activation. In experiment III, solely performed with metabolic activation, toxic effects were noted at 80.0 µg/mL and above.

No substantial or reproducible dose dependent increase in mutant colony numbers was observed at acceptable levels of cytotoxicity. Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced mutant colonies, indicating that the tests were sensitive and valid.

In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported SYN545974 did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation. Therefore, SYN545974 is considered to be non-mutagenic in this mouse lymphoma assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off white, solid
Lot/Batch number:	SMU2EP12007
Molecular weight:	426.7 g/mol
Purity	98.5% w/w
CAS#:	1228284-64-7
Stability of test compound:	Not indicated by the sponsor
Control Materials:	
Negative:	-
Solvent control (final concentration):	DMSO (0.5 %)
Positive control:	Absence of S9 mix: Methyl methanesulphonate, 19.5 µg/mL
	Presence of S9 mix: Cyclophosphamide (CPA), 3.0 / 4.5 µg/mL

Mammalian metabolic system: S9 derived

X	Induced		Aroclor 1254	X	Rat	X	Liver
	Non-induced	X	Phenobarbitol		Mouse		Lung
			None		Hamster		Other
		X	Other β-naphthoflavone		Other		

X indicates those that apply

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to give a final protein concentration of 0.75 mg/mL in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM MgCl₂
 33 mM KCl
 5 mM glucose-6-phosphate
 4 mM NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4. The concentration in the final test medium was 5 % (v/v).

Test cells: mammalian cells in culture

X	Mouse lymphoma L5178Y cells		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells		List any others
Media: RPMI 1640			
Properly maintained?	X	Yes	No
Periodically checked for Mycoplasma contamination?	X	Yes	No
Periodically checked for karyotype stability?	X	Yes	No
Periodically “cleansed” against high spontaneous background?	X	Yes	No

X indicates those that apply

Locus Examined:		Thymidine kinase (TK)		Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT)		Na ⁺ /K ⁺ ATPase
Selection agent:		Bromodeoxyuridine (BrdU)		8-azaguanine (8-AG)		ouabain
		Fluorodeoxyuridine (FdU)		6-thioguanine (6-TG)		
	X	Trifluorothymidine (TFT)				

X indicates those that apply

Test compound concentrations used:

Absence of S9 mix 7.5; 15.0; 22.5; 30.0; 60.0 µg/mL (experiment I)
 7.5; 15.0; 30.0; 45.0; 60.0 µg/mL (experiment II))
 Presence of S9 mix 7.5; 15.0; 30.0; 45.0; 60.0 µg/mL (experiment I)
 7.5; 15.0; 30.0; 60.0; 90.0 µg/mL L (experiment II)
 40.0; 80.0; 90.0; 100.0; 110.0 µg/mL (experiment III)

Study Design and Methods:

In-life dates: Start: 05 September 2012, End: 11 December 2012

Test performance:

Cell treatment: Cells were exposed to test compound, negative/solvent or positive controls for 4 hours in both the presence and absence of S9 mix.

After washing, cells were cultured for 2 days (expression period) before cell selection.

After expression, cells from each experimental group were seeded into 2 microtiter plates so that each well contained approximately 4×10^3 cells in selective medium. Dilutions of the cultures to approximately 2 cells/well were cultured for 10-15 days without selective agent to determine cloning efficiency.

Cell growth in individual microwell plates was assessed after 10-15 days using a dissecting microscope. The survival plates and viability plates were scored for the number of wells containing no cell growth (negative wells). The mutation plates were scored so that each well contained either a small colony (considered to be associated with clastogenic effects), a large colony (considered to be associated with gene mutation effects), or no colony.

Statistical Methods:

A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 (SYSTAT Software, Inc., 501, Canal Boulevard, Suite C, Richmond, CA 94804, USA) statistics software. The number of mutant colonies obtained for the groups treated with the test item was compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. However, both, biological relevance and statistical significance were considered together.

Evaluation Criteria: Each well of the mutation plates (those containing TFT) was scored as containing either, a small colony, a large colony or no colony according to the following criteria:

Colonies were counted manually. In accordance with their size the colonies were classified into two groups. The colony size distribution was determined in the controls and at all concentrations of the test item. Criteria to determine colony size were the absolute size of the colony (more than 1/3 of a well for large colonies) and the optical density of the colonies (the optical density of the small colonies is generally higher than the large colonies).

RESULTS

Preliminary toxicity assay:

The pre-experiment was performed in the presence and absence of metabolic activation with a treatment time of 4 hours. Test substance concentrations between 15.6 µg/mL and 2000 µg/mL were used. The highest concentration in the pre-experiment was chosen with regard to the solubility of the test substance in organic solvents and aqueous medium.

Toxic effects leading to RSG values below 50% were observed following 4 hours treatment at 31.3 µg/mL and above without and at 15.6 µg/mL and above with metabolic activation.

The test medium was checked for precipitation at the end of the treatment period (4 hours) before the test substance was removed. Precipitation was observed at 250 µg/mL and above in the absence of metabolic activation and at 125 µg/mL and above in the presence of metabolic activation.

Both, pH value and osmolarity was determined at the maximum concentration of the pre-experiment and in the solvent control without metabolic activation. No relevant change of the osmolarity or pH value was observed (solvent control: 365 mOsm, pH 7.65 versus 341 Osm and pH 7.57 at 2000 µg/mL).

The concentrations used in the main experiments were selected based on the results obtained in the pre-experiment.

To overcome problems with possible deviations of the solubility the main experiments were started with more than four concentrations. The individual concentrations were generally spaced by a factor of 2. Narrower spacing was used at high concentrations to cover the cytotoxic range more closely. The upper concentrations of the third experiment were very narrowly spaced to cover the recommended cytotoxic range of approximately 10-20% relative total growth.

Following the expression phase of 48 hours the cultures at 1.9 and 3.8 µg/mL in experiment I and II without metabolic activation were not continued since a minimum of only four analysable concentrations are required by the test guidelines. The cultures at 3.8 µg/mL in experiment I and II with metabolic activation were not continued for the same reason. The cultures at the highest concentration of 120 µg/mL in experiment I and II with metabolic activation were not continued due to exceedingly severe cytotoxic effects. In experiment III, solely performed with metabolic activation, the cultures at 120 µg/mL were not continued based on exceedingly severe cytotoxic effects. The cultures at 20 µg/mL were not continued since a minimum of only four analysable concentrations are required by the test guidelines.

Mutation assay:

The study was performed to investigate the potential of SYN545974 to induce mutations at the mouse lymphoma thymidine kinase locus of the cell line L5178Y.

The assay was performed in three independent experiments, using two parallel cultures each. Experiments I and II were performed with and without liver microsomal activation and a treatment period of 4 hours. Experiment III was solely performed in the presence of metabolic activation with a treatment period of 4 hours.

The main experiments were evaluated at the following concentrations:

Experiment I:

without S9 mix:	7.5; 15.0; 22.5; 30.0; and 60.0 µg/mL
with S9 mix:	7.5; 15.0; 30.0; 45.0; and 60.0 µg/mL

Experiment II:	
without S9 mix:	7.5; 15.0; 30.0; 45.0; and 60.0 µg/mL
with S9 mix:	7.5; 15.0; 30.0; 60.0; and 90.0 µg/mL

Experiment III:	
with S9 mix:	40.0; 80.0; 90.0; 100.0; and 110.0 µg/mL

No precipitation of the test item was noted in experiment I, II, or III.

Relevant cytotoxic effects indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50% in both cultures occurred in experiment I at 60.0 µg/mL without metabolic activation.

In experiment II cytotoxic effects in both parallel cultures were noted at 45.0 µg/mL and above in the absence of metabolic activation.

In experiment III cytotoxic effects in both parallel cultures were noted at 80.0 µg/mL and above. The recommended cytotoxic range of approximately 10-20% relative total growth or relative cloning efficiency I was covered with and without metabolic activation.

No substantial or reproducible dose dependent increase of the mutation frequency exceeding the threshold of 126 above the corresponding solvent control was observed in the main experiments up to the maximum concentration tested with and without metabolic activation at acceptable levels of cytotoxicity.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies using SYSTAT®11 statistics software. A significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was detected in the second culture of the first experiment without metabolic activation and in the first culture of the second experiment with metabolic activation. These trends however, were judged as irrelevant since they actually were reciprocal, going down versus increasing concentrations.

In this study the range of the solvent controls was from 46 up to 80 mutant colonies per 10P^{6P} cells; the range of the acceptable groups treated with the test substance was from 20 up to 126 mutant colonies per 10P^{6P} cells.

The lowest solvent controls fell just short of the lower limit of the spontaneous mutation frequency of the acceptance criteria (46 and 48 versus 50 colonies per 10⁶ cells). These deviations were judged as biologically irrelevant as they were minor and the solvent controls of the parallel cultures met the acceptance criteria. The cloning efficiency 2 (viability) exceeded the upper limit of 120% with 131%. However, the corresponding value of the parallel culture (84%) remained well within the acceptable range. Viability values above 120% occasionally occur as the cells do not really form an ideal solution even though they grow in suspension. The cells transiently form aggregates that are counted as individual cells and thus may push the viability to values above 100%. As the measured values of the viability are used to calculate the mutation frequency no bias is caused by viability values above the upper limit of 120%. The viability fell just short of the lower limit of 65% in the solvent control of the second culture of the second experiment with metabolic activation (62%). The data are acceptable however, as the deviation was minor and the viability of the parallel culture was 77% (Table 6.4.1-2).

MMS (19.5 µg/mL) and CPA (3.0 and 4.5 µg/mL) were used as positive controls and showed a distinct increase in induced total mutant colonies at acceptable levels of toxicity with at least one of the concentrations of the controls. The CPA control with 4.5 µg/mL in experiment I was rejected as the RTG values fell short of the 10% limit, however the data at 3.0 µg/mL were acceptable.

Table 6.4.1-2: summary of Results

Experiment I and II

			relative	relative	mutant		relative	relative	mutant	
	conc. µg	S9	cloning	total	colonies/		cloning	total	colonies/	
	per mL	mix	efficiency I	growth	10 ⁶ cells	threshold	efficiency I	growth	10 ⁶ cells	threshold
Column	1	2	3	4	5	6	7	8	9	10
Experiment I / 4 h treatment			culture I				culture II			
Solv. control with DMSO		-	100.0	100.0	48	174	100.0	100.0	69	195
Pos. control with MMS	19.5	-	98.1	37.2	236	174	88.4	54.0	287	195
Test item	1.9	-	75.1	culture was not continued [#]			98.2	culture was not continued [#]		
Test item	3.8	-	112.8	culture was not continued [#]			62.2	culture was not continued [#]		
Test item	7.5	-	91.3	74.0	59	174	49.5	94.2	82	195
Test item	15.0	-	98.1	54.4	45	174	80.2	63.7	47	195
Test item	22.5	-	82.6	45.7	83	174	86.9	116.0	41	195
Test item	30.0	-	88.2	54.0	60	174	89.9	107.7	36	195
Test item	60.0	-	12.9	5.3	50	174	4.5	7.6	20	195
Solv. control with		+	100.0	100.0	56	182	100.0	100.0	60	186
Pos. control with CPA	3.0	+	22.6	16.2	264	182	34.9	17.0	370	186
Pos. control with CPA	4.5	+	4.6	2.3	740	182	7.3	2.8	688	186
Test item	3.8	+	98.3	culture was not continued [#]			116.6	culture was not continued [#]		
Test item	7.5	+	81.1	75.7	39	182	111.2	79.1	67	186
Test item	15.0	+	101.7	64.0	62	182	100.0	69.9	72	186
Test item	30.0	+	98.3	73.1	37	182	118.5	73.3	81	186
Test item	45.0	+	118.1	80.1	54	182	109.5	60.8	58	186
Test item	60.0	+	66.1	51.1	30	182	86.4	25.7	64	186
Test item	120.0	+	0.3	culture was not continued ^{##}			4.8	culture was not continued ^{##}		
Experiment II / 4 h treatment			culture I				culture II			
Solv. control with DMSO		-	100.0	100.0	56	182	100.0	100.0	46	172
Pos. control with MMS	19.5	-	39.2	9.8	890	182	47.5	12.1	450	172
Test item	1.9	-	112.8	culture was not continued [#]			120.1	culture was not continued [#]		
Test item	3.8	-	101.9	culture was not continued [#]			132.4	culture was not continued [#]		
Test item	7.5	-	89.8	66.1	98	182	148.5	75.0	60	172
Test item	15.0	-	77.5	68.3	79	182	103.8	91.2	49	172
Test item	30.0	-	9.6	81.6	68	182	89.9	118.8	35	172
Test item	45.0	-	13.6	2.6	68	182	29.9	10.9	73	172
Test item	60.0	-	10.6	6.3	74	182	11.8	12.7	52	172
			culture I				culture II			
Solv. control with DMSO		+	100.0	100.0	80	206	100.0	100.0	70	196
Pos. control with CPA	3	+	48.7	12.4	811	206	50.8	34.9	401	196
Pos. control with CPA	4.5	+	12.1	7.8	673	206	19.7	12.0	553	196
Test item	3.8	+	94.9	culture was not continued [#]			105.2	culture was not continued [#]		
Test item	7.5	+	84.7	56.5	93	206	98.4	224.4	61	196
Test item	15.0	+	91.8	119.8	75	206	103.4	227.2	48	196
Test item	30.0	+	88.8	97.9	57	206	93.7	73.9	71	196
Test item	60.0	+	83.3	71.6	40	206	96.8	40.3	126	196
Test item	90.0	+	79.6	15.5	21	206	85.4	57.9	42	196
Test item	120.0	+	2.0	culture was not continued ^{##}			6.2	culture was not continued ^{##}		

Experiment III

			relative	relative	mutant		relative	relative	mutant	
	conc. µg	S9	cloning	total	colonies/		cloning	total	colonies/	
	per mL	mix	efficiency I	growth	10 ⁶ cells	threshold	efficiency I	growth	10 ⁶ cells	threshold
Column	1	2	3	4	5	6	7	8	9	10
Experiment III / 4 h treatment			culture I				culture II			
Solv. control with DMSO		+	100.0	100.0	63	189	100.0	100.0	58	184
Pos. control with CPA	3	+	62.4	38.4	401	189	40.6	28.6	507	184
Pos. control with CPA	4.5	+	13.5	10.0	707	189	8.9	4.8	1743	184
Test item	20.0	+	114.5	culture was not continued [#]			98.1	culture was not continued [#]		
Test item	40.0	+	85.3	90.8	62	189	75.7	73.2	94	184
Test item	80.0	+	46.2	14.7	25	189	45.5	23.2	102	184
Test item	90.0	+	53.1	2.6	47	189	32.0	10.9	70	184
Test item	100.0	+	10.2	1.7	78	189	16.9	2.4	63	184
Test item	110.0	+	24.2	7.3	47	189	23.9	16.6	59	184
Test item	120.0	+	6.7	culture was not continued ^{##}			4.8	culture was not continued ^{##}		

Threshold = number of mutant colonies per 106 cells of each solvent plus 126

culture was not continued since a minimum of only four analysable concentrations is required

culture was not continued due to exceedingly severe cytotoxic effects

The values printed in bold are judged as invalid, since the acceptance criteria are not met (RTG<10%)

CONCLUSION:

In this GLP and OECD test guideline compliant in vitro mammalian cell gene mutation test (█, 2013), mouse lymphoma L5178Y cells were exposed to pydiflumetofen in the presence and absence of mammalian metabolic activation at the following concentrations:

Metabolic activation	Experiment	Concentration of test compound (µg/mL)
Absence of S9 mix	Experiment I	7.5; 15.0; 22.5; 30.0; 60.0
	Experiment II	7.5; 15.0; 30.0; 45.0; 60.0
Presence of S9 mix	Experiment I	7.5; 15.0; 30.0; 45.0; 60.0
	Experiment II	7.5; 15.0; 30.0; 60.0; 90.0
	Experiment III	40.0; 80.0; 90.0; 100.0; 110.0

The concentration range in the main experiments was limited by cytotoxic effects. There was no precipitation of the test item observed up to the maximum concentration with and without metabolic activation.

In experiment I at 60 µg/mL without metabolic activation, relevant cytotoxic effects (indicated by a relative cloning efficiency 1 (survival) or a relative total growth (RTG) of less than 50%) occurred in both cultures. In experiment II, cytotoxic effects were observed at 45.0 µg/mL and above in the absence of metabolic activation. In experiment III, which was only performed with metabolic activation, cytotoxic effects were seen at 80.0 µg/mL and above.

There were no substantial or reproducible dose dependent increase in mutant colony numbers observed at acceptable levels of cytotoxicity. Appropriate reference mutagens (methyl methanesulphonate, 19.5 µg/mL and cyclophosphamide, 3.0 and 4.5 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies, indicating that the tests were sensitive and valid.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y with or without metabolic activation up to concentrations causing cytotoxicity. Therefore, pydiflumetofen is considered to be non-mutagenic in this mouse lymphoma assay.

(█, 2013)

Photomutagenicity

SYN545974 does not meet the trigger for a photomutagenicity study according to the criteria laid down in Commission Regulation (EU) No 283/2013. The Ultraviolet/visible molar extinction/absorption coefficient of SYN545974 is less than $1000 \text{ L} \times \text{mol}^{-1} \times \text{cm}^{-1}$ ⁷, and therefore the photomutagenicity testing is not required.

B.6.4.2. In vivo studies in somatic cells

Two in vivo mouse micronucleus studies (one using the standard batch of the material and one using a batch spiked with impurities to support the technical specification) and one chromosome aberration study in the rat are available.

Report:	K-CA 5.4.2/01 [REDACTED] (2012). SYN545974 – Micronucleus Assay in Bone Marrow Cells of the Mouse. [REDACTED] [REDACTED]. Laboratory Report No. 1498904, issue date: 20 December, 2012, Unpublished. Syngenta File No. SYN545974_10045.
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Guidelines: Mouse bone marrow micronucleus test OECD 474 (1997): OPPTS 870.5395 (1998): 2000/32/EC 440/2008 B.12 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

HSE Comment: the study was considered acceptable.

Deviation:

During the acclimation phase of the animals used for the pre-experiments the relative humidity was between 45 – 75 % for a maximum of 6 hours.

This deviation did not affect the validity of the study.

EXECUTIVE SUMMARY

This study was performed in order to investigate the potential of SYN545974 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test item was suspended in 1% carboxymethylcellulose, which was also used as the vehicle control. The volume administered orally was 20 mL/kg body weight (bw). At 24 and 48 hours after a single administration of the test item, the bone marrow cells were collected for micronuclei analysis. Seven males per test group (except the control groups with 5 males only) were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes (PCEs) were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined per slide and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

24 hour preparation interval: 500, 1000, and 2000 mg/kg bw.

48 hour preparation interval: 2000 mg/kg bw.

⁷ [REDACTED] (2014). Report on the UV/Vis spectra for SYN545974. Report no. 300021097. Syngenta File No. SYN545974_10172. See MCA Section 2.4

The highest dose was estimated to be a suitable maximum tolerated dose based on a pre-experiment. After treatment with the test item the number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the mean value of PCEs per 2000 erythrocytes of the vehicle control, thus indicating that SYN545974 did not exert any significant cytotoxic effects in the bone marrow. In comparison to the corresponding vehicle controls there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item with any dose level used. The mean values of micronuclei observed after treatment with SYN545974 were below or near to the value of the vehicle control group and within the historical vehicle control range. A dose of 40 mg/kg bw cyclophosphamide administered orally was used as the positive control, which showed a substantial increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg bw.

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore, SYN545974 is considered to be non-mutagenic in this bone marrow micronucleus assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974		
Description:	Off white, solid		
Lot/Batch number:	SMU2EP12007		
Purity:	98.5 % w/w Dose calculation was not adjusted to purity		
CAS#:	1228284-64-7		
Stability of test compound:	Not indicated by the sponsor		
Control Materials:			
Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	1% carboxymethylcellulose (CMC)	Final Volume: 20 mL/kg	Route: oral
Positive control :	Cyclophosphamide	Final Doses: 40 mg/kg	Route: oral
Test Animals:			
Species	Mouse		
Strain	NMRI		
Age/weight at dosing	10 – 11 weeks (at start of experiment); mean value 38.8 g (Standard Deviation \pm 2.5 g), range 34.9-44.9 g		
Source	[REDACTED]		
Housing	1/cage		
Acclimatisation period	At least 5 days		
Diet	Pelleted standard diet, ad libitum		
Water	Tap water, ad libitum		
Environmental conditions	Temperature: 20-24°C Humidity: 45-75% Air changes: 15/hour Photoperiod: 12hours dark/12 hours light		

Test compound administration:			
	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg bw	20 mL/kg bw	oral
Main Study:	2000, 1000, 500 mg/kg bw	20 mL/kg bw	oral

Study Design and Methods:

In-life dates: Start: 01 August 2012

End: 16 August 2012

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethality or severe toxicity observed over a two-day observation period following a single oral dose.

Micronucleus Test:

Experimental design

Treatment	Dose	Number of Males /Time of kill	
		24 hours	48 hours
Vehicle control	20 ml/kg	5	5
Positive control	40 mg/kg	5	
Test substance	500 mg/kg	7	
Test substance	1000 mg/kg	7	
Test substance	2000 mg/kg	7	7

Slide Preparation: All animals designated for bone marrow smears were killed by over-exposure to halothane followed by cervical dislocation. The animals were sacrificed using CO₂ followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 ×g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained with May-Grünwald /Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.

Slide Analysis: Slides were coded and scored blind. Two thousand immature erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes per 2000 erythrocytes.

RESULTS

Preliminary toxicity assay: In a pre-experiment 2 male and 2 female animals received a single oral dose of SYN545974 (2000 mg/kg bw) suspended in 1% CMC (20 mL/kg bw). The treated animals displayed signs of toxicity as shown in the table below:

Table 6.4.2-3: Clinical observations recorded post dose at 2000 mg/kg bw in the preliminary toxicity assay

Signs of Toxicity	hours post-treatment					
	male / female					
	1 h	2-4 h	6 h	24 h	30 h	48 h
Reduction of spontaneous activity	1/1	0/0	0/0	0/0	0/0	0/0
Abdominal posture	1/1	0/0	0/0	0/0	0/0	0/0

No substantial gender specific differences in toxicity were observed, thus, the main study was performed using male animals only, as permitted by the Guideline. Following the described pre-experiments 2000 mg/kg bw was judged to be the maximum tolerable dose.

Micronucleus test: In the main experiment for the highest dose group 14 males received a single oral dose of 2000 mg/kg bw SYN545974 formulated in 1 % CMC. The volume administered was 20 mL/kg bw.

The animals treated with 2000 mg/kg bw displayed signs of toxicity as shown in the table below, which included reduced spontaneous activity abdominal posture and ruffled fur.

Table 6.4.2-4: Clinical observations recorded post dose at 2000 mg/kg bw in the main experiment

Signs of Toxicity	hours post-treatment				
	males (no of animals with clinical signs)				
	1 h	2-4 h	6 h	24 h	48 h*
Reduction of spontaneous activity	6	0	0	0	0
Abdominal posture	6	0	0	0	0
Ruffled fur	0	4	0	0	0

*: data from 7 males only.

For the mid dose group 7 males received orally a single dose of 1000 mg/kg bw SYN545974 suspended in 1% CMC, respectively. The volume administered was 20 mL/kg bw.

The animals treated with 1000 mg/kg bw displayed signs of toxicity as shown in the table below which included reduced spontaneous activity abdominal posture and ruffled fur only.

Table 6.4.2-5: Clinical observations recorded post dose at 1000 mg/kg bw in the main experiment

Signs of Toxicity	hours post-treatment			
	Males (no of animals with clinical signs)			
	1 h	2-4 h	6 h	24 h
Reduction of spontaneous activity	2	0	0	0
Abdominal posture	2	0	0	0
Ruffled fur	0	2	0	0

For the low dose group 7 males received orally a single dose of 500 mg/kg bw SYN545974 suspended in 1% CMC, respectively. The volume administered was 20 mL/kg bw.

The animals treated with 500 mg/kg bw did not express any signs of toxicity.

The animals of the vehicle control groups (1% CMC) for both sampling times also did not show any clinical symptoms.

The test item SYN545974 was assessed in the micronucleus assay for its potential to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test item was suspended in 1% CMC, which was also used as vehicle (negative) control. The volume administered orally was 20 mL/kg bw. At 24 h and 48 h after a single administration of the test item, the bone marrow cells were collected for micronuclei analysis. Seven males per test group (except the control groups with five males only) were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes

(PCEs) were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

24 h preparation interval: 500, 1000, and 2000 mg/kg bw.

48 h preparation interval: 2000 mg/kg bw.

The mean number of polychromatic erythrocytes was not decreased after treatment with the test item as compared to the mean value of PCEs of the vehicle control, indicating that SYN545974 did not have any significant cytotoxic properties in the bone marrow. In comparison to the corresponding vehicle control values there was no biologically relevant enhancement and no statistically significant increase in the frequency of the detected micronuclei after administration of the test item. The mean value of micronuclei observed after treatment with SYN545974 was below or near to the value of the respective vehicle control group and within the historical vehicle control range. A dose of 40 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg bw (Table 6.4.1-6). Statistical significance at the five per cent level ($p < 0.05$) for the incidence of micronuclei was evaluated by means of the non-parametric Mann-Whitney test (Table 6.4.1-7)

Table 6.4.1-6 Summary of Micronucleus test results

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythrocytes
vehicle	0	24	0.070	1 - 3	1225
test item	500	24	0.064	0 - 2	1311
test item	1000	24	0.086	0 - 3	1308
test item	2000	24	0.129	1 - 5	1383
positive control	40	24	1.450	19 - 42	1395
vehicle	0	48	0.070	1 - 2	1215
test item	2000	48	0.079	0 - 5	1277

Table 6.4.1-7 : Biometry

Vehicle control versus test group	Significance	p
500 mg SYN545974 /kg b.w.; 24 h	n.t.	-
1000 mg SYN545974 /kg b.w.; 24 h	-	0.4116
2000 mg SYN545974 /kg b.w.; 24 h	-	0.1149
40 mg CPA/kg b.w.; 24 h	+	0.0040
2000 mg SYN545974 /kg b.w.; 48 h	-	0.7020

+ = significant ;

- = not significant ;

n.t.= not tested, as the mean micronucleus frequency was not above the vehicle control value

CONCLUSION:

In this GLP and OECD test guideline compliant in vivo micronucleus test (████████, 2012), male mice were orally administered 20 mL/kg bw pydiflumetofen suspended in 1 % carboxymethylcellulose. The dose levels tested were 500, 1000 and 2000 mg/kg bw at the 24-hour preparation interval and 2000 mg/kg bw at the 48-hour preparation interval. The top dose was estimated to be a suitable maximum tolerated dose (MTD) based on a pre-experiment.

After treatment, the number of polychromatic erythrocytes (PCE) per 2000 erythrocytes was not substantially decreased compared to the mean number of PCEs per 2000 erythrocytes in the vehicle control. This indicates that pydiflumetofen did not exert significant cytotoxic effects in the bone marrow.

Compared to the corresponding vehicle controls, no biologically relevant or statistically significant increase in the frequency of micronuclei was observed at any preparation interval at any dose level. The mean values of micronuclei observed in the treatment groups were below or near to the values in the vehicle control group and within the historical vehicle control range. The positive control (cyclophosphamide, 40 mg/kg bw) displayed a statistically significant increase in induced micronucleus frequency, indicating that the test was sensitive and valid.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen did not induce micronuclei in the bone marrow cells of the mouse. Therefore, pydiflumetofen is considered to be non-mutagenic in this bone marrow micronucleus assay.

The weakly positive/equivocal in vitro clastogenicity finding for pydiflumetofen is therefore not corroborated in vivo.

HSE notes that the MTD was reached in the study, with signs of toxicity including reduced spontaneous activity, abdominal posture and ruffled fur displayed in animals treated with the top dose. Therefore, the genotoxic potential of pydiflumetofen was adequately investigated in this micronucleus assay.

This test did not include an assessment of bone marrow exposure to pydiflumetofen, but the clinical signs observed at the highest dose are indicative of systemic availability of the test material. As the bone marrow is widely perfused, it can be predicted that the test substance was distributed to the bone marrow. (████████, 2012)

Report:	K-CA 5.4.2/02 ██████████ (2014). SYN545974 - Micronucleus Assay in Bone Marrow Cells of the Mouse. ██████████ ██████████. Laboratory Report No. 1648702, issue date: 12 December 2014, Unpublished. Syngenta File No. SYN545974_10141.
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Guidelines: Mouse bone marrow micronucleus test OECD 474 (1997); OPPTS 870.5395 (1998); 2000/32/EC 440/2008 B.12 (2008)

GLP: Signed and dated GLP and Quality Assurance statements were provided.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

This study was performed in order to investigate the potential of SYN545974 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test substance was suspended in 1% carboxymethylcellulose, which was also used as the vehicle control. The volume administered orally was 10 mL/kg body weight (bw). At 24 and 48 hours after a single administration of the test substance, the bone marrow cells were collected for micronuclei analysis. Seven males per test group (except the negative and positive control groups with five males only) were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes (PCEs) were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test substance the ratio between polychromatic and normochromatic erythrocytes was determined per slide and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test item were investigated:

24 h preparation interval: 500, 1000, and 2000 mg/kg bw.

48 h preparation interval: 2000 mg/kg bw.

The highest dose was estimated to be a suitable maximum tolerated dose based on a pre-experiment. After treatment with the test substance the number of PCEs per 2000 erythrocytes was not substantially decreased as compared to the mean value of PCEs per 2000 erythrocytes of the vehicle control, thus indicating that SYN545974 did not exert any significant cytotoxic effects in the bone marrow. In comparison to the corresponding vehicle controls, there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test substance with any dose level used. For all treatment groups the mean values of micronuclei observed after treatment with SYN545974 were well within the historical vehicle control range. Additionally, no dose dependency was observed. A dose of 40 mg/kg bw cyclophosphamide administered orally was used as the positive control, which showed a substantial increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg bw.

In conclusion, it can be stated that under the experimental conditions reported, the test substance did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore, SYN545974 is considered to be non-mutagenic in this bone marrow micronucleus assay.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Product Code:	SYN545974 technical
Batch No:	SMU4FL762
Purity:	96.7 % w/w Dose calculation was not adjusted to purity
CAS#:	1228284-64-7
Physical state and appearance:	White powder
Stability in Solvent:	Not indicated by the sponsor
Storage (provided by the Sponsor):	Room temperature, light and moisture protected
Recertification Date:	End of July 2016

Control Materials:			
Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	1% w/v aqueous CMC	Final Volume: 10 mL/kg	Route: oral
Positive control :	Cyclophosphamide (CPA)	Final Doses: 40 mg/kg	Route: oral

Test Animals:	
Species	Mouse
Strain	NMRI
Age/weight at dosing	9 – 10 weeks (at start of experiment); mean value 37.2 g (Standard Deviation \pm 2.6 g), range 32.4 – 41.7 g
Source	
Housing	1/cage
Acclimatisation period	At least 5 days
Diet	Pelleted standard diet, ad libitum
Water	Tap water, ad libitum
Environmental conditions	Temperature: 22 \pm 2°C Humidity: 45 - 65% (Aim of 50-60%) Photoperiod: 12 hours dark/12 hours light

Test compound administration:			
	Dose Levels	Final Volume	Route
Preliminary:	2000 mg/kg bw	10 mL/kg bw	oral
Main Study:	2000, 1000, 500 mg/kg bw	10 mL/kg bw	oral

Study Design and Methods:

In-life dates: Start: 19 August 2014

End: 29 September 2014

Preliminary Toxicity Assay: A maximum tolerated dose (MTD) was determined, based on patterns of lethality or severe toxicity observed over a two-day observation period following a single oral dose.

Micronucleus Test:

Experimental design

Treatment	Dose	Number of Animals /Time of kill	
		24 hours	48 hours
Vehicle control	10 ml/kg	5	5
Positive control	40 mg/kg	5	
Test substance	500 mg/kg	7	
Test substance	1000 mg/kg	7	
Test substance	2000 mg/kg	7	7

Slide Preparation: The animals were sacrificed using CO₂ followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out with fetal calf serum using a syringe. The cell suspension was centrifuged at 1500 rpm (390 \times g) for 10 minutes and the supernatant was discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-

dried and then stained with May-Grünwald /Giemsa. Cover slips were mounted with EUKITT. At least one slide was made from each bone marrow sample.

Slide Analysis: Slides were coded and scored blind. Two thousand immature erythrocytes were examined for the presence of micronuclei for each animal. The slides were also examined for evidence of cytotoxicity, which may be manifest by alterations in the ratio of different cell types in the bone marrow. This was assessed by counting the ratio of immature to mature erythrocytes.

RESULTS

Preliminary toxicity assay: In the pre-experiment 2 male and 2 female animals received a single oral dose of SYN545974 (2000 mg/kg bw) suspended in 1% CMC (10 mL/kg bw). The animals treated with 2000 mg/kg bw displayed signs of toxicity, including abdominal posture, partially closed eyes, ruffled fur, and slightly reduced spontaneous activity (Table 6.4.1-8). Hence the maximum dose level used was determined to be the limit dose for the assay, 2000 mg/kg bw, along with two lower dose levels, 1000 and 500 mg/kg bw. No differences between sexes in toxicity were observed, so that only male animals were used in the main experiment.

Table 6.4.1-8: Pre-Experiment for toxicity: 2000 mg/kg bw SYN545974

Signs of Toxicity	hours post-treatment male / female					
	0-1 h	2-4 h	5-6 h	24 h	30 h*	48 h
Abdominal posture	2/0	0/0	0/0	0/0	-	0/0
Partially closed eyes	2/0	0/0	0/0	0/0	-	0/0
Ruffled fur	0/0	2/2	2/2	2/2	-	0/0
Slightly reduced spontaneous activity	2/0	0/0	0/0	0/0	-	0/0

* The 30 h time point was not assessed

Micronucleus test: In the main experiment for the high dose groups 14 males (2 × 7 males per group) received orally a single dose of 2000 mg/kg bw SYN545974 suspended in 1% CMC. The volume administered was 10 mL/kg bw. The animals treated with 2000 mg/kg bw displayed signs of toxicity; initially abdominal posture and reduced activity, later ruffled fur (Table 6.4.1-9).

Table 6.4.1-9: Signs of toxicity in the main Experiment:

2000 mg/kg bw SYN545974

Signs of Toxicity	hours post-treatment male				
	0-1 h	2-4 h	5-6 h	24 h	48 h*
Abdominal posture	14	0	0	0	0
Ruffled fur	0	14	14	14	7
Slightly reduced spontaneous activity	14	0	0	0	0

* data from 7 males only

1000 mg/kg bw SYN545974

Signs of Toxicity	hours post-treatment male			
	0-1 h	2-4 h	5-6 h	24 h
Ruffled fur	0	0	0	3

For the mid and low dose group 7 males each received orally a single dose of 1000 mg/kg bw and 500 mg/kg bw SYN545974 suspended in 1% CMC, respectively. The volume administered was 10 mL/kg bw. The animals treated with 1000 mg/kg bw displayed ruffled fur at the 24 h observation.

The animals treated with 500 mg/kg bw as well as the animals of the vehicle control groups (1% CMC) for both sampling times also did not show any clinical symptoms.

The test substance SYN545974 was assessed in the micronucleus assay for its potential to induce micronuclei in PCEs in the bone marrow of the mouse. The test substance was suspended in 1% CMC, which was also used as the vehicle control. The volume administered orally was 10 mL/kg body weight (bw). At 24 h and 48 h after a single administration of the test substance, the bone marrow cells were collected for micronuclei analysis. Seven males per test group (except the negative and positive control groups with five males only) were evaluated for the occurrence of micronuclei. Per animal 2000 PCEs were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test substance the number of polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

The following dose levels of the test substance were investigated:

24 h preparation interval: 500, 1000, and 2000 mg/kg bw.

48 h preparation interval: 2000 mg/kg bw.

As determined by a pre-experiment in male and female mice, 2000 mg SYN545974 per kg bw was suitable as the highest dose. Since no obvious substantial gender-specific differences in the sensitivity to the test substance were observed and as requested by the Sponsor, the main experiment was performed using male animals only. The mean number of polychromatic erythrocytes was not substantially decreased after treatment with the test substance as compared to the mean value of PCEs of the vehicle control, indicating that SYN545974 did not have any significant cytotoxic properties in the bone marrow. However, systemic exposure to SYN545974 has been demonstrated in mice previously, refer to section 6.1.

In comparison to the corresponding vehicle control values there was no biologically relevant enhancement or statistically significant increase in the frequency of the detected micronuclei at any preparation interval and dose level after administration of the test substance. The mean values of micronuclei observed after treatment with SYN545974 were for all dose groups well within the historical vehicle control range. Additionally no dose dependence was observed. A dose of 40 mg/kg bw cyclophosphamide administered orally was used as positive control which showed a statistically significant increase of induced micronucleus frequency. The volume of the positive control administered was 10 mL/kg bw (Table 6.4.1-10). Statistical significance at the five per cent level ($p < 0.05$) for the incidence of micronuclei was evaluated by means of the non-parametric Mann-Whitney test (Table 6.4.1-11)

Table 6.4.1-10: Summary of Micronucleus test results

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE per 2000 erythrocytes
negative control	0	24	0.080	0 -4	1305
test substance	500	24	0.171	0 -9	1254
test substance	1000	24	0.114	0 -6	1353
test substance	2000	24	0.136	2 -4	1273
positive control	40	24	2.690	37 -88	1201
negative control	0	48	0.080	0 -4	1471
test substance	2000	48	0.100	0 -8	1287

Table 6.4.1-11 : Biometry

Negative control versus test group	Significance	p
500 mg SYN545974 /kg b.w.; 24 h	-	0.285
1000 mg SYN545974 /kg b.w.; 24 h	-	0.618
2000 mg SYN545974 /kg b.w.; 24 h	-	0.126
40 mg CPA/kg b.w.; 24 h	+	0.012
2000 mg SYN545974 /kg b.w.; 48 h	-	0.802

+ = significant ;

- = not significant

CONCLUSION:

In this GLP and OECD test guideline compliant in vivo micronucleus test (■■■■■, 2014), male mice were orally administered 10 mL/kg bw pydiflumetofen suspended in 1 % carboxymethylcellulose. The batch (SMU4FL762) tested in this study was spiked with potential impurities to support the technical specification of pydiflumetofen. The dose levels tested were 500, 1000 and 2000 mg/kg bw at the 24-hour preparation interval and 2000 mg/kg bw at the 48-hour preparation interval. The top dose was estimated to be a suitable maximum tolerated dose (MTD) based on a pre-experiment.

After treatment, the number of polychromatic erythrocytes (PCE) per 2000 erythrocytes was not substantially decreased compared to the mean number of PCEs per 2000 erythrocytes in the vehicle control. This indicates that pydiflumetofen did not exert significant cytotoxic effects in the bone marrow.

Compared to the corresponding vehicle controls, no biologically relevant or statistically significant increase in the frequency of micronuclei was observed at any preparation interval at any dose level. The mean values of micronuclei observed in the treatment groups were well within the historical vehicle control range. The positive control (cyclophosphamide, 40 mg/kg bw) displayed a statistically significant increase in induced micronucleus frequency, indicating that the test was sensitive and valid.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen (batch spiked with impurities) did not induce micronuclei in the bone marrow cells of the mouse. Therefore, pydiflumetofen is considered to be non-mutagenic in this bone marrow micronucleus assay.

The weakly/equivocal positive *in vitro* clastogenicity finding for pydiflumetofen is therefore not corroborated *in vivo*.

HSE notes that the MTD was reached in the study, with signs of toxicity including reduced spontaneous activity, abdominal posture and ruffled fur displayed in animals treated with the top dose. Therefore, the genotoxic potential of pydiflumetofen was adequately investigated in this micronucleus assay.

This test did not include an assessment of bone marrow exposure to pydiflumetofen, but the clinical signs observed at the highest dose are indicative of systemic availability of the test material. As the bone marrow is widely perfused, it can be predicted that the test substance was distributed to the bone marrow. (██████████ 2014)

During the commenting period, EFSA has requested to the applicant to provide the new *in vivo* genotoxicity study (rat bone marrow chromosome aberration assay) conducted on SYN545974 (EFSA Request for additional information (February 2018), Question 20). The applicant explained that this second *in vivo* genotoxicity study on SYN545974 has been conducted to support regulatory requirements in other regions. This requested *in vivo* genotoxicity study has been assessed below.

Report:	K-CA 5.4.2/03 ██████████ (2017). Rat Bone Marrow Chromosome Aberration Assay. ██████████. Laboratory Report No. 8359217, issue date: 11 October 2017, Unpublished. Syngenta File No. SYN545974_10513.
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Guidelines: Genetic Toxicology: Mammalian Bone Marrow Chromosome Aberration Test OECD 475 (2016).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

HSE Comment: the study was considered acceptable.

EXECUTIVE SUMMARY

SYN545974 was tested for its ability to induce chromosome aberrations in the bone marrow of treated rats.

Strain / Species:	Han Wistar rats.
Vehicle:	0.5% Tween 80/0.5% CMC in distilled water.
Administration route:	Oral by gavage.
Dosing regimen:	Single administration at 0 hours (Day 1).
Gender:	Males only (no gender differences observed in the Range-Finder Experiment).
Dose levels:	500, 1000 or 2000 mg/kg.
Maximum dose:	Regulatory maximum recommended dose of 2000 mg/kg.
Positive control:	Cyclophosphamide (CPA) 30 mg/kg.
Animals per group:	Six (three for the positive control group).
Dose volume:	10 mL/kg.
Clinical signs of toxicity:	None.
Bone marrow sampled:	16 hours (Subgroup 1) or 42 hours (Subgroup 2) after administration.

Assay validity:

For both 16 and 42 hour data, the proportion of cells with structural aberrations (excluding gaps) in the vehicle control animals fell within the laboratory's historical vehicle control ranges.

The positive control induced a statistically significant increase in the frequency of chromosome aberrations (excluding gaps) that was comparable with the laboratory's positive control ranges. All animals exhibited aberrant cell frequencies (excluding gaps) that were clearly elevated and fell within the laboratory's historical positive control ranges. The assay was therefore accepted as valid.

Results 16 hour data

For dose levels of 500, 1000 and 2000 mg/kg, cytotoxicity (as measured by mitotic inhibition [%MIH]) was 6%, 24% and 27% respectively providing weak evidence of bone marrow toxicity at 2000 mg/kg.

At the 16 hour sample time, the mean percentage of cells with aberrations (excluding gaps) was 0.42%, 0.42% and 0.72% at 500, 1000 and 2000 mg/kg respectively compared to 0.42% in the concurrent vehicle control. Group mean frequencies of cells with structural chromosome aberrations (excluding gaps) were generally similar to and not significantly ($p \leq 0.05$) higher than those observed in the concurrent vehicle controls (for all dose groups). At 2000 mg/kg there was a slight increase in mean aberrant cell frequency which fell outside of the historical vehicle control 95% reference range. However, the increase was mainly attributable to a single animal in the group (Animal 302). Individual aberration frequencies were similar to those observed in the vehicle control group. In the absence of statistical significance or a statistically significant linear trend the increase was considered to be not biologically relevant.

For dose groups of 500, 1000 and 2000 mg/kg, mean numerical aberration frequencies were 0.9%, 0.6% and 0.6% respectively compared to 0.4% in the concurrent vehicle control. Overall, frequencies of cells with numerical aberrations in all treated groups fell within the expected distribution of the historical control range with the exception of a small elevation in polyploidy restricted to a single animal in each group (Animals 101, 203 and 302). Since the group mean values were within the historical vehicle control 95% reference range, these isolated individual increases were considered not biologically relevant.

Results 42 hour data

At 42 hours cytotoxicity (as measured by %MIH) at 2000 mg/kg was 17%.

At the 42 hour sample time, the mean percentage of cells with aberrations (excluding gaps) was 0.58% at 2000 mg/kg compared to 0.33% in the concurrent vehicle control. The majority of individual animals exhibited aberrant cell frequencies (excluding gaps) that fell within the historical vehicle control 95% reference range. As the group mean aberrant cell frequency at 2000 mg/kg also fell within the historical vehicle control 95% reference range and in the absence of statistical significance these data were considered to indicate no evidence of a test article related effect on chromosome aberration frequency.

At 2000 mg/kg, the mean numerical aberration frequency was 0.2% compared with 0.7% for the concurrent vehicle control. Frequencies of cells with numerical aberrations at 2000 mg/kg fell within the historical vehicle control 95% reference range.

It is concluded that SYN545974 did not induce chromosome aberrations in the bone marrow cells of rats when tested up to 2000 mg/kg (the recommended maximum dose for this assay according to current regulatory guidelines) under the experimental conditions employed.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off-white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 % (w/w)
Stability of test compound:	Retest date : 30 April 2020

Control Materials:

Negative control (if not vehicle) :	N/A	Final Volume: N/A	Route: N/A
Vehicle:	0.5% Tween 80 and 0.5% (w/v) carboxymethylcellulose in distilled water	Final Volume: 10 mL/kg	Route: oral
Positive control :	Cyclophosphamide monohydrate	Final Doses: 30 mg/kg	Route: oral

Test Animals:

Species	Rats
Strain	WI(Han)
Age/weight at dosing	7-8 weeks (at start of experiment); Main study: range 207 - 254 g
Source	
Housing	3/cage
Acclimatisation period	At least 5 days
Diet	Pelleted standard diet, <i>ad libitum</i>
Water	Tap water, <i>ad libitum</i>
Environmental conditions	Temperature: 20-24 °C Humidity: 45 % to 65 % Photoperiod: 12 hours dark/12 hours light

Test compound administration:

	Dose Levels	Final Volume	Route
Preliminary:	Range-finding phase: 2000 mg/kg/day males and females	10 mL/kg b.w.	oral
Main Study:	500, 1000, 2000 mg/kg/day males only	10 mL/kg b.w.	oral

Study Design and Methods:

Study initiation date: 19 December 2016
 Experimental start date: 03 January 2017
 Experimental termination date: 24 Feb 2017.

Preliminary Toxicity Assay: Dosing was once by oral (gavage) administration. The animals were observed periodically for up to 24 hours after dosing.

Chromosomal Aberration Test: The test article, vehicle and positive control were given as a single administration by oral gavage, at 0 hours. Animals were sampled at an early time point 16 hours (Subgroup 1) or late time point 42 hours (Subgroup 2) after administration.

These sampling times are based on a cell cycle time of 9 hours for proliferating rodent bone marrow cells and consistent with regulatory guidance.

Approximately 2 hours prior to sampling, animals were injected intraperitoneally with colchicine to arrest dividing cells in metaphase.

Slide Preparation: Both femurs were removed and bone marrow isolated from all Main Experiment animals at necropsy. Cells were pelleted and resuspended in a minimal amount of fresh fixative so as to give a milky suspension. Several drops of 45% (v/v) aqueous acetic acid were added to each suspension to enhance chromosome spreading, and several drops of suspension were transferred on to clean microscope slides which had been dipped in water. Multiple slides were made from each animal.

After the slides had dried on a warm plate and allowed to cool, the cells were stained in 4% (v/v) filtered Giemsa stain in Gurr's pH 6.8 buffer for 5 minutes. The slides were then rinsed, dried and mounted with coverslips.

Slide Analysis: Scoring was carried out using a light microscope at an appropriate magnification. Slides from vehicle and positive control animals were checked to for quality and/or response prior to analysis. All slides were allocated a random code and analysed by an individual not connected with the dosing of the study. All animals per group were analysed.

Mitotix Index was measured in at least 1000 cells per animal (including animals from the positive control group) to assess any evidence of toxicity.

Where possible, 200 metaphases from each animal were analysed for chromosome aberrations. Only cells with 40-42 chromosomes were considered acceptable for analysis of structural aberrations. Any cell with more than 42 chromosomes, that was polyploidy or endoreduplicated observed during this search were noted and recorded separately (hyperdiploid cells were not scored).

Data Evaluation: The experimental unit of exposure for *in vivo* studies is the animal, and all analysis was based on individual animal responses.

After completion of microscopic analysis and decoding of the data the aberrant cells were categorised as follows:

1. Cells with structural aberrations including gaps
2. Cells with structural aberrations excluding gaps
3. Polyploid and endoreduplicated cells.

The totals in Category 2 in vehicle control animals were used to determine whether or not the study was acceptable.

The mean of each category for each group was calculated, and the frequency of cells with aberrations (\pm standard deviation) determined.

For each group, inter individual variation in the proportion of aberrant cells was estimated by means of a heterogeneity chi-square calculation.

The proportion of cells with structural chromosome aberrations excluding gaps were compared with the proportion in vehicle controls by using Fisher's exact test. In addition, a Cochran-Armitage Trend Test

was performed to aid determination of concentration response relationships. Probability values of $p \leq 0.05$ were accepted as significant (see Deviations).

The proportions of cells in categories 1 and 2 for each treated group were examined in relation to historical negative control ranges.

This assay was considered valid if the following criteria were met:

1. The proportion of cells with structural aberrations (excluding gaps) in vehicle control animals fell within the laboratory's historical vehicle control ranges
2. The positive control induced a statistically significant increase in the frequency of chromosome aberrations (excluding gaps) that was comparable with the laboratory's positive control ranges
3. Adequate numbers of cells and doses were analysed
4. The high dose was considered to be the MTD, the maximum recommended dose, the maximum practicable dose or one that demonstrated cytotoxicity to the target cells.

For valid data, the test article was considered to induce clastogenic / aneugenic damage if:

1. A statistically significant increase in the proportion of cells with structural chromosome aberrations (excluding gaps) occurred at one or more concentration and/or sample time
2. The proportion of cells with structural aberrations in individual animals at such a point exceeded the 95% reference range
3. A dose-response trend in the proportion of cells with structural chromosome aberrations (where more than two dose levels were analysed) was observed.

The test article was considered positive in this assay if all of the above criteria were met.

The test article was considered negative in this assay if none of the above criteria were met and target tissue exposure has been confirmed.

Results which only partially satisfied the above criteria were dealt with on a case-by-case basis. Evidence of a dose-related effect was considered useful but not essential in the evaluation of a positive result. Biological relevance was taken into account, for example consistency of response within and between dose levels.

RESULTS

Bioanalysis: The Sponsor confirmed that Bioanalysis was not required within the current study to confirm proof of exposure as this has been previously confirmed at dose levels up to 1000 mg/kg (1).

Range-finding phase: No clinical signs of toxicity or notable effect of treatment on body weights was observed.

From these results the regulatory maximum dose level of 2000 mg/kg was considered to be well tolerated in males and females and was selected as the maximum dose level for the Main Experiment. Intermediate and low dose levels of 1000 and 500 mg/kg were also selected. In the absence of a

difference in tolerability between males and females, the Main Experiment was conducted in male animals only.

Chromosome Aberration Analysis:

16 hour data

For dose levels of 500, 1000 and 2000 mg/kg, cytotoxicity (as measured by mitotic inhibition [%MIH]) was 6%, 24% and 27% respectively providing weak evidence of bone marrow toxicity at 2000 mg/kg

Table 6.4.1-12: SYN545974: Summary of cells with structural aberrations excluding gaps, 16 hours

Treatment (mg/kg)	Cytotoxicity (%) §	Cells Scored	Aberrant Cells Excluding Gaps*	Aberrant Cells Excluding Gaps (%)	Fisher's Exact Test	Statistical Significance
Vehicle	-	1200	5	0.42	-	-
500	6	1200	5	0.42	0.500	NS
1000	24	1183	5	0.42	0.491	NS
2000	27	1114	8	0.72	0.175	NS
CPA 30	78	554	165	29.78	0.000	p≤0.001

* Historical vehicle control 95% reference range (excluding gaps) range 0 to 0.5%

§ Cytotoxicity based on mitotic inhibition

Binomial Dispersion Test χ^2 : 17.840 DF: 20 p-value: 0.598 Significance: NS
Cochran-Armitage Linear Trend p-value: 0.165 Significance: NS

Table 6.4.1-13: Cells with structural aberrations – Subgroup 1, 16 hours

Group / Dose Level (mg/kg)	Animal Number	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Statistical Significance §	Mitotic Index % (Cytotoxicity %)
1M / Vehicle (0)	R0001	200	3 #	2 #		6.1
	R0002	200	1	0		12.4
	R0003	200	0	0		7.1
	R0004	200	0	0		5.0
	R0005	200	1	1		6.0
	R0006	200	2 #	2 #		15.4
	Total	1200	7 (0.58)	5 (0.42)	-	(-)
2M / SYN545974 (500)	R0101	200	1	1		6.5
	R0102	200	0	0		10.3
	R0103	200	0	0		8.6
	R0104	200	2 #	1		10.1
	R0105	200	1	1		7.0
	R0106	200	2 #	2 #		6.5
	Total	1200	6 (0.50)	5 (0.42)	NS	(6%)
3M / SYN545974 (1000)	R0201	200	0	0		6.7
	R0202	200	2 #	1		7.0
	R0203	200	0	0		5.8
	R0204	183	2 #	2 #		2.8
	R0205	200	1	1		8.0
	R0206	200	1	1		9.0
	Total	1183	6 (0.51)	5 (0.42)	NS	(24%)
4M / SYN545974 (2000)	R0301	200	2 #	1		9.1
	R0302	162	3 #	3 #		2.2
	R0303	200	2 #	2 #		8.4
	R0304	152	1	1		2.4
	R0305	200	1	0		8.6
	R0306	200	1	1		7.1
	Total	1114	10 (0.90)	8 (0.72)	NS	(27%)
5M / CPA (30)	R0401	172	64	62		2.2
	R0402	199	55	54		1.9
	R0403	183	49	49		1.6
	Total	554	168 (30.32)	165 (29.78)	p≤0.001	(78%)

§ = statistical significance (Fishes Exact Test)

= Numbers exceed historical vehicle control range (Table 6.4.1-20)

NS = Not significant

At the 16 hour sample time, the mean percentage of cells with aberrations (excluding gaps) was 0.42%, 0.42% and 0.72% at 500, 1000 and 2000 mg/kg respectively compared to 0.42% in the concurrent vehicle control. Group mean frequencies of cells with structural chromosome aberrations (excluding gaps) were generally similar to and not significantly ($p \leq 0.05$) higher than those observed in the concurrent vehicle controls (for all dose groups) (Table 6.4.1-12).

At 2000 mg/kg there was a slight increase in mean aberrant cell frequency which fell outside of the historical vehicle control 95% reference range (see Table 6.4.1-20). However, the increase was mainly

attributable to a single animal in the group (Animal 302). Individual aberration frequencies were similar to those observed in the vehicle control group (Table 6.4.1-13).

Table 6.4.1-14: Statistical analysis of test article data – Subgroup 1, 16 hours

Dose Level (mg/kg)	Cells Scored	Aberrant Cells	Proportion	Fisher's Exact Test	Significance
Vehicle	1200	5	0.004	-	-
500	1200	5	0.004	0.500	NS
1000	1183	5	0.004	0.491	NS
2000	1114	8	0.007	0.175	NS
CPA, 30	554	165	0.298	0.000	p ≤ 0.001

Binomial Dispersion Test χ^2 : 17.840
Cochran-Armitage Linear Trend

DF: 20

p-value: 0.598
p-value: 0.165

Significance: NS
Significance: NS

DF = Degrees of freedom
NS = Not significant

Table 6.4.1-15: Summary of numerical aberrations observed, Subgroup 1, 16 hours

Group / Dose Level (mg/kg)	Animal Number	Cells **	E	P	Total Abs	% With Numerical Aberrations
1M / Vehicle (0)	R0001	201	0	1	1	0.5
	R0002	201	0	1	1	0.5
	R0003	201	0	1	1	0.5
	R0004	200	0	0	0	0.0
	R0005	200	0	0	0	0.0
	R0006	202	0	2	2	1.0
	Total	1205	0	5	5	0.4
2M / SYN545974 (500)	R0101	203	0	3#	3#	1.5
	R0102	201	0	1	1	0.5
	R0103	201	0	1	1	0.5
	R0104	202	0	2	2	1.0
	R0105	202	0	2	2	1.0
	R0106	202	0	2	2	1.0
	Total	1211	0	11	11	0.9
3M / SYN545974 (1000)	R0201	200	0	0	0	0.0
	R0202	201	0	1	1	0.5
	R0203	203	0	3#	3#	1.5
	R0204	184	0	1	1	0.5
	R0205	202	0	2	2	1.0
	R0206	200	0	0	0	0.0
	Total	1190	0	7	7	0.6
4M / SYN545974 (2000)	R0301	200	0	0	0	0.0
	R0302	167	0	5#	5#	3.0
	R0303	200	0	0	0	0.0
	R0304	153	0	1	1	0.7
	R0305	201	0	1	1	0.5
	R0306	200	0	0	0	0.0
	Total	1121	0	7	7	0.6
5M / CPA (30)	R0401	173	0	1	1	0.6
	R0402	199	0	0	0	0.0
	R0403	184	0	1	1	0.5
	Total	556	0	2	2	0.4

** Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control rang (Table 6.4.1-20)

E = endoreduplicated

P = polyploid (greater than 62 chromosomes)

In the absence of statistical significance or a statistically significant linear trend (Table 6.4.1-14), the increase in mean aberration frequency was considered to be not biologically relevant.

For dose groups of 500, 1000 and 2000 mg/kg, mean numerical aberration frequencies were 0.9%, 0.6% and 0.6% respectively compared to 0.4% in the concurrent vehicle control (Table 6.4.1-15).

Overall, frequencies of cells with numerical aberrations in all treated groups fell within the expected distribution of the historical control range with the exception of a small elevation in polyploidy restricted to a single animal in each group (Animals 101, 203 and 302). Since the group mean values were within the historical vehicle control 95% reference range these isolated individual increases were considered not biologically relevant.

42 hour data

At 42 hours cytotoxicity (as measured by %MIH) at 2000 mg/kg was 17%.

Table 6.4.1-16: SYN545974: Summary of cells with structural aberrations excluding gaps, 42 hours

Treatment (mg/kg)	Cytotoxicity (%) §	Cells Scored	Aberrant Cells Excluding Gaps*	Aberrant Cells Excluding Gaps (%)	Fisher's Exact Test	Statistical Significance
Vehicle	-	1200	4	0.33	-	-
2000	17	1200	7	0.58	0.193	NS

* Historical vehicle control 95% reference range (excluding gaps) 0 to 1.03%

§ Cytotoxicity based on mitotic inhibition

Binomial Dispersion Test χ^2 : 10.908 DF: 10 p-value: 0.365 Significance: NS
Cochran-Armitage Linear Trend p-value: 0.182 Significance: NS

Table 6.4.1-17: Cells with structural aberrations – subgroup 2, 42 hours

Group / Dose Level (mg/kg)	Animal Number	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations Excluding Gaps	Statistical Significance §	Mitotic Index % (cytotoxicity %)
1M / Vehicle (0)	R0007	200	2	2		8.3
	R0008	200	0	0		8.9
	R0009	200	2	1		13.9
	R0010	200	0	0		10.8
	R0011	200	0	0		11.3
	R0012	200	1	1		9.1
	Total	1200	5 (0.42)	4 (0.33)	-	(-)
4M / SYN545974 (2000)	R0307	200	1	1		11.0
	R0308	200	1	0		9.6
	R0309	200	2	1		2.6
	R0310	200	1	0		10.6
	R0311	200	3 #	3 #		14.0
	R0312	200	3 #	2		4.1
	Total	1200	11 (0.92)	7 (0.58)	NS	(17%)

§ = statistical significance (Fishes Exact Test)

= Numbers exceed historical vehicle control range (Table 6.4.1-)

NS = Not significant

At the 42 hour sample time, the mean percentage of cells with aberrations (excluding gaps) was 0.58% at 2000 mg/kg compared to 0.33% in the concurrent vehicle control (Table 6.4.1-16).

The majority of individual animals exhibited aberrant cell frequencies (excluding gaps) that fell within the historical vehicle control 95% reference range (Table 6.4.1-17) and Table 6.4.1-20).

Table 6.4.1-18: Statistical analysis of test article data – subgroup 2, 42 hours

Dose Level (mg/kg)	Cells Scored	Aberrant Cells	Proportion	Fisher's Exact Test	Significance
Vehicle	1200	4	0.003	-	-
2000	1200	7	0.006	0.193	NS

Binomial Dispersion Test χ^2 : 10.908 DF: 10 p-value: 0.365 Significance: NS
Cochran-Armitage Linear Trend p-value: 0.182 Significance: NS

DF = Degrees of freedom
NS = Not significant

Table 6.4.1-19: summary of numerical aberrations observed – subgroup 2, 42 hours

Group / Dose Level (mg/kg)	Animal Number	Cells **	E	P	Total Abs	% With Numerical Aberrations
1M / Vehicle (0)	R0007	205	0	5#	5#	2.4
	R0008	201	0	1	1	0.5
	R0009	200	0	0	0	0.0
	R0010	201	0	1	1	0.5
	R0011	200	0	0	0	0.0
	R0012	201	0	1	1	0.5
	Total	1208	0	8	8	0.7
4M / SYN545974 (2000)	R0307	200	0	0	0	0.0
	R0308	200	0	0	0	0.0
	R0309	202	0	2	2	1.0
	R0310	200	0	0	0	0.0
	R0311	200	0	0	0	0.0
	R0312	201	0	1	1	0.5
	Total	1203	0	3	3	0.2

** Total cells examined for numerical aberrations

Numbers highlighted exceed historical negative control range (Table 6.4.1-)

As the group mean aberrant cell frequency at 2000 mg/kg also fell within the historical vehicle control 95% reference range and in the absence of statistical significance (Table 6.4.1-18) these data were considered to indicate no evidence of a test article related effect on chromosome aberration frequency.

At 2000 mg/kg, the mean numerical aberration frequency was 0.2% compared with 0.7% for the concurrent vehicle control (table 6.4.1-19). Frequencies of cells with numerical aberrations at 2000 mg/kg fell within the historical vehicle control 95% reference range.

These data confirm that SYN545974 did not induce chromosome aberrations in the bone marrow of treated male rats.

Table 6.4.1-20: Historical data for chromosome aberrations in rat bone marrows: males

Data generated from studies performed within the GLP laboratory, by GLP trained staff, whether a claim of GLP compliance was made or not, were included in the compilation of the historical control ranges without bias.

16 Hour Males - Vehicle

	% Frequency of Structural Aberrations		%Numerical	
	Including Gaps	Excluding Gaps	Poly	Total
Number of Animals	48	48	48	48
Number of Expts	8	8	8	8
Mean	0.1	0.1	0.3	0.34
SD	0.21	0.19	0.45	0.92
Median	0.0	0.0	0.0	0.13
Observed	0 to 0.7	0 to 0.7	0 to 4	0 to 4
95% Reference Range	Lower	0	0	0
	Upper	0.5	1.4	1.4

Individual Distribution	Count	Cells Exc Gaps	Frequency
Counts cells excluding gaps	0	40	83%
	>0, <or=1	8	17%
	>1	0	0%

Poly		Frequency
Counts		
0	27	56%
>0, <or=1	14	29%
>1, <or=2	5	10%
>2, <or=3	1	2%
>3, <or=4	1	2%

Numerical		Frequency
Counts		
0	24	50%
>0, <or=1	16	33%
>1, <or=2	6	13%
>2, <or=3	1	2%
>3, <or=4	1	2%

Data generated in November 2016 from studies started between September 2007 and January 2016.

CONCLUSION:

In this GLP and OECD test guideline compliant in vivo bone marrow chromosome aberration test (██████████, 2017), male rats were orally administered 10 mL/kg bw pydiflumetofen suspended in 0.5 % Tween 80/0.5 % CMC in distilled water. The dose levels tested were 500, 1000 and 2000 mg/kg bw. The top dose tested was the regulatory maximum recommended dose (2000 mg/kg bw).

The bone marrow was sampled either 16 hours (subgroup 1) or 42 hours (subgroup 2) after administration of the test item. At both time points, the mean percentage of cells with aberrations (excluding gaps) was not substantially different compared to the vehicle controls.

The positive control (cyclophosphamide, 30 mg/kg bw) induced a statistically significant increase in the frequency of chromosome aberrations (excluding gaps) that was comparable with the laboratory's positive control ranges. This indicated that the test was sensitive and valid.

At 16 hours, at dose levels of 500, 1000 and 2000 mg/kg bw, cytotoxicity (mitotic inhibition) was observed at 6, 24 and 27 % respectively compared to controls, providing weak evidence of bone marrow toxicity at 2000 mg/kg bw. The mean percentage of cells with aberrations (excluding gaps) was 0.42, 0.42 and 0.72 % at 500, 1000 and 2000 mg/kg bw, respectively, compared to 0.42 % in the concurrent vehicle control (not statistically significant). The mean numerical aberration frequencies were 0.9, 0.6 and 0.6 % at 500, 1000 and 2000 mg/kg bw, respectively, compared to 0.4 % in the concurrent vehicle control. The frequencies of cells with numerical aberrations for all dose groups were within the expected historical control range.

At 42 hours, cytotoxicity at 2000 mg/kg bw was 17 % compared to controls. The mean percentage of cells with aberrations (excluding gaps) was 0.58 % at 2000 mg/kg bw, compared to 0.33 % in the concurrent vehicle control (not statistically significant). The mean numerical aberration frequencies were 0.2 % at 2000 mg/kg bw, compared to 0.7 % in the concurrent vehicle control. The frequencies of cells with numerical aberrations at 2000 mg/kg bw were within the historical vehicle control 95 % reference range.

In conclusion, under the reported experimental conditions of this GLP and OECD study, pydiflumetofen did not induce chromosome aberrations in the bone marrow cells of the rat when tested up to 2000 mg/kg bw (the recommended maximum dose for this assay). Therefore, pydiflumetofen is considered to be non-mutagenic in this bone marrow chromosome aberration assay in the rat.

This *in vivo* rat bone marrow chromosome aberration test did not include an assessment of bone marrow exposure to pydiflumetofen. However, it is known that pydiflumetofen is systemically available in the rat, following oral gavage dosing, as demonstrated in the studies reported in Section B.6.1 and therefore bone marrow will have been exposed to pydiflumetofen in this study.

(██████████ 2017)

B.6.4.3. In vivo studies in germ cells

SYN545974 is non-genotoxic in somatic cells. Given that there is no evidence that SYN545974 is genotoxic in somatic cell systems, further *in vivo* studies in germ cells are not required.

B.6.4.4. Summary of genotoxicity

The genotoxic potential of pydiflumetofen was tested in a range of *in vitro* and *in vivo* tests.

The *in vitro* tests included two Ames tests (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*), an *in vitro* mammalian cell gene mutation assay (in mouse lymphoma L5178Y cells) and an *in vitro* chromosome aberration test (in human lymphocytes). The two Ames tests were conducted on different batches of pydiflumetofen: one on a standard toxicology batch (SMU2EP12007) and one on a batch spiked with potential impurities (SMU4FL762) to support the technical specification of pydiflumetofen. Both Ames tests and the mammalian cell gene mutation assay gave negative results. The *in vitro* chromosome aberration test gave a weakly positive/equivocal result, indicative of potential clastogenicity.

The *in vivo* tests included two mouse bone marrow micronucleus tests and a rat bone marrow chromosome aberration assay. The two mouse bone marrow micronucleus tests were conducted on

different batches of pydiflumetofen: one on a standard toxicology batch (SMU2EP12007) and one on a batch spiked with potential impurities (SMU4FL762) to support the technical specification of pydiflumetofen. The in vivo mouse studies gave negative results, meaning the weakly positive/equivocal in vitro clastogenicity finding was not corroborated in vivo. The rat bone marrow chromosome aberration assay was conducted on the same batch (SMU2EP12007) that gave a positive result in the in vitro chromosome aberration test. Pydiflumetofen gave a negative result when tested in this in vivo chromosome aberration test in rats. Therefore, pydiflumetofen is not considered to be genotoxic in vivo. Classification for mutagenicity is not required (see [GB MCL Technical Report](#)).

The in vivo bone marrow tests in the rat and mouse did not include assessments of bone marrow exposure to pydiflumetofen. However, in the mouse studies, the observed clinical signs indicated that the test material had been systemically available, reaching the bone marrow. Additionally, pydiflumetofen is known to be systemically available in the mouse and rat after oral gavage dosing, as demonstrated in the ADME studies reported in section 6.1. Therefore, the bone marrow will have been exposed to pydiflumetofen in these in vivo mouse bone marrow micronucleus tests and the in vivo rat bone marrow chromosome aberration assay.

The following table summarises the genotoxicity investigation of pydiflumetofen:

Test system and Acceptability	Concentration/ dose levels	Purity (%)	Result	Reference
In vitro studies				
Bacterial mutation assay (Ames) <i>S. typhimurium</i> strains (TA 1535, TA 1537, TA 98 and TA 100). <i>E. coli</i> strains (WP2 uvrA pKM101 and WP2 pKM101) +/- S9 <i>Acceptable modern study</i>	3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Batch: SMU2EP12007	98.5	Negative	(2012) Harlan (CCR) Report No. 1498901
Bacterial mutation assay (Ames) <i>S. typhimurium</i> strains (TA 1535, TA 1537, TA 98 and TA 100). <i>E. coli</i> strains (WP2 uvrA pKM101 and WP2 pKM101) +/- S9 <i>Acceptable modern study</i>	3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate Batch: SMU4FL762 (spiked with impurities)	96.7	Negative	(2014) Harlan (CCR) Report No. 1648701

In vitro cytogenetics in human lymphocytes +/- S9 <i>Acceptable modern study</i>	Exp I (4 hrs, with and without S9): With S9 – 16.1, 28.1, 49.2 µg/mL Without S9 – 16.1, 28.1, 150.8 µg/mL Exp IIA (22 hrs, with and without S9): With S9 – 9.2, 16.1, 2475.4, 4332.0 µg/mL Without S9 – 5.3, 9.2, 16.1 µg/mL Exp IIB (22 hrs, without S9): 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 15.0, 20.0, 40.0 µg/mL Batch: SMU2EP12007	98.5	Weakly positive/equivocal in absence of metabolic activation	(2013) Harlan (CCR) Report No. 1498902
Mammalian cell mutation assay (mouse lymphoma L5178Y TK +/-) +/- S9 <i>Acceptable modern study</i>	Exp I (4 hrs, with and without S9): With S9 – 7.5, 15.0, 30.0, 45.0, 60.0 µg/mL Without S9 – 7.5, 15.0, 22.5, 30.0, 60.0 µg/mL Exp II (4 hrs, with and without S9): With S9 – 7.5, 15.0, 30.0, 60.0, 90.0 µg/mL Without S9 – 7.5, 15.0, 30.0, 45.0, 60.0 µg/mL Exp III (4 hrs, without S9): 40.0, 80.0, 90.0, 100.0, 110.0 µg/mL Batch: SMU2EP12007	98.5	Negative	(2013) Harlan (CCR) Report No. 1498903
In vivo studies				
Micronucleus assay in mouse-bone marrow <i>Acceptable modern study</i>	24-hour preparation interval: 500, 1000, and 2000 mg/kg bw. 48-hour preparation interval: 2000 mg/kg bw. Batch: SMU2EP12007	98.5	Negative	(2012) Report No. 1498904
Micronucleus assay in mouse bone marrow <i>Acceptable modern study</i>	24-hour preparation interval: 500, 1000, and 2000 mg/kg bw. 48-hour preparation interval: 2000 mg/kg bw. Batch: SMU4FL762 (spiked with impurities)	96.7	Negative	(2014) Report No. 1648702
Rat Bone Marrow Chromosome Aberration Assay <i>Acceptable modern study</i>	500, 1000 and 2000 mg/kg Batch: SMU2EP12007	98.5	Negative	(2017) Report No. 8359217

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

SYN545974 has been evaluated for chronic toxicity in the rat and for carcinogenic potential in the rat and the mouse.

B.6.5.1. Two-year rat study

During the commenting period, EFSA requested the following data requirement: “Applicant to provide additional HCD for thyroid cell adenomas and thyroid follicular cell hypertrophy in the 2-yr rat study”. This additional HCD for thyroid cell adenomas and thyroid follicular cell hyperplasia were subsequently submitted by the applicant and assessed by the EU RMS (FR) within the summary [REDACTED] (2015a) study below.

Report:	K-CA 5.5/01 [REDACTED] (2015a). SYN545974: 104 Week Rat Dietary Carcinogenicity Study with a Combined 52 Week Toxicity Study. [REDACTED] [REDACTED]. Laboratory Report No. 36248, 30 July 2015. Report Amendment 1, 2 March 2016. Unpublished. Syngenta File No. SYN545974_10245.
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Report:	K-CA 5.5/02 [REDACTED], 2016 SYN545974 - Historical Control Data for SYN545974_10245, [REDACTED], Syngenta File No. SYN545974_10464
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Guidelines: Combined chronic toxicity/carcinogenicity. OECD guideline reference 453 (2009): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.4300 (1998): EU Directive 96/54/EC B.33 Combined Chronic Toxicity/Carcinogenicity Annex V to Directive 67/548/EEC, (2004): Appendix to Director General Notification, No. 12-Nousan-8147, JMAFF (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rodent diet has been assessed and validated.

EXECUTIVE SUMMARY

In a combined chronic toxicity / carcinogenicity study, groups of 52 male and 52 female Han Wistar [REDACTED]: WI (Han) rats were fed diets containing 200, 1000 or 6000 ppm and 0, 150, 450 or 1500 ppm of SYN545974, to males and females respectively, for 104 weeks to assess the carcinogenicity potential of SYN545974. In addition, a toxicity study comprising a further 4 groups of 12 males and 12 females were included and dosed in an identical fashion for a period of 52 consecutive weeks.

Animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at pre-determined intervals from pretrial up until the completion of treatment. Prior to terminal kill, blood samples were collected from all surviving animals for haematological analysis. Blood smears and clinical pathology samples were obtained from all surviving toxicity animals during Weeks 14, 27 and 52. Blood films were made from all surviving carcinogenicity animals during Weeks 52, 79 and prior to termination; however, blood cell morphology was not performed as no treatment related effects were seen on haematological parameters at termination.

Toxicity study animals were euthanized and subjected to a detailed necropsy examination after completion of 52 weeks of treatment. All surviving carcinogenicity animals were killed and subjected to a detailed necropsy examination after completion of 104 weeks of treatment. Tissues from all animals were subject to a comprehensive histological evaluation.

There were no treatment-related clinical observations noted during treatment.

Dietary administration of SYN545974 at 0, 200/150, 1000/450 and 6000/1500 ppm (males/females), for a period of at least 104 weeks, was associated with lower body weights and body weight gains for males treated at ≥ 1000 ppm and in females treated at ≥ 450 ppm. Food consumption and food utilisation was considered to be lower than controls for males and females that received 6000 or 1500 ppm, respectively while food consumption was noted to be generally lower when compared to controls in males at 1000 ppm or females at 450 ppm.

There were no treatment-related ophthalmoscopy findings.

There were no differences in qualitative or quantitative functional observations or motor activity assessments noted during the neurotoxicity assessment.

There were no toxicologically significant treatment-related differences in haematological, coagulation or blood chemistry parameters.

The proportion of unscheduled deaths in the control, 200/150, 1000/450 and 6000/1500 ppm SYN545974 dose groups was 14/52, 23/52, 7/52 and 10/52 in males, and 23/52, 13/52, 10/52 and 16/52 in females, respectively.

There were no treatment-related neoplastic findings observed following dietary administration of SYN545974 for 52 weeks (toxicity study) or 104 weeks (carcinogenicity study) at doses of 200, 1000 and 6000 ppm to males and 150, 450 and 1500 ppm to females.

In the toxicity study, hepatocellular hypertrophy in the liver was observed in males at ≥ 1000 ppm and in females at 1500 ppm, and correlated with the increased liver weights (≥ 1000 ppm in males and at 1500 ppm in females). Increased gamma glutamyl transferase activity was noted in males that received 6000 ppm. Grossly prominent lobular architecture was seen at 6000 ppm in males.

At 104 weeks hepatocellular hypertrophy was observed in males at ≥ 1000 ppm and in females at 1500 ppm. In the males at 6000 ppm this finding was associated with hepatocyte cytoplasmic eosinophilic inclusions and grossly, prominent liver lobular architecture.

The No Observed Adverse Effect Level (NOAEL) for this study was considered to be 200 ppm in males, equating to an achieved dose of 9.9 mg SYN545974/kg/day and 450 ppm in females equating to 31 mg SYN545974/kg/day.

In males, the LOAEL was considered to be 1000 ppm (equating to an achieved dose of 51.0 mg SYN545974/kg/day) based on reduced body weight and body weight gains ($>10\%$), decrease food utilization, liver weight increase and hepatocellular hypertrophy. In females, the LOAEL was considered to be 1500 ppm based on reduced body weight, body weight gains and food utilization, slight liver weight increase associated with minimal hepatocellular hypertrophy.

A 6000 ppm, the liver effects increased in severity (hepatocellular hypertrophy associated with grossly, prominent liver lobular architecture and cytoplasmic eosinophilic inclusions, increase blood GGT).

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Technical, off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 %
CAS#:	1228284-64-7
Stability of test compound:	Expiry date 30 June 2016 (stored at <30°C)

Vehicle and/or positive control: The test substance was administered via Rat and Mouse (Modified) No.1 Maintenance Diet SQC Expanded (Ground) supplied by [REDACTED].

Test Animals:	
Species	Rat
Strain	Han Wistar ([REDACTED] WI(Han))
Age/weight at dosing	Approximately 6-8 weeks / 116-183 g (males), 106-160 g (females)
Source	[REDACTED]
Housing	4 per cage by sex in polycarbonate cages with stainless steel grid tops and solid bottoms.
Acclimatisation period	Up to 3 weeks
Diet	Rat and Mouse (Modified) No.1 Maintenance Diet SQC Expanded (Ground) <i>ad libitum</i> (except during a 4 hour period for urine collection in toxicity animals in weeks 13, 26 and 51). Supplied by [REDACTED].
Water	Water from the public water supply <i>ad libitum</i> (except during a 4 hour period for urine collection in toxicity animals in weeks 13, 26 and 51).
Environmental conditions	Temperature: 19-22°C Humidity: 12-77% Air changes: Not reported Photoperiod: Not reported

Study Design and Methods:

In-life dates: Start: 07 November 2012, End: 14 July 2015

In a combined chronic toxicity / carcinogenicity study, groups of 52 male and 52 female Han Wistar [REDACTED] WI (Han) rats were fed diets containing 200, 1000 or 6000 ppm and 0, 150, 450 or 1500 ppm of SYN545974, to males and females respectively, for 104 weeks to assess the carcinogenicity potential of SYN545974. In addition, a toxicity study comprising a further 4 groups of 12 males and 12 females were included and dosed in an identical fashion for a period of 52 consecutive weeks. Control animals received blank diet only *i.e.* diet not containing the test substance.

Dose level selection: Dose levels were selected after evaluation of previous subchronic (90 day) and pharmacokinetic studies of the test substance ([REDACTED] Study Nos. 520843 and 195073) in this test model. These studies demonstrated a clear non-linear exposure in males and females and were indicative of a saturation of absorption as the dose increased. Statistical analysis to assess the proportional relationship between pharmacokinetic parameters and dose demonstrated exposure/dose proportional relationship between 5-300 mg/kg bw/day for males and 5-100 mg/kg bw/day in females. Consequently, doses between 200-6000 ppm were selected for the males (approximately 10-300 mg/kg bw/day) and 150-1500 ppm for the females (approximately 10-100 mg/kg bw/day). Although, typical biological stress indicators in the sub-chronic studies at a dose level of 300 mg/kg bw/day in males and 100 mg/kg bw/day in females are insufficient to establish a Maximum Tolerated Dose, toxicokinetic evidence of loss of dose proportionality is considered to be a scientifically robust approach for the chronic dose selection.

Animal assignment: On arrival from the suppliers, the animals were allocated to cages on racks. Cages were racked by treatment group and vertically throughout the rack. The control animals were housed on a separate rack from the treatment groups. During detailed functional observation assessments, control and treated groups were housed on the same rack. Approximately every 4 weeks, each rack was moved one position in the room, with the first move in Week 24. Group mean body weights were within $\pm 20\%$ (with a few small exceptions) indicating acceptable homogeneity of body weight.

Animals were allocated to 4 treatment groups as follows:

Study design

Test group	Treatment	Dietary concentration (ppm)	Number of animals			
			Carcinogenicity study		Toxicity study	
			Males	females	Males	Females
1	control	0	52	52	12	12
2	low dose	200	52		12	
		150		52		12
3	intermediate dose	1000	52		12	
		450		52		12
4	high dose	6000	52		12	
		1500		52		12

Diet preparation and analysis: Diet formulations were prepared as required, as serial dilutions from a more concentrated stock of at least 200 g. The stock was prepared by firstly mixing the test substance with the required amount of untreated control diet in an automated mortar and pestle and mixed until visibly homogeneous. Diet for the high dose group at a concentration of 6000 ppm was prepared by adding the stock prepared to a suitably sized diet bin, adding an appropriate amount of untreated diet, and this was then blended for 20 minutes in a diet mixer (Winkworth). For Day 1 dose preparations, Group 3 and Group 4 formulations were prepared and lower concentrations were prepared from these stocks. For subsequent dose preparations, male diets at lower concentrations (1000 and 200 ppm) were prepared as a dilution from the male high dose group. Female diets at lower concentrations were prepared as a dilution from the female high dose group. Diet formulations were prepared at appropriate intervals, stored and used within the conditions established during [REDACTED] Study No. 429624.

Diet formulation samples from all groups, were collected for concentration and homogeneity analysis during weeks 1, 13, 27, 39, 52, 65, 79, 91 and 104. Analyses were performed by HPLC using a validated analytical procedure. Duplicate top, middle and bottom samples were collected for analysis. Stability analyses performed previously in [REDACTED] Study No. 429624 demonstrated that the test substance is stable in the diet when prepared and stored under the same conditions as this study at concentrations of 10 to 16000 ppm.

Analytical results: Analysed concentrations of test item within the diet were found to be within $\pm 8.7\%$ of the theoretical concentrations on all occasions, indicating acceptable accuracy of formulation. The coefficient of variation was generally low (8.3% or lower) indicating satisfactory homogeneity. However in Week 13 the 200 ppm test diet showed a coefficient of variance of 10.7% (the high coefficient of variation was confirmed on re-analysis); as this was a minor deviation and an isolated incident it was considered not to affect the integrity of the study. SYN545974 was not detected in the control diet.

Observations: All animals were checked early morning and as late as possible each day for signs of viability. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and hair condition, eyes and mucous membranes, respiration and excreta.

Body weight: Body weights were recorded once during pretrial and then once a week until Week 14 of treatment, and approximately once every 2 weeks from Week 15 up until the end of treatment.

Food consumption, utilisation and achieved dose: The quantity of food consumed by each cage of animals was measured and recorded once weekly during pretrial, daily during Week 1 and weekly up until Week 14 of treatment. From Week 15, food consumption was measured once every 2 weeks from up until the end of treatment.

Food utilisation was calculated for Weeks 1-4, 5-8, 9-13 and 1-13 as follows: (cage mean weight gain x 100)/cage total food consumption.

The amount of test substance ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{nominal concentration (ppm)} \times \text{food consumption (g/day)}}{\text{Mid-point body weight (g)}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Ophthalmoscopy: All carcinogenicity study and spare animals were examined once during pretrial. On weeks 50 and 102, carcinogenicity study control and high dose animals were examined. There were no observations seen in the control group or high dose group that required the remaining groups to be evaluated. The eyes were examined using an indirect ophthalmoscope after the application of a mydriatic agent (1% Tropicamide Mydricyl®).

Detailed functional observations: These examinations were conducted for all toxicity study animals during Week 51/52. The observations were made by one observer who was 'blind' with respect to the animals' treatment and comprised the following:

Cageside observations

- Posture/condition on first approach (animal undisturbed), checked for: Prostration; Lethargy; Writhing; Circling; Breathing abnormalities; Gait abnormalities; Tremor; Fasciculation; Convulsions; Biting (of cage components or self-mutilating); Vocalisations; Piloerection.
- Ease of removal from the cage.
- Body temperature (rectal).
- Condition of the eyes, checked for: Pupillary function; Miosis; Mydriasis; Exophthalmos; Encrustation; Lachrymation.
- Condition of the coat.
- Presence of salivation.
- Overall ease of handling.

Observations in a standardised area (2 min observation period)

- Latency (time to first locomotory movement)
- Level of mobility.
- Rearing.
- Grooming.

- Urination/defecation.
- Arousal (level of alertness).
- Posture, tremor/convulsions, vocalisation, piloerection – recorded as for cageside observations.
- Palpebral closure.
- Gait abnormalities.
- Stereotypy (excessive repetition of behaviours) and/or unusual behaviours.

Functional tests

The following additional function tests were performed for all animals during Week 51/52.

- Reaction to sudden sound (click above the head).
- Reaction to touch on the rump with a blunt probe.
- Grip strength. The procedure was repeated 3 times for the forelimbs and 3 times for the hindlimbs, and the mean fore and hind grip strengths calculated.
- Pain perception. This was assessed by measurement of the tail flick response
- Landing foot splay. The procedure was repeated 3 times and the average measurement recorded.
- Motor activity. Each animal was placed in an individual cage held within a Smartfame utilising infrared pyroelectric detectors. Movement was detected in 2 dimensions anywhere in the cage, and was differentiated into basic and fine movements, and X and Y ambulation. Each animal was monitored for one session of 1 h, with activity counts being recorded over successive period of 5 min each (periods 1-12).

Other physical/functional abnormalities

Any other abnormality not already recorded in the above screening battery.

Haematology and coagulation: A blood film was taken from the orbital sinus of all surviving toxicity animals in Weeks 14, 27 and 52 and from all surviving carcinogenicity animals in Weeks 52, 79 and at termination. The films were not examined, however, as haematological findings indicated that evaluation would not yield any further information.

Blood was collected from all surviving toxicity animals (Weeks 14, 27 and 52) and all surviving carcinogenicity animals (at termination) via a capillary tube from the orbital sinus under isoflurane anaesthesia. The rats were not starved overnight. The following parameters were examined:

haemoglobin	total white cell count
haematocrit	differential white cell count
red blood cell count	reticulocytes
mean cell volume	reticulocyte count (absolute)
mean cell haemoglobin	fibrinogen
mean cell haemoglobin concentration	activated partial thromboplastin time
red cell distribution width	prothrombin time
platelets	

Clinical chemistry: Blood samples were obtained from all surviving toxicity animals (Weeks 14, 27 and 52) via the orbital sinus under isoflurane anaesthesia. The rats were not starved overnight. The following parameters were examined:

urea	albumin
glucose	globulin
aspartate aminotransferase	AG ratio
alanine aminotransferase	cholesterol
alkaline phosphatase	creatinine
lactate dehydrogenase	total bilirubin
creatine phosphokinase	sodium
gamma glutamyl transferase	potassium
total protein	chloride
triglycerides	calcium
	inorganic phosphate

Urinalysis: Urine was collected during weeks 13, 26 and 51 from toxicity animals individually housed in metabolism cages over a 4 h period of food and water deprivation. The following parameters were evaluated:

colour	glucose
turbidity	bilirubin
specific gravity	ketones
volume	leucocytes
pH	blood pigments
protein	microscopy of spun deposit
urobilinogen	

Bone smear evaluation: Bone marrow smears were taken at necropsy and stored for possible evaluation. However, they were not examined as haematological findings indicated that evaluation would not yield any further information.

Bioanalysis evaluation: Blood samples were obtained via the tail vein (orbital sinus week 52 only) from all surviving toxicity animals at the following timepoints: Day 2, Weeks 4, 14, 27 and 52. The blood was stored, deep frozen (-70 to -90°C) pending future analysis.

Investigations *post mortem*:

Unscheduled euthanasia: Animals were weighed, a necropsy undertaken, specified tissues stored and samples for evaluation of clinical pathology parameters and toxicokinetic analysis were obtained where possible. Clinical pathology results were not reported.

Termination: Animals surviving until scheduled euthanasia, after completion of 52 weeks of treatment (toxicity study animals) or 104 weeks (carcinogenicity animals) had a terminal body weight recorded and were killed by exposure to rising concentration of carbon dioxide followed by exsanguination. Animals were not fasted before necropsy.

Macroscopic examination: Each animal was subject to a detailed necropsy which consisted of a complete external and internal examination including body orifices and cranial, thoracic and abdominal organs and tissues.

Organ weights: The organs below were removed and weighed from all scheduled termination animals. Paired organs were weighed together.

brain	liver
epididymides	ovaries
adrenal glands	spleen
thyroid glands	testes
heart	uterus/cervix
kidneys	

Tissue submission: The following tissues from all animals were examined *in situ*, removed and examined and fixed in an appropriate fixative:

aorta (from thoracic segment)	liver
blood smear (from animals killed prematurely)	lung
bone marrow smear (femur)	lymph node mandibular
bone marrow (femur)	lymph node mesenteric
bone marrow (sternum)	skeletal muscle (from thigh)
bone (femur)	nasal cavity (only histopathologically examined for carcinogenicity study)
bone (sternum)	optic nerves
brain (forebrain, midbrain, cerebellum and medulla oblongata)	sciatic nerve
cervix (examined and collected toxicity study only)	oesophagus
epididymides	ovaries
eyes	oviducts
adrenal glands	pancreas
Harderian glands	pharynx
lacrimal glands	skin
mammary glands (with inguinal skin)	duodenum
parathyroid glands (examined only if present in the routine section of thyroid gland)	ileum
pituitary gland	jejunum
prostate gland	spinal cord (cervical, thoracic and lumbar)
salivary glands (mandibular)	spleen
seminal vesicles and coagulating glands	stomach (glandular and non-glandular regions)
thyroid gland	testes
gut-associated lymphoid tissue	thymus
heart	tongue
kidneys	trachea
caecum	urinary bladder
colon	uterus with cervix
rectum	vagina
larynx	gross lesions and/or masses

Liver sampling: After samples were collected for histopathological evaluation, multiple samples of liver (*ca* 150 mg each) were taken from two 5 mm sections of the left lateral lobe. The samples were snap frozen in liquid nitrogen in individual RNA-ase free tubes. All samples were taken as quickly as possible and stored at *ca* -70°C pending possible future analysis.

A representative section from the left lateral lobe, the right median lobe and the caudate lobe were taken and fixed in formalin (fixed for 48 hours). The liver was processed after 48 hours to a paraffin wax block and stored for possible future analysis. A small piece of duodenum was incorporated into each block.

The remainder of the liver was sectioned into 4-5 pieces and snap frozen in liquid nitrogen. Samples were stored frozen at *ca* -70°C pending possible future analysis.

Microscopic examination: All processed tissues from all animals (with the exception of blood and bone marrow smears and, tongue) were examined by light microscopy.

Statistics: All statistical tests were performed using appropriate computing devices or programs. All statistical tests were two-sided and performed at the 5% and 1% significance level. All means were presented with standard deviations. Males and females were analysed separately. If the variances were clearly heterogeneous, log or square root transformations were used in an attempt to stabilise the variances.

Quantitative data: Body weight, cumulative body weight gain, food consumption, food utilisation, haematology, coagulation, clinical chemistry, quantitative urinalysis values, quantitative FOB measurements (grip strength, landing foot splay and/or time to tail flick), and motor activity data at each measurement interval and overall activity were analysed using a parametric ANOVA. Organ weights were analysed using ANOVA as above and by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. Group summaries (mean, standard deviation and number of observations) and individual values for organ weights as a percentage of body weight were not evaluated for statistical significance. Pairwise comparisons were only performed against the control group.

For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant. Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Mortalities which were the result of animals killed following accidents or at scheduled termination were considered to be censored observations. Intergroup comparisons of mortality comparing each treatment group with the control group and an overall test for trend was performed using a log rank test, separately for males and females. Macroscopic findings are presented as total incidences per sex and treatment group, but were not evaluated for statistical significance.

For non-neoplastic micropathology incidence data, the total incidence (combining all severity grades) was analysed using Fisher's Exact Probability Test. For neoplastic micropathology incidence data, pairwise comparisons of the incidence of tumours were made using the Fisher's Exact Probability test function within Nevis 2012. Further analyses were performed using Peto's time adjusted methods. However, where only a limited number of animals in each group were examined, no statistical analysis was performed. For non-palpable tumours, the study pathologist classified each of the tumours as either fatal, probably fatal, probably incidental or incidental. For the purposes of statistical analysis, tumours classified as either fatal or probably fatal were considered fatal and those tumours classified as probably non-fatal or non-fatal were considered non-fatal. All tumours detected in animals that died in a planned sacrifice were automatically classified as non-fatal. For the purposes of statistical analysis, all palpable tumours were considered to be fatal and the time of first detection was used in the age-adjusted analysis ("onset rate method" of analysis). For palpable tumours not detected in-life, the time to death was used in the analysis. Methods used for the age-adjusted analysis of non-palpable tumours (including fatal tumours (death rate analysis) and non-fatal tumours (prevalance analysis)), and age-adjusted analysis of palpable tumours (onset rate analysis) was based on the IARC guidelines. All age-adjusted analyses of tumour data was two-sided and performed at the 5% significance level using SAS (v8.2). The analysis of non-fatal tumours was conducted by dividing the experimental period into the following fixed time intervals: 1-52 weeks and 53-78 weeks, 79-92 weeks and over 92 weeks and single intervals for any

planned sacrifices. For each considered dataset, the significance of a linear dose related increase in tumour incidence was evaluated using a two-sided trend test. For the purposes of the trend tests, the group numbers were used. If the overall Peto trend was significant at the 5% level for any given tumour incidence, then the overall Peto trend was re-analysed for Groups 1-3; if still significant it was re-analysed for Groups 1-2. Males and females were analysed separately.

For each statistical test performed on a dataset containing 10 or less tumours, the discrete permutation distribution was used to calculate the corresponding p-value.

Qualitative data (e.g. possible values of 0, 1, 2 or present/absent): Qualitative functional observational battery parameters or any other parameters not specifically mentioned above that yield qualitative data were presented as summary data, but were not analysed statistically.

RESULTS

Mortality:

Toxicity study: There were no treatment-related mortalities.

Carcinogenicity study: Various neoplastic and non-neoplastic causes of death were reported. None of these were considered to be related to SYN545974 administration. In males, an increase in mortality was observed in the low (200 ppm) dose group, but the difference from control did not attain statistical significance and in the absence of an effect at the higher doses and an overall statistically significant decreasing trend in male mortality with increasing dose, the mortality at 200 ppm is considered incidental to administration of SYN545974. In females, administration of SYN545974 did not result in an increase in mortality.

Table 6.5-3: Overall % survival in carcinogenicity study

Group	Dietary concentration (ppm)	Males		Females	
		number	%	number	%
1	0	38/52	73	29/52	56
2	200/150	29/52	56	40/52	77
3	1000/450	45/52	87	41/52	79
4	6000/1500	42/52	81	36/52	69

Clinical observations and palpable masses: There were no treatment-related clinical observations during the study. The number of palpable masses in animals assigned to the carcinogenicity study was equivalent between control and treated groups in both sexes

Body weight: Males and females that received 6000/1500 ppm, respectively, showed statistically significantly lower body weights compared to their respective controls throughout the treatment period. Similarly, for males and females that received 1000/450 ppm, respectively, group mean body weights were statistically significantly lower throughout the treatment period when compared with the concurrent control. There were no differences from control noted for animals that received 200/150 ppm, respectively.

Changes in group mean body weights in animals that received 1000/450 or 6000/1500 ppm were reflected in lower body weight gain over the duration of the treatment period (Days 1-722). The difference in overall body weight change (Days 1-722) from control was $\geq 10\%$ for males and females at 1000/450 ppm and 6000/1500 ppm respectively.

Table 6.5-4: Intergroup comparison of body weights (g) and body weight gains (g) (carcinogenicity and toxicity combined) - selected timepoints

Day	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
1	153	152	154	148*	129	128	125*	128
29	295	299	282**	256**	194	195	188*	186**
64	382	381	362**	324**	230	231	223*	220**
120	443	448	420*	383**	252	254	243*	240**
232	513	517	484**	448**	277	275	263**	259**
358	573	578	538**	496**	307	303	285**	276**
484	633	628	576**	535**	345	339	314**	305**
610	671	645	600**	554**	372	375	341*	324**
722	704	664	628**	576**	395	402	365	359*
1-92	266±40	268±32	245±34**	213±26**	118±14	116±16	111±14	106±12*
1-722	550±103	513±79	476±87**	429±47**	267±64	274±63	241±58	231±45*

* Statistically significant difference from control group mean, p<0.05 (Dunnett)

** Statistically significant difference from control group mean, p<0.01(Dunnett)

Food consumption, utilisation and achieved dose: For males that received 6000 ppm, the quantity of food consumed was statistically significantly lower during the first week of treatment (measured daily due to proven stability at the time of treatment) when compared to concurrent controls indicating an initial palatability effect. Although the food consumption values progressively returned to levels similar to concurrent controls thereafter, they were consistently slightly lower and achieved statistical significance on several occasions during treatment. A similar pattern was observed for females that received 1500 ppm, where an initial palatability effect was observed, with the quantity of food consumed per cage of animals returning to levels similar to the concurrent control. Again, values were consistently slightly lower throughout treatment achieving statistical significance on several occasions.

For males and females that received 1000/450 ppm, respectively, the quantity of food consumed was also noted to be generally lower when compared to the concurrent control groups, although to a lesser degree observed for 6000/1500 ppm treated animals. Statistical significance was observed on several occasions throughout the treatment period.

There were no differences noted for animals that received 200/150 ppm, respectively.

Table 6.5-5: Intergroup comparison of food consumption (g/animal/day) (carcinogenicity and toxicity combined) - selected timepoints

Day	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
1	19.0	18.8	18.9	18.4	15.5	15.4	14.8	15.3
29	23.9	24.0	22.3*	21.0**	17.9	17.6	16.9*	17.0
64	21.4	21.0	20.5*	19.0**	18.2	17.5	17.2	17.7
127	22.7	22.4	23.0	21.8	19.0	18.1	17.1**	17.4*
239	24.0	24.1	23.1	22.8*	19.6	20.0	18.8	17.4**
365	22.6	23.7	23.0	21.7	17.7	19.0	16.8	16.5
477	22.8	23.0	22.7	21.8	19.0	18.1	18.7	18.0
617	23.8	23.5	20.6**	22.3	20.2	20.9	19.9	17.4**
729	24.9	24.0	23.0	22.9	19.3	19.3	18.7	20.9

* Statistically significant difference from control group mean, $p < 0.05$ (Dunnett)

** Statistically significant difference from control group mean, $p < 0.01$ (Dunnett)

Males that received 6000 ppm had statistically significantly lower food utilisation during weeks 1-4 and 5-8, and overall for weeks 1-13 in comparison to controls while statistical significance was observed for males that received 1000 ppm during weeks 1-4 and overall for weeks 1-13, suggesting that these differences reflect an effect of treatment. Although no incidences of statistical significance were observed for females that received 1500 ppm, it was considered that food utilisation was slightly lower than concurrent controls during weeks 1-4 and overall for weeks 1-13. These changes were consistent with the effects on food consumption profiles.

Table 6.5-6: Intergroup comparison of food utilisation (g body weight gain/100g food consumed) (carcinogenicity and toxicity combined) - selected timepoints

Weeks	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
1-4	22.3	22.9	20.7**	18.8**	14.2	14.6	14.4	13.7
5-8	11.6	11.0	11.6	10.5**	6.1	6.0	6.4	6.3
1-13	13.0	13.2	12.4*	11.6**	7.3	7.2	7.2	6.9

* Statistically significant difference from control group mean, $p < 0.05$ (Dunnett)

** Statistically significant difference from control group mean, $p < 0.01$ (Dunnett)

Estimated achieved dose: Dose rates (based on nominal dietary levels) were calculated in terms of mg SYN545974/kg body weight. Mean values are shown below:

Table 6.5-7: Mean Dose Received (mg/kg/day)

Dietary concentration (ppm)	200/150	1000/450	6000/1500
Males	9.9	51.0	319
Females	10.2	31.0	102

Water consumption: There were no treatment-related effects on water consumption.

Ophthalmoscopic examinations: There were no treatment-related effects.

Functional Observational Battery: There were no treatment related differences in the functional observation battery parameters following administration of SYN545974 at dose levels up to 6000ppm in males or 1500 ppm in females.

Detailed clinical observations: There were no treatment-related effects.

Quantitative functional observations: There were no treatment-related effects.

Motor activity: There were no notable inter-group differences in motor activity following administration of SYN545974 at dose levels up to 6000ppm in males or 1500 ppm in females.

Haematology and coagulation: There were no toxicologically significant differences noted in any haematology or coagulation parameters at any timepoint evaluated throughout treatment with SYN545974 at dose levels up to 6000 ppm in males or 1500 ppm in females.

There were minor isolated differences observed in the haematology parameters but these were considered to be unrelated to the administration of SYN545974 as they were within the range of the historical control data, or lacked true dose-relationship and were therefore considered not to be test item related.

Blood clinical chemistry: There were no toxicologically significant differences noted in any clinical chemistry parameter at any timepoint evaluated throughout treatment with SYN545974 at dose levels up to 6000 ppm in males or 1500 ppm in females. For parameters where a statistically significant change from control was observed, the values are presented in Table 6.5-8.

However, Gamma glutamyl transferase levels were statistically significantly higher in males that received 6000 ppm when compared with controls on all occasions (Weeks 14, 27 and 52).

Table 6.5-8: Intergroup comparison of selected blood clinical chemistry parameters

		Dietary Concentration (ppm)							
		Males				Females			
		0	200	1000	6000	0	150	450	1500
Week 14	Gamma glutamyl transferase (GGT)	2.00	2.00	2.00	7.08**	2.18	2.25	2.09	2.17
	Potassium	4.2	4.2	4.5	4.6**	3.6	3.5	3.6	3.9*
	Glucose	10.70	11.41	10.36	10.45	9.13	8.07	8.98	7.90*
	Chlorine	104	104	103	104	104	103	103*	105
Week 27	Gamma glutamyl transferase (GGT)	2.00	2.00	2.00	8.08**	2.00	2.00	2.00	2.09
	Glucose	11.81	11.06	10.69	12.41	10.67	10.59	8.55*	10.14
	Albumin	42	40	41	43	47	48	50*	47
Week 52	Gamma glutamyl transferase (GGT)	2	2	2	11**	2	2	2	2
	Glucose	11.36	10.81	10.99	9.59*	8.77	8.99	9.51	8.82
	Triglycerides	2.20	2.46	2.16	1.67*	1.98	1.74	1.34	1.49
	Chlorine	103	103	103	104	103	102*	102*	103
	Calcium	2.68	2.71	2.69	2.72	2.68	2.75*	2.73	2.73

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

There were other minor isolated differences from controls observed in the clinical chemistry parameters which were within the range of the overall control group data, or were judged to be due to individual changes, or due to biological variation or lacked true dose-relationship and were therefore considered not to be test item-related.

Urinalysis: There were no notable inter-group differences in any urinalysis parameters following administration of SYN545974 at any timepoint throughout treatment at dose levels up to 6000 ppm in males or 1500 ppm in females.

Sacrifice and pathology:

Macroscopic findings:

Toxicity study: Prominent lobular architecture in the liver (3/12) were observed in males at 6000 ppm and considered related to the administration of SYN545974. Other findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to administration of SYN545974 (Table 6.5-9).

Table 6.5-9: Intergroup comparison of selected macroscopic findings – toxicity study

Finding	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver (no. examined)	12	12	12	12	12	12	12	12
Prominent lobular architecture	0	0	0	3	0	0	0	0
Thyroid gland (no. examined)	12	12	12	12	12	12	12	12
Enlargement, unilateral	0	0	0	2	0	0	0	0

Carcinogenicity study: In males, prominent lobular architecture in the liver was observed at incidences of 5, 15, 14 and 17/52 (in Groups 1-4 respectively); in some animals this correlated with the microscopic finding of hepatocyte hypertrophy. In females, gross observations in the liver were considered incidental, of the nature commonly observed in this strain and age of rats and/or were of similar incidence in control and treated animals and, therefore considered unrelated to administration of SYN545974 (Table 6.5.10).

In males there was an apparent increase in the incidence of masses in the thyroid gland between controls and treated groups (0, 3, 2 and 3/52 in Groups 1-4 respectively). However, in the absence of an increased incidence of thyroid tumours in the treated groups, the apparent increase in masses is considered incidental. In females, the incidence of masses in the thyroid gland showed an apparent higher incidence at 1500 ppm (2 versus 0/52 controls). The masses correlated to follicular cell adenomas; however, the incidence was considered incidental to treatment.

Other findings observed were of similar incidence in control and treated animals, and/or of the nature commonly observed in this strain and age of rat. These findings were, therefore, considered incidental and unrelated to administration of SYN545974.

Table 6.5-10: Intergroup comparison of selected macroscopic findings – carcinogenicity study

Finding	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver (no. examined)	52	52	52	52	52	52	51	52
Prominent lobular architecture	5	15	14	17	7	0	5	3
Prominent lobular architecture associated with hepatocyte hypertrophy	0	0	1	14	0	0	0	0
Thyroid gland (no. examined)	52	52	52	52	52	52	51	52
Mass	0	3	2	3	0	0	0	2
Enlargement, unilateral	1	0	0	0	0	0	0	0

Organ weights:

Toxicity study: Organ weights were increased relative to controls in the liver at ≥ 1000 ppm in males and ≥ 450 ppm in females. No other test substance-related organ weight changes were noted. There were other isolated organ weight values that were different from their respective controls. There were, however, no patterns, trends, or correlating data to suggest these values were toxicologically relevant. Thus, other organ weight differences observed were considered incidental and unrelated to administration of SYN545974 (Table 6.5-11).

Table 6.5- 11: Intergroup comparison of organ weights (toxicity study)

	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver								
Absolute value (g)	16.064	16.240	18.763 (+17%)	20.125* (+25%)	9.331	9.194	9.875	9.866 (+6%)
Absolute (covariance analysis)	15.902	15.280	18.397 (+16%)	21.559** (+36%)	9.011	9.141	9.785	10.379* (+15%)
% of body weight†	2.9113	2.8549	3.3673 (+16%)	4.0279 (+38%)	3.0481	3.1350	3.3315	3.6202 (+17%)
Thyroid								
Absolute value (g)	0.0305	0.0295	0.0311	0.0329	0.0218	0.0223	0.0215	0.0227
Absolute (covariance analysis)	0.0303	0.0286	0.0307	0.0343	0.0208	0.0222	0.0212	0.0243
% of body weight†	0.0057	0.0051	0.0056	0.0067	0.0071	0.0076	0.0073	0.0083

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

† no statistical analysis performed

Carcinogenicity Study: Organ weight data from animals at 104/105 weeks are compromised due to the geriatric changes and development of spontaneous tumours which confound the interpretation of the organ weight data. However, following covariate analysis, liver weights were higher at ≥ 1000 ppm in males and 1500 ppm in females, which is consistent with findings in the toxicity phase and with the histopathology finding of hypertrophy (Table 6.5-12).

There were other isolated organ weight values that were different from their respective controls, however, there were no patterns, trends, or correlating data to suggest these values were toxicologically relevant.

Table 6.5-12: Intergroup comparison of organ weights (carcinogenicity study)

	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver								
Absolute value (g)	19.426	19.964	19.707	20.840 (+7%)	11.065	11.370	11.403	11.478 (+4%)
Absolute (covariance analysis)	17.929	19.531	20.009* (+12%)	22.169** (+24%)	10.780	11.017	11.588	11.871* (+10%)
% of body weight†	2.8203	3.1157	3.2219 (+14%)	3.6671 (+30%)	2.8704	2.9391	3.1963	3.3029 (+15%)
Thyroid								
Absolute value (g)	0.0480	0.0670	0.0452	0.0505	0.0293	0.0305	0.0298	0.0552
Absolute (covariance analysis)	0.0519	0.0681	0.0444	0.0471	0.0288	0.0299	0.0301	0.0559
% of body weight†	0.00710	0.1160	0.00733	0.00893	0.00767	0.00793	0.00835	0.01593

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

† No statistical analysis performed

Microscopic findings:

Toxicity study:

Non-neoplastic findings: Hepatocellular hypertrophy was observed in males at ≥ 1000 ppm. The incidence and severity of the hypertrophy increased with dose and as associated with cytoplasmic eosinophilic inclusions. This correlated to the prominent lobular architecture observed grossly in the liver in males. In females, hepatocyte hypertrophy in the liver was observed at 1500 ppm (Table 6.5-13).

The incidence of erythrophagocytosis in the mesenteric lymph node was increased in Group 4 males when compared with controls, achieving statistical significance. This is a common background finding attributed to haemorrhage occurring during euthanasia. There were no findings in the intestines or other tissues draining to this lymph node to suggest this was related to haemorrhage occurring prior to euthanasia. Therefore this was considered not to be related to administration of SYN545974. In the eye, retinal atrophy was observed at an increased incidence in males at 6000 ppm (3/12 compared with 0/11 in controls). However, since a similar trend was not observed at the end of the carcinogenicity study (the incidence at 6000 ppm was lower than in controls), this finding was considered not to be related to administration of SYN545974.

Other non-neoplastic microscopic findings observed were considered incidental, of the nature commonly observed in this strain and age of rats, and/or were of similar incidence and severity in control and treated animals and, therefore, were considered unrelated to administration of SYN545974

Table 6.5-13: Intergroup comparison of selected microscopic findings (toxicity study)

Finding	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver (no. examined)	11	12	12	12	11	12	11	10
Hepatocellular hypertrophy total	0	0	5*	11**	0	0	0	4
minimal	0	0	5	1	0	0	0	4
mild	0	0	0	5	0	0	0	0
moderate	0	0	0	5	0	0	0	0
Mesenteric lymph node (no. examined)	11	12	12	12	11	12	11	10
Erythrophagocytosis total	1	0	1	7*	1	1	0	0
minimal	0	0	1	5	0	1	0	0
mild	1	0	0	2	0	0	0	0
moderate	0	0	0	0	1	0	0	0
Thyroid (no. examined)	12	12	12	12	12	12	12	12
Hyperplasia; Focal, C-cell	0	1	0	1	0	0	0	0

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Neoplastic findings: No test substance-related neoplastic findings were noted.

Carcinogenicity study:

Non-neoplastic findings: Hepatocellular hypertrophy was observed in males at ≥ 1000 ppm. The incidence and severity of the hypertrophy increased with dose and at 6000 ppm was associated with cytoplasmic eosinophilic inclusions. These findings correlated with the prominent lobular architecture observed grossly in the liver in males. In females, hepatocyte hypertrophy in the liver was observed at 1500 ppm (Table 6.5-14).

Some non-neoplastic microscopic findings were present at a lower incidence with respect to controls and achieved statistical significance by Fisher's exact test, these decreases are considered to be of no biological significance and a consequence of biological variation in aged animals. Other non-neoplastic microscopic findings observed were of a similar incidence in control and treated animals, and/or of the nature commonly observed in this strain and age of rat. These findings were, therefore, considered incidental and unrelated to administration of SYN545974.

Following a request of the EU RMS on May 2018, the applicant submitted historical control data for thyroid follicular cell hyperplasia in female rats from 104-week studies performed at [REDACTED] (see Table 6.5-16). The incidence of 3/52 (5.8%) observed for follicular cell hyperplasia in females at the highest dose of 1500 ppm is within the historical control data (Range: 0- 6%).

Table 6.5-14: Intergroup comparison of selected non-neoplastic microscopic findings (carcinogenicity study)

Finding	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Liver (no. examined)	52	51	52	52	52	52	51	52
hepatocellular hypertrophy total	0	0	3	39**	0	0	0	3
minimal	0	0	3	13	0	0	0	2
mild	0	0	0	20	0	0	0	1
moderate	0	0	0	6	0	0	0	0
eosinophilic inclusions	0	0	0	19**	0	0	0	0
Thyroid (no. examined)	52	50	50	52	51	52	51	51
Hyperplasia; Focal, Follicular cell	4	3	3	7	1	3	0	3
Hyperplasia; Diffuse, C-cell	45	38	36	42	34	28	27	28
Hyperplasia; Focal, C-cell	6	5	7	6	11	5	7	7
Infiltration Inflammatory cell	2	1	0	0	0	0	0	0

** Statistically significant difference from control group mean, $p < 0.01$ (Fisher's exact test)

Neoplastic findings: There were no test substance-related neoplastic findings.

In females there was a slight numerical increase in the incidence of thyroid follicular cell adenomas at 1500 ppm, which did not reach statistical significance on pairwise comparison, or show a dose-related trend (Peto $p = 0.16$), and was within the historical control range of 0-10%. Therefore, this was considered by the applicant not to be related to administration of SYN545974 (Tables 6.5-15 and 16). The HCD range of 0-10% corresponds to the background incidence of thyroid cell adenomas in females from 19 carcinogenicity (104-weeks) dietary rat studies provided by the performing laboratory between 2001 and 2013. One study performed in 2005 showed an incidence of 5/50 (10%). Following a request of the EU RMS on May 2018, the applicant submitted updated historical control data including two additional 104-week studies performed in 2013 and 2015 in female Hans Wistar rats at [REDACTED]. If we take into account the 5-year time frame only (7 studies; 2009-2013), the background incidence is 0-5.8% (3/52) (as presented in Table 6.5-16). Thus, the increase in the incidence of thyroid follicular cell adenomas observed at 1500 ppm in females (3/51 (5.9%) vs 1/51 (2%) in control) can be considered within the historical control provided by the performing laboratory in the 5-year time frame. To support that the thyroid follicular cell adenomas observed in females at the higher dose can be considered not treatment related, it should be noted that there were no preneoplastic lesions observed neither in the toxicity study nor in the carcinogenicity study. In addition, the incidence of thyroid follicular adenomas

in female rats after administration of pydiflumetofen was not statistically significantly different to controls and there was no dose response (peto analysis). It was also shown that pydiflumetofen does not have a direct effect on thyroid peroxidase in the rat (*in vitro*) and therefore, pydiflumetofen is not acting via a direct effect on the thyroid (data presented in DAR B6.8.2).

Table 6.5-15: Intergroup comparison of selected neoplastic microscopic findings in the thyroid (carcinogenicity study)

Finding	Dietary Concentration (ppm)							
	Males				Females			
	0	200	1000	6000	0	150	450	1500
Thyroid (no. examined)	52	50	50	52	51	52	51	51
C-cell Adenoma; Benign, incidental	5	3	2	2	3	5	4	4
C-cell Carcinoma	0	0	1	1	0	0	0	0
Follicular Cell Adenoma; Benign, incidental	8	3	4	5	1	0	0	3

p>0.05(Peto test)

Table 6.5-16: Historic Control Data - 104-Week Studies in Female Han Wistar Rats at [REDACTED] (April 2007 2001 – March 2015)

Study identifier	Start	No. Examined	Follicular Cell Adenoma (a)		Follicular Cell Carcinoma (b)		Hyperplasia; Focal follicular cell (c)	
			Incidence	%	Incidence	%	Incidence	%
371	2001	50	1	2	0	0		
895	2002	100	0	0	0	0		
921	2002	100	1	1	1	1		
223	2002	49	3	6	0	0		
811	2002	50	1	2	1	2	0	0
894	2003	100	1	1	0	0	4	4
679	2003	129	1	0.8	0	0		
993	2004	100	6	6	2	2	2	2
137	2004	55	0	0	0	0		
384	2005	50	5	10	0	0	0	0
666	2005	110	5	4.5	0	0	0	0
304	2007	52	0	0	3	5.8	0	0
325	2007	52	0	0	0	0	3	5.8
930	2007	110	4	3.6	0	0	1	0.9
580	2009	50	0	0	0	0	3	6
072	2009	119	0	0	0	0	5	4.2
911	2010	65	1	1.5	0	0		
612	2012	51	2	3.9	0	0		
261	2013	52	1	1.9	0	0	1	1.9
312	2013	50	2	4.0	0	0	3	6
727	2015	52	3	5.8	0	0	0	0
All studies (2001-2015)								
TOTAL		544 1546 (a; b) 947 (c)	8 37		3 7		22	
RANGE				0-3.9 0-10		0-5.8		0-6
MEAN				1.36 2.4		0.7 0.45		2.3
N (studies)				8 21		8 21		13
5-year time frame with respect to completion date of the study (2009-2015)								
TOTAL		439 (a; b) 323 (c)	9		0		12	
RANGE				0-5.8		-		0-6
MEAN				2		0		3.7
N (studies)				7		7		5

Shaded cells indicate the studies that have been conducted within a 5 year period from when this study was conducted

CONCLUSION:

Overall, in a GLP and guideline chronic toxicity/carcinogenicity study in rats given doses of 0, 200, 1000 or 6000 ppm to males (9.9, 51, 319 mg/kg bw/d) and doses of 0, 150, 450 or 1500 ppm to females (10.2, 31 and 102 mg/kg bw/d), no tumours were observed in both sexes up to the top dose tested. Although thyroid follicular adenomas were increased in females at the top dose (3/51 (5.9%) vs 1/51

(2%) in controls), the increase was within the laboratory contemporary (5-years) HCD (0 – 5.8%), they were not statistically significant and there was no association with pre-neoplastic lesions. Therefore the NOAEL for carcinogenicity was 6000 ppm (319 mg/kg bw/d) in males and 1500 ppm (102 mg/kg bw/d) in females.

In males, pydiflumetofen caused decreases in body weight (10.8%), body weight gain (13%), food consumption and food utilisation, an increase in liver weight (16%) with associated hypertrophy at the mid dose of 1000 ppm. These effects became more severe at the top dose of 6000 ppm (e.g. ↓18.2% in body weight; ↓22% body weight gain; ↑36% liver weight) at which an increase in GGT was also seen. In females, adverse effects were only observed at the top dose of 1500 ppm (↓ 9.1 % body weight; ↓ 13% body weight gain, reduced food consumption, ↑15% liver weight; hepatocyte hypertrophy). Therefore a chronic toxicity NOAEL of 200 ppm (9.9 mg/kg bw/d) was identified in males and a chronic toxicity NOAEL of 450 ppm (31 mg/kg bw/d) was identified in females.

HSE notes that the MTD (maximum tolerated dose) was reached in the study, with reductions in body weight gain of 22% in males and 13% in females at the top dose. Therefore, the carcinogenic potential of pydiflumetofen was adequately investigated in the rat.

(██████████, 2015a)

B.6.5.2. 18-month mouse study

During the commenting period, EFSA requested the following additional information regarding the long-term mouse study:

Applicant to provide new historical control data from ██████████ for the liver findings in the long term mouse study.

Applicant to amend data regarding liver weights in Table 6.5-21 in vol.3, in the carcinogenicity study in the mouse and also liver weight values in Table 16 of Appendix 19 of the original study reports.

The applicant provided additional HCD for eosinophilic foci of cellular alteration in the liver of male mice and these additional data have been integrated within the summary ██████████ (2015b). In addition, the absolute values for intergroup comparison of liver weights have been amended in Table 6.5-21, along with the original study report.

Report:	K-CA 5.5/03 ██████████ (2015a). SYN545974: 80 Week Mouse Dietary Carcinogenicity Study. Report Amendment 1. ██████████. Laboratory Report No. 35914, 17 July 2015. Report Amendment 1, 18 September 2015. Report Amendment 2, 29 February 2016. Report Amendment 3, 16 January 2017. Unpublished. Syngenta File No. SYN545974_10237.
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Guidelines: Carcinogenicity. OECD guideline reference 451 (2009): United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.4200 (1998): Annex V to Council Directive 67/548/EEC, published in the Ninth Adaptation, Commission Directive 88/302/EEC B.32 (1987) OJEC, L133, 32-36, 1988: Appendix to Director General Notification, No. 12-Nousan-8147, JMAFF (2000).

GLP: Signed and dated GLP and Quality Assurance statements were provided.

There were no deviations from the current regulatory guideline considered to compromise the scientific validity of the study.

HSE Comment: the study was considered acceptable. The analytical method for determination of pydiflumetofen in rodent diet has been assessed and validated (see DAR Vol. 3 B. 5.1.2.2).

EXECUTIVE SUMMARY

In a carcinogenicity study, groups of 50 male and 50 female CD-1 (ICR) mice were fed diets containing 0, 75, 375 and 2250 ppm of SYN545974 for a period of at least 80 weeks.

Animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at pre-determined intervals from pretrial up until the completion of treatment. At Week 80, prior to terminal kill, blood samples were collected from all surviving animals for haematological analysis. Blood smears were obtained from all surviving animals during Week 52/53 and at Week 80; however, blood cell morphology was not performed as no treatment related effects were seen on total and differential white cell haematological parameters at termination.

All surviving animals were killed and subjected to a detailed necropsy examination after completion of treatment. Tissues from all animals were subject to a comprehensive histological evaluation.

Dietary administration of SYN545974 at 0, 75, 375 and 2250 ppm, for a period of at least 80 weeks, was associated with lower body weights and body weight gains for males and females treated at 2250 ppm. The difference from control for body weight from Day 1 to Day 561 was -14% in males and -24% in females. Food intake was lower for males and females receiving 2250 ppm which was consistent with the effects on body weight. However, food utilisation values were only lower for males receiving 2250 ppm.

There were no treatment-related effects on mortality, clinical observations or haematological parameters in male and female treated groups. Liver weight (adjusted for terminal weights) was increased in males and females receiving 2250 ppm.

Neoplastic and non-neoplastic findings attributable to the administration of SYN545974 were observed in the liver. A higher incidence of hepatocellular carcinomas and adenomas was observed in males administered SYN545974 at ≥ 2250 ppm compared with the control group which correlated with a higher incidence of liver masses observed at necropsy at ≥ 375 ppm. The number of animals with multiple adenomas in the liver was also increased by treatment at ≥ 375 ppm in male mice. There was a numerical increase in eosinophilic foci of cellular alteration in the liver of males treated with SYN545974. The incidence was statistically higher than the control group only at 2250 ppm. Centrilobular hepatocellular hypertrophy was observed in males administered SYN545974 at ≥ 375 ppm.

SYN545974 was considered to cause carcinogenic effects in males that received ≥ 375 ppm where higher incidences of liver tumours (hepatocellular carcinomas and adenomas) coupled with a higher incidence of liver masses, eosinophilic foci of cellular alteration, centrilobular hypertrophy and liver weight were observed. With the exception of increased liver weights in females at 2500 ppm, these findings were not present in female mice at dose levels up to 2250 ppm.

Dietary administration of 2250 ppm was associated with a decrease in body weight and body weight gain in males and females, and a decrease in food utilisation during the early stages of the study in males only.

For males, the No Observed Effect Level (NOAEL) for this study was 75 ppm, equating to achieved dose levels of 9.2 mg SYN545974/kg/day. For females, the No Observed Effect Level (NOAEL) for this study was 375 ppm equating to 48.4 mg SYN545974/kg/day.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Technical, off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5 %
CAS#:	1228284-64-7
Stability of test compound:	Expiry date 30 June 2016 (stored at <30°C)

Vehicle and/or positive control: The test substance was administered via Rat and Mouse (Modified) No.1 Maintenance Diet SQC Expanded (Ground). Supplied by [REDACTED].

Test Animals:	
Species	Mouse
Strain	[REDACTED] CD-1(ICR)
Age/weight at dosing	6-7 weeks / 29.4-43.5 g (males), 20.5-30.5 g (females)
Source	[REDACTED]
Housing	Males were housed individually and females up to 3 per cage in suspended polypropylene cages with stainless steel grid tops
Acclimatisation period	16 Days
Diet	[REDACTED] Rat and Mouse No. 1 Diet [REDACTED] <i>ad libitum</i>
Water	Public supply water <i>ad libitum</i>
Environmental conditions	Temperature: 19-22°C Humidity: 12-77% Air changes: ≥ 10/hour Photoperiod: 12 hours light / 12 hours dark

Study Design and Methods:

In-life dates: Start: 07 March 2013, End: 16 July 2015.

Dose level selection: Dose levels were selected following evaluation of pharmacokinetic studies with the test substance in this test model. An evaluation of the toxicology and toxicokinetic data following oral (gavage/diet) administration of SYN545974 to mice demonstrated a clear non-linear exposure in males and females and indicated saturation of absorption, which limits exposure with increasing doses. Assessment of the relationship between exposure and dose demonstrates proportionality (linear kinetics) between 10-300 mg/kg bw/day for males and females. Consequently, doses between 75 and 2250 ppm were selected (estimated to be approximately 10-300 mg/kg bw/day).

Animal assignment: On arrival from the suppliers, the animals were allocated to cages on racks (1 male per cage or up to 3 females per cage). Cages were racked by treatment group and vertically throughout the rack. The control animals were housed on a separate rack from the treatment groups. Approximately every 4 weeks, each rack was rotated in the room, with the first move in Week 7. During pretrial, group mean body weights were checked to ensure the weight variation of all animals did not exceed ±20% of all animals within the study, separately for each sex. Animals were allocated to dose groups as in the table below:

Study design

Test group	Dietary concentration (ppm)	Approximate mg/kg bw/day equivalent	# male	# female
Control	0	0	50	50
Low dose	75	10	50	50
Mid dose	375	50	50	50
High dose	2250	300	50	50

Diet preparation and analysis: Diet formulations were prepared based on a method established at the Test Facility at appropriate concentrations to meet dosage level requirements. Diet formulations were prepared as required, approximately every two weeks, as serial dilutions from a more concentrated stock of at least 200 g. The stock was prepared by firstly mixing the test substance with the required amount of untreated control diet in an automated mortar and pestle and mixed until visibly homogeneous. Diet for the high dose group at a concentration of 2250 ppm was prepared by adding the stock prepared to a suitably sized diet bin, adding an appropriate amount of untreated diet, and this was then blended for 20 minutes in a diet mixer (Winkworth). The diets at lower concentrations (375 ppm and 75 ppm) were prepared as a dilution from the high dose group. Diet formulations were prepared at appropriate intervals, stored and used within the conditions established during [REDACTED] Study No. 429624.

Diet formulation samples, from all groups, were collected for concentration and homogeneity analysis during weeks 1, 13, 25, 39, 51, 65 and 79. Analyses were performed by HPLC using a validated analytical procedure. Duplicate top, middle and bottom samples were collected for concentration and homogeneity analysis. Stability analyses performed previously in [REDACTED] Study No. 429624 demonstrated that the test substance is stable in the diet for 15 days when prepared and stored under the same conditions as this study at concentrations of 10 to 16000 ppm.

Analysis results: The results of all samples were found to be within or equal to the acceptance criteria of $\pm 10\%$ of their theoretical concentrations. The analysis performed periodically showed that the formulations were accurately prepared. For homogeneity, the RSD of concentrations for all samples in each group tested was within the acceptance criteria of $\leq 5\%$ with the exception of Week 39, Group 3 samples which was greater than the $\leq 5\%$ specification (5.3%).

Observations: All animals were checked twice daily for signs of viability and regularly for signs of reaction to treatment. Once each week all animals received a detailed clinical examination, including appearance, movement and behaviour patterns, skin and fur condition, eyes and mucous membranes, respiration and excreta.

Body weight: Body weights were recorded once during pretrial and then once a week until Week 14 of treatment, and approximately once every 2 weeks from Week 15 up until the end of treatment.

Food consumption, utilisation and achieved dose: The quantity of food consumed by each cage of animals was measured and recorded once weekly during pretrial and until Week 14 of treatment. From Week 15, food consumption was measured once every 2 weeks until the end of treatment.

Food utilisation was calculated for Weeks 1-4, 5-8, 9-13 and 1-13 as follows: (cage mean weight gain x 100)/cage total food consumption.

The amount of test substance ingested was calculated at regular intervals during treatment using the following formula:

$$\text{Achieved intake (mg/kg/day)} = \frac{\text{nominal concentration (ppm)} \times \text{food consumption (g/day)}}{\text{Mid-point body weight (g)}}$$

Water consumption: Water consumption was qualitatively monitored by visual inspection of the water bottles on a weekly basis throughout the study.

Haematology and clinical chemistry: Blood samples were obtained from all animals via the orbital sinus under isoflurane anaesthesia, prior to the terminal kill. The animals were not deprived of food overnight prior to sampling. The following parameters were assessed:

total white cell count (WBC)	Eosinophils (Eos)
Neutrophils (Neut)	Basophils (Baso)
Lymphocytes (Lymph)	Large Unclassified Cells (LUC)
Monocytes (Mono)	

A blood film was taken from the saphenous vein of all surviving mice using a capillary tube containing K2EDTA, in Weeks 52/53, and stained for possible examination. Femoral bone marrow smears were taken at necropsy and stored for possible evaluation. Blood and bone marrow smears were not evaluated as it was considered that examination would not yield any further information.

Toxicokinetic Sampling: Blood samples were obtained from all surviving toxicity animals, via the saphenous vein, for possible whole blood analysis, at the following times: Day 180 (07:00, 11:00 and 15:00 hours) and once during weeks 52/53. The blood was stored, deep frozen (-70 to -90°C) pending future analysis.

Investigations *post mortem*:

Unscheduled euthanasia: Animals were weighed, a necropsy undertaken, specified tissues stored and samples for evaluation of clinical pathology parameters and toxicokinetic analysis were obtained, if possible.

Termination: After at least 80 weeks of treatment all surviving animals were killed by exposure to carbon dioxide and had their terminal body weight recorded followed by exsanguination. Animals were not fasted before their necropsy.

Macroscopic examination: Each animal was subject to a detailed necropsy which consisted of a complete external and internal examination including body orifices and cranial, thoracic and abdominal organs and tissues.

Organ weights: The organs below were removed and weighed from all scheduled termination animals. Paired organs were weighed separately.

adrenal glands	ovaries
brain	spleen
epididymides	testes
heart	uterus (with cervix)
kidneys	thyroid glands
liver	

Tissue submission: The following tissues were examined *in situ*, removed and examined and fixed in

an appropriate fixative:

aorta (from thoracic segment)	lung
gross lesions and/or masses	liver
bone marrow smear (femur)	lymph nodes mandibular
bone marrow (femur)	lymph nodes mesenteric
bone marrow (sternum)	skeletal muscle (from thigh)
bone (femur)	nasal cavity
bone (sternum)	optic nerves
brain (forebrain, midbrain, cerebellum and medulla oblongata)	sciatic nerve
epididymides	oesophagus
eyes	ovaries
gallbladder	oviducts
adrenal glands	pancreas
Harderian glands	pharynx
lacrimal glands	skin
mammary glands (females only)	duodenum
parathyroid glands (examined only if present in the routine section of thyroid gland)	ileum
pituitary gland	jejunum
prostate gland	spinal cord (cervical, thoracic and lumbar)
salivary glands (mandibular)	spleen
seminal vesicles and coagulating glands	stomach (glandular and non-glandular region)
thyroid gland	testes
gut-associated lymphoid tissue	thymus
heart	tongue
kidneys	trachea
caecum	ureter
colon	urinary bladder
rectum	uterus with cervix
larynx	vagina

Liver sampling: After samples were collected for histopathological evaluation, multiple samples of liver (*ca* 150 mg each) were taken from two 5 mm sections of the left lateral lobe. The samples were snap frozen in liquid nitrogen in individual RNA-ase free tubes. All samples were taken as quickly as possible and stored at *ca* -70°C pending possible future analysis.

A representative section from the left lateral lobe, the right median lobe and the caudate lobe were taken and fixed in formalin (fixed for 48 hours). The liver was processed after 48 hours to a paraffin wax block. A small piece of duodenum was incorporated into each block. The remainder of the liver was snap frozen in liquid nitrogen. Samples were stored frozen at *ca* -70°C pending possible future analysis.

Microscopic examination: All processed tissues on all animals (with the exception of bone marrow smears, tongue and ureters) were examined by light microscopy.

Statistics: Body weight, cumulative body weight gain, food consumption, food utilisation, haematology and organ weight data were analysed using a parametric ANOVA and pairwise comparisons made using the Dunnett's t-test. Organ weights were also analysed by analysis of covariance (ANCOVA) using terminal kill body weight as covariate. Statistical comparisons to control values were not performed for relative organ weight (%), because the ANCOVA results with terminal body weight as covariate provide a more robust statistical determination of this parameter. The Dunnett's test was performed for all

continuous data parameters, regardless of whether the initial ANOVA or ANCOVA was statistically significant. Macroscopic findings are presented as total incidences per sex and treatment group, but were not evaluated for statistical significance.

For non-neoplastic micropathology incidence data, the total incidence (combining all severity grades) were analysed using Fisher's Exact Probability Test. Kaplan-Meier survival estimates were calculated separately for each sex and treatment group. Mortalities resulting from animals killed following accidents or scheduled termination were considered censored observations. Intergroup comparisons of mortality comparing each treatment group with the control group and an overall test for trend was performed separately for males and females using a logrank test.

For neoplastic micropathology incidence data, pairwise comparisons of the incidence of tumours were made using the Fishers Exact Probability test. Further analyses were performed using Peto's time adjusted methods. For non-palpable tumours, the study pathologist classified each of the tumours as fatal, probably fatal, probably incidental or incidental. For the purposes of statistical analysis of non-palpable tumours, tumours classified either as fatal or probably fatal were considered fatal and those classified as probably non-fatal were considered non-fatal. All tumours detected in animals that died in a planned sacrifice were automatically classed as incidental. For the purposes of statistical analysis, all palpable tumours were considered to be fatal and the time of first detection was used in the age-adjusted analysis ('onset rate method' of analysis). For palpable tumours not detected in-life, the time to death was used in the analysis. Methods used for the age-adjusted analysis of non-palpable tumours (including fatal tumours (death rate analysis) and non-fatal tumours (prevalence analysis)), and age-adjusted analysis of palpable adjusted analyses of tumour data was two-sided and performed at the 5% significance level using SAS (v8.2 or later). The analysis of non-fatal tumours was conducted by dividing the experimental period into the following fixed time intervals: 1-52 weeks, and 53-80 weeks and single intervals for any planned sacrifice. For each considered dataset, the significance of a linear dose related trend in tumour incidence was evaluated using a two-sided trend test. For the purposes of the trend tests, group numbers were used. (Peto test and survival trend test analysed the trend on group number (1-4) and not on dose level). If the overall Peto trend was significant at the 5% level for any given tumour incidence, then overall Peto trend was re-analysed for Groups 1-3; if still significant it was re-analysed for Groups 1-2. Males and females were analysed separately. For each statistical test performed on a dataset containing 10 or less tumours, the discrete permutation distribution was used to calculate the corresponding p-value.

RESULTS

Mortality: There were no treatment-related mortalities.

Doses	Overall % survival	
	Males	Females
0	37/50 (74%)	26/50 (52%)
75 ppm	38/50 (76%)	32/50 (64%)
375 ppm	41/50 (82%)	40/50 (80%)
2250 ppm	45/50 (90%)	35/50 (70%)

Clinical observations and palpable masses: There were a variety of clinical observations recorded in control and treated mice but these were either commonly seen observations in this age and strain of mouse or were seen in small numbers of animals. None of the observations seen were considered to be related to treatment. There were no treatment-related effects on the number of palpable masses.

Body weight and weight gain: Males and females receiving 2250 ppm showed lower group mean body weights and body weight change when compared to their control. Statistically significant differences were noted at multiple timepoints and the decreases were consistent throughout the treatment period.

There were no effects of treatments on body weight for animals receiving 75 or 375 ppm.

Table 6.5-17: Intergroup comparison of body weights (g) - selected timepoints

	Dietary Concentration (ppm)							
	Males				Females			
day	0	75	375	2250	0	75	375	2250
1	34.5	34.4	34.4	33.6	25.0	25.0	24.7	24.7
8	36.0	35.5	35.5	34.3**	26.6	26.8	26.2	26.0
15	37.0	36.7	36.6	35.9	27.3	28.1	27.3	27.0
85	43.1	43.6	43.8	41.2	33.2	34.1	32.9	32.1
169	47.3	47.8	47.0	44.3*	36.3	37.4	37.5	35.3
239	50.4	51.2	50.1	46.7*	40.5	41.2	41.4	37.5*
323	52.0	52.8	51.9	47.9*	42.1	44.2	43.5	39.8
407	52.6	54.5	52.8	49.1	45.1	46.6	44.0	40.9*
561	53.9	54.1	52.8	50.2	46.7	46.8	44.0	41.3*
1-92	9.1±3.8	10±4.2	9.5±3.5	8.1±2.7	7.8±3.1	9.4±3.4*	9.0±3.3	7.5±2.7
1-561	19.6±7.3	19.6±6.1	18.8±7.3	16.8±6.2	21.4±8.5	22.1±6.5	19.5±6.3	16.2±6.2**

* Statistically significant difference from control group mean, p<0.05 (Dunnett)

** Statistically significant difference from control group mean, p<0.01 (Dunnett)

Food consumption, utilisation and compound intake: For females receiving 375 ppm and 2250 ppm, lower food intakes were noted most weeks during the treatment period compared with control, attaining statistical significance on several occasions. The effect at 2250 ppm was consistent with the changes noted in body weight change at this dose level. Males receiving 2250 ppm showed slightly lower food consumption, which was consistent with the effect on body weight, but statistical significance was attained only on a few occasions.

Table 6.5-18: Intergroup comparison of food consumption (g/animal/day) - selected timepoints

	Dietary Concentration (ppm)							
	Males				Females			
day	0	75	375	2250	0	75	375	2250
1	6.5	6.0**	5.9**	6.2	4.6	4.3	4.3	4.5
8	5.9	6.1	5.6	5.8	4.4	4.6	4.4	4.5
15	5.9	6.1	6.3*	5.9	4.7	4.9	4.8	4.7
85	5.7	6.0	5.6	5.9	5.1	4.8	4.7	4.9
344	5.8	5.6	5.7	5.6	5.2	5.1	4.9	4.8
456	5.3	5.8*	5.4	5.4	4.7	4.8	4.6	4.8
554	5.7	5.7	5.8	5.6	5.5	5.2	4.8	4.9

* Statistically significant difference from control group mean, p<0.05 (Dunnett)

** Statistically significant difference from control group mean, p<0.01 (Dunnett)

Lower overall food utilisation for Weeks 1-13, for males receiving 2250 ppm, was generally observed during measurement intervals, suggesting that these differences reflect an effect of treatment. Food utilisation for males receiving 75 or 375 ppm and all groups of females were unaffected by treatment.

Table 6.5-19: Intergroup comparison of food utilisation (g body weight gain/100g food consumed)

Weeks	Dietary Concentration (ppm)							
	Males				Females			
	0	75	375	2250	0	75	375	2250
1-4	2.7	2.6	2.7	2.3	3.2	3.8	3.3	3.2
5-8	1.6	1.5	1.5	1.3	1.4	1.7	1.7	1.7
9-13	1.0	1.5**	1.3	1.1	1.0	1.2	1.5	0.7
1-13	1.7	1.9	1.8	1.5	1.8	2.1	2.1	1.8

** Statistically significant difference from control group mean, $p < 0.01$ (Dunnett)

Dose rates (based on nominal dietary levels) were calculated in terms of mg SYN545974/kg body weight. Mean values are shown below:

Table 6.5-20: Mean Dose Received (mg/kg/day)

Dietary concentration (ppm)	75	375	2250
Males	9.2	45.4	287.9
Females	9.7	48.4	306.2

Haematology: There were no differences in haematology parameters which were considered to be attributable to treatment with SYN545974.

Sacrifice and pathology:

Macroscopic findings: An increase in the incidence of liver masses was observed in males administered SYN545974 at ≥ 375 ppm when compared with the controls. The incidence was as follows: 5/50, 9/50, 14/50 and 20/50 in the control, 75, 375 and 2250 ppm groups, respectively. Most of the masses correlated with microscopic findings of hepatocellular carcinoma or adenoma at doses of 375 and 2250 ppm in males.

Other gross findings observed were considered incidental, of the nature commonly observed in this strain and age of mice, and/or were of similar incidence in control and animals dosed with SYN545974 and, therefore, were considered unrelated to administration of SYN545974.

Organ weights: Liver weights, adjusted for covariance and terminal body weight, were statistically significantly increased in males and females that received 2250 ppm. Adjusted liver weights were also higher (12% above control) in males that received 375 ppm, however, did not achieve statistical significance; the difference is considered to be due to the weight of the liver from histopathological evaluation.

Table 6.5-21: Intergroup comparison of liver weights

	Dietary Concentration (ppm)							
	Males				Females			
	0	75	375	2250	0	75	375	2250
Absolute value (g)	5.412 2.943	5.541 2.974	5.925 3.189 (+8%)	7.953 4.034** (+37%)	2.638 2.356	2.215 2.275	2.372 2.337	2.637 2.562 (+9%)
Absolute (covariance analysis)	2.804	2.877	3.144 (+12%)	4.269** (+52%)	2.272	2.227	2.344 (+5%)	2.660** (+17%)
% of body weight†	5.4116	5.5408	5.9249 (+9.5%)	7.9527 (+47%)	5.0186	4.9237	5.3468 (+6.5%)	6.3306 (+26%)

** Statistically significant difference from control group mean, $p < 0.01$

† no statistical analysis performed

Other slight variances from the control organ weights were noted in either sex, some of which were seen to be statistically significant. However, due to a lack of corroborating data, evidence of a dose related response or consistency between sexes, it is considered that these findings were not treatment related.

Microscopic findings:

Non-neoplastic: In the liver, centrilobular hepatocellular hypertrophy was observed in males administered SYN545974 at ≥ 375 ppm.

Other non-neoplastic findings observed were considered incidental, of the nature commonly observed in this strain and age of mice, and/or were of similar incidence and severity in control animals and therefore were considered unrelated to administration of SYN545974.

Table 6.5-22: Intergroup comparison of selected non-neoplastic findings in males

Finding	Dietary Concentration (ppm)			
	Males			
	0	75	375	2250
Liver (no. examined)	50	50	49	50
hepatocellular hypertrophy total	0	0	6*	18**
minimal	0	0	2	2
mild	0	0	3	10
moderate	0	0	1	6

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Neoplastic: A statistically significant increased incidence of liver hepatocellular carcinomas and adenomas was observed in males administered SYN545974 at 2250 ppm. 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 Group 4 animals (2250 ppm) were excluded from the analysis (summarized in table below). In this study, the incidence of hepatocellular carcinomas and adenomas in animals dosed at 75 or 375 ppm were within historical control data. Hepatocellular carcinomas and adenomas correlated with liver masses observed at necropsy at ≥ 375 ppm. Although a higher incidence of adenomas and/or carcinomas was also noted in males that received 75 ppm, this was not only within the historical control data for the performing laboratory, but was towards the lower end of the spectrum and did not show any statistical

significance (pairwise or Peto trend test). Furthermore, this group has only single occurring tumours as can be expected with spontaneous liver tumours, whereas in males that received ≥ 375 ppm a clear increase in multiplicity was observed for adenomas (see text table).

Table 6.5-23: Summary of hepatocellular neoplastic and proliferative findings in the liver in males

Finding	Dietary Concentration (ppm)				
	Males				Historical control data
	0	75	375	2250	
Liver (no. examined)	50	50	49	50	250
hepatocellular carcinoma	2 (4%)	3 (6%)	4 (8.2%)	10* (20.0%)	19 (7.6%) range 6-10%
hepatocellular adenoma	4 (8.0%)	6 (12.0%)	9 (18.4%)	22** (44.0%)	45 (18.0%) range 10-28%
focus of cellular alteration, eosinophilic	1 (2.0%)	4 (8.0%)	6 (12.2%)	10** (20.0%)	7 (2.8%) range 0-6%
total					
minimal	0	0	1	1	
mild	1	2	1	3	
moderate	0	2	4	6	

* Statistically significant difference from control group mean, $p < 0.05$ (Fisher's exact test)

** Statistically significant difference from control group mean, $p < 0.01$ (Fisher's exact test)

Table 6.5-24: Historical control data (2007-2013) for eosinophilic foci of cellular alteration in the liver of male mice

Study Start	Eosinophilic foci		
	No. Examined	Incidence	%
2007	50	1	2
2007	50	3	6
2007	50	0	0
2007	50	3	6
2009	50	0	0
2012	50	0	0
2013	50	1	2
2015	50	0	0
TOTAL	400	9	
RANGE (%)			0-6 %
MEAN (%)			2.0
N (studies)			9

Table 6.5-24: Peto analysis of hepatocellular tumours in liver in males

Tumour type	Sex	p value for linear trend groups 1-4	p value for linear trend including groups 1-3	direction of trend
hepatocellular adenoma	male	$<0.001^*$	0.14	positive
hepatocellular carcinoma	male	0.018*	0.48	positive

* $p < 0.05$ by Peto Trend Test

Table 6.5-25: Summary of adenomas and carcinomas in the liver in males

Group		Males				HCD (minimum-maximum) 250
		1	2	3	4	
Dose (ppm)		0	75	375	2250	
No. Examined		50	50	49	50	
Hepatocellular carcinoma (multiple)	N	2 (0)	3 (0)	4 (0)	10* (2)	3-5 [#]
Hepatocellular adenoma (multiple)	N	4 (0)	6 (0)	9 (7*)	22** (14**)	5-14 [#]

* Statistically significant difference from control group mean, $p < 0.05$ (Fisher's exact test)

** Statistically significant difference from control group mean, $p < 0.01$ (Fisher's exact test)

[#] Incidence range (min-max). HCD from five 80-week carcinogenicity in mice performed by the conducting laboratory [REDACTED] between 2007 and 2009

There was a numerical increase in eosinophilic foci of cellular alteration in the liver of males (summarised in Table 6.5-23). The incidence was only significantly higher in males dosed at 2250 ppm compared to the control group; and the incidence of eosinophilic foci at 75 ppm was only marginally higher than the [REDACTED] historical control data ($n=4$ (8.0%) at 75 ppm versus $n=3$ (6.0%) in historical control data). These HCD provided in the study report, were from five 80-week mouse studies performed between 2007 and 2009. Following an EFSA request, the applicant submitted new HCD for eosinophilic foci of cellular alteration in the liver of male mice from long-term studies performed during a larger period from 2007 to 2013 (total of nine 80-week mouse studies). With this new data, the incidence of eosinophilic foci at 75 ppm (8%) is still outside the range of this HCD (0-6%). When limiting the number of studies to a time frame period of 5 years from the completion date of the study, the remaining four studies give a smaller HCD range of 0-2%. With this restricted data, the 8% incidence of eosinophilic foci observed at 75 ppm is still above the historical control data range.

However, it should be noted that the increase in eosinophilic foci in liver of male mice treated with 75 ppm is not statistically significant compared to the control group, the increase being statistically significant only at the higher dose of 2250 ppm. No peto trend analysis was performed by the applicant regarding the total incidence for this parameter which leads difficult the interpretation of this effect slightly above the HCD at 75 ppm. Foci of cellular alteration represents small to large aggregates of tinctorially distinct hepatocytes within the hepatic parenchyma and are sometimes considered putative preneoplastic lesions. Foci of hepatocellular alteration increase with age in mice and may be induced by treatment in younger rodents. All types of foci (eosinophilic, basophilic and clear cell foci) are more common in male than in female mice. In this study, the eosinophilic foci were the only type of foci observed and they were only seen in males. They occurred in terminal animals and not in preterminal death/decedent animals, which could be indicating that there is no early occurrence or reduction in latency. In addition, it is noteworthy that eosinophilic foci were not observed in the 90 day mouse study where only an increase in hepatic centrilobular hypertrophy was noted with no evidence of hepatic necrosis. Thus, the biologic relevance of this effect slightly above the HCD observed at 75 ppm at the end of the experiment is questionable. In the mouse database, a very weak proliferative signal after exposure to 75 ppm SYN545974 was observed in the 28 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) and no increases in mitosis (see Table 6.5-27). In addition, no alterations in liver weight or histopathology in the 90-d study were observed at the low dose of 100 ppm. In this present study (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 this low dose level (75 ppm).

Taking into account all this element, HSE is of opinion that that the marginal increase of eosinophilic foci observed at 75 ppm can be considered not related to treatment.

Other neoplastic and proliferative findings observed were considered incidental, of the nature commonly observed in this strain and age of mice, and/or were of similar incidence and severity in control and

animals administered SYN545974 and, therefore, were considered unrelated to administration of SYN545974.

CONCLUSION:

Overall, in a GLP and guideline carcinogenicity study in mice given doses of 0, 75, 375 or 2250 ppm (0, 9.2/9.7, 45.4/48.4, or 288/306 mg/kg bw/d in males/females), there was a dose-related increase in liver adenomas (18% and 44% at mid- and top-dose vs 8% in controls) and carcinomas (8% and 20% at mid- and top-dose vs 4% in controls) in males from the mid dose, which reached statistical significance at the highest dose. The incidences at the top dose were also above the laboratory historical control ranges from 5 studies conducted between 2007-2009 (10-28% for adenoma; 6-10% for carcinoma) and tumour multiplicity was also noted. In addition, pre-neoplastic lesions (eosinophilic foci of cellular alterations: 12.2% and 20% at the mid and top dose respectively vs 2% in controls; above HCD) occurred in males from the mid dose. Overall, pydiflumetofen was clearly carcinogenic in the liver of male mice up to a dose which was not excessively toxic to the animals (7% and 11.6% reduction in terminal body weight in males and females respectively). Therefore the NOAEL for carcinogenicity was 75 ppm (9.2 mg/kg bw/d) in males and the top dose of 2250 ppm (306 mg/kg bw/d) in females.

In both sexes, pydiflumetofen caused decreases in terminal body weight (7% and 11.6% in males and females), body weight gain (14% and 24% in males and females) and food consumption at the top dose. Food utilisation was also decreased (by 12%) in top dose males. A statistically significant increase in liver weight (by 52% and 17% in males and females respectively) was observed at the top dose in both sexes. In addition, in males, liver weight was increased by 12% at the mid dose. In males, increased liver weight was associated with hepatocyte hypertrophy from the mid dose (6/49 and 18/50 at 375 and 2250 ppm respectively vs 0/50 in controls). Therefore a chronic toxicity NOAEL of 75 ppm (9.2 mg/kg bw/d) was identified in males and a chronic toxicity NOAEL of 375 ppm (48.4 mg/kg bw/d) was identified in females.

(██████████, 2015b)

B.6.5.3. Mechanistic studies

A number of mechanistic studies were performed in male mice and culture of mouse and human hepatocytes to investigate a possible non-genotoxic mode of action for the liver tumours observed in male mice involving liver stimulation via constitutive androstane receptor (CAR) induction.

Report:	K-CA 5.5/05 ██████████ (2015). SYN545974: A 28-Day Dietary Liver Mode of Action Study in Male CD-1 Mice. ██████████ ██████████. Laboratory Report No. ██████████, 24 August 2015, Unpublished. Syngenta File No. SYN545974_10267.
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Guidelines: Oral (Dietary) Mechanistic Study to Evaluate Effects on the Liver in the Male Mouse.

GLP: This study has not been subjected to any study specific Quality Assurance procedures. Study activities at the Test Facility were conducted according to relevant SOPs. There were no deviations from the protocol, or from company procedures, to affect the quality, integrity or achievement of the study objectives. This study was not conducted according to Good Laboratory Practice standards as defined by 40 CFR part 160. No claim of GLP compliance was made for this study.

HSE comment: as a mechanistic study, this study was considered supportive

EXECUTIVE SUMMARY

The aim of this study was to evaluate effects on the liver of male CD-1 mice in response to SYN545974 administered via the diet. These evaluations were made following 2, 7 or 28 days administration at dietary inclusion levels of 0 (control), 75 and 2250 ppm.

Male CD-1 mice were treated with SYN545974 at dietary inclusion levels of 75 ppm or 2250 ppm (corresponding to average achieved doses of 10.0 mg/kg/day and 324.0 mg/kg/day) for 2, 7 or 28 days.

SYN545974 had no impact on terminal bodyweights in either the 75 ppm or 2250 ppm treatment groups.

Increased absolute liver weights and liver bodyweight ratios were observed in animals administered 2250 ppm for 7 and 28 days.

The plasma clinical chemistry measurements were essentially unchanged in both treatment groups throughout the study indicating a lack of overt cytotoxicity due to SYN545974 administration via the diet. Only AST levels were statistically lower than the controls, reaching 70% and 60% of control values following 28 days administration of 75 ppm and 2250 ppm SYN545974-containing diet, respectively. This decrease in AST levels was not considered to be toxicologically relevant.

The total cytochrome P450 levels were unaffected by treated at 75 ppm SYN545974, and pentoxyresorufin-O-depentylation (PROD) activities were increased minimally (1.6 to 2.4-fold), but this did not achieve statistical significance at 75 ppm SYN545974 treatment at all timepoints evaluated. At 2250 ppm, SYN545974 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.

Treatment with 75 ppm SYN545974 did not induce any changes in the morphology of the liver.

Exposure of male mice to SYN545974 at a dose level of 2250 ppm induced centrilobular hypertrophy that increased in severity with time up to the terminal end point (28 days of dietary SYN545974 administration). After 2 days of treatment at 2250 ppm, an increase in mitosis in the liver was also observed, but no increases in mitoses were observed following 7 or 28 days dietary administration.

Finally, the hepatocellular labelling index (S-phase) was slightly increased at 75 ppm SYN545974 by 3.1, 2.3- and 5.6-fold relative to time-matched controls after 2, 7 and 28 days, respectively and these differences were statistically significant after 7 and 28 days. At 2250 ppm SYN545974, substantial and statistically significant increases in the hepatocellular labelling index (S-phase) were observed, with a maximal increase after 2 days of dietary administration (13.6-fold) and lower magnitude changes after 7 and 28 days administration (5.4 and 5.6-fold of control values).

SYN545974 caused minimal impact on the liver when administered via the diet at 75 ppm. When male mice were exposed to the higher dose of 2250 ppm, SYN545974 caused increased liver weights, which were accompanied by hepatocellular hypertrophy, increased cytochrome P450 levels, and increased PROD activity after 2, 7 and 28 days of treatment. In addition, increased DNA-synthesis (S-phase) was maximal after 2 days of treatment and accompanied by a histopathology finding of increased mitosis. The latter observation was only evident at the earliest time-point assessed.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	White powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	Not reported
Stability of test compound	Expiry date end June 2016 (stored 30 °C)

Vehicle control: The test substance was administered via RM1 powdered diet (supplied by [REDACTED]).

Test Animals:	
Species:	Mouse
Strain:	CD-1 ([REDACTED])
Age/weight at dosing:	5 to 6 weeks / 27 to 31 g
Source:	[REDACTED]
Housing:	Animals were housed in groups of five in grid-floor cages suspended over paper-lined trays
Acclimatisation period:	The animals were examined on arrival and found to be outwardly healthy. The animals were acclimatised within the study room for at least 11 days before the start of treatment.
Diet:	<i>ad libitum</i>
Water:	<i>ad libitum</i>
Environmental conditions:	Temperature: 19 °C to 23 °C Humidity: 40% to 70% Air changes: not stated Photoperiod: 12 hours light and 12 hours dark

In-life dates: Start: 25 November 2013 End: 09 December 2013

Study Design and Methods:

Rationale for dose level selection and route of administration

The dose levels of 0, 75 and 2250 ppm were selected by the Sponsor to reflect dose levels used on an 80 week carcinogenicity study in the CD-1 mouse ([REDACTED], 2015). The oral (dietary) route of administration was selected as a potential route of human exposure.

Animals:

Ninety (90) male CD-1 mice (aged 6 to 7 weeks upon arrival at Medical School Resource Unit (MRSU)) were obtained from [REDACTED].

Diet:

RM1 powdered diet was used. Test diets containing SYN545974 at nominal concentrations of 75 and 2250 ppm were prepared by [REDACTED] (in accordance with [REDACTED] standard operating procedures). The diet was deemed to be homogeneous and the average achieved concentrations of the 75 ppm and 2250 ppm SYN545974 diets were 72.6 ppm (96.8% of target) and 2222.2 ppm (98.8% of target), respectively. SYN545974-containing test diet was prepared without purity correction and was stored at ambient temperature, in the dark, throughout the study.

Study design

Animals were allocated to groups by a stratified randomization scheme to achieve similar group mean body weights. Mice were uniquely numbered by tail marking. The animals were aged 7 – 8 weeks at start of study.

Prior to start of dosing, body weights were checked to ensure all groups were within $\pm 20\%$ of the mean weight of all male mice.

The study consisted of 3 groups. Control animals received powdered RM1 diet ad libitum for 2, 7 and 28 days. The test groups were administered 75 and 2250 ppm SYN545974 ad libitum in the diet for 2, 7 and 28 days. All animals were administered 5 mL/kg bromodeoxyuridine solution (BrdU) by subcutaneous (SC) injection 2 h \pm 10 min before scheduled termination.

Experimental Procedures

Bodyweight: The bodyweight of each mouse was recorded manually at the start of the study, once weekly and on the day of termination.

Clinical observations: Prior to the start of the study, all mice were observed to ensure that they were physically normal and that they exhibited normal activity. Each mouse was observed at least once daily during the study. The study diary was kept in the MSRU until completion of the in-life phase of the study and then transferred to the Study File for retention and archiving.

Food consumption: Food consumption was measured daily for the 2 day time-point, and a minimum of once weekly for the 7- and 28-day time-points. Diet hoppers were weighed and refilled as necessary.

Achieved dosage: Achieved dosage was calculated retrospectively after food consumption and body weight measurements were taken.

Post Mortem & Subsequent Investigations

On the day of termination the mice were weighed and terminated by exposure to a rising concentration of CO₂.

Preparation of plasma: Venous blood was taken via cardiac puncture and dispensed into lithium/heparin-coated tubes and plasma prepared.

Liver processing: The gall bladder was removed from each animal prior to removal of the liver. Each liver was weighed. Two pieces of liver (5 mm³) were removed from the left lobe for possible TaqMan® analysis. These liver pieces were placed in the same cryovial, flash frozen in liquid nitrogen and then stored at approximately -70°C until required for possible toxicogenomics work. Two samples of liver, approximately 4 mm strips, were taken from the right medial and left lateral lobes from terminations on days 2 and 7. From the termination on day 29, three samples of liver, approximately 4 mm strips, were taken, one from the right medial, one from the left lateral and one from the caudate lobe. These samples (from 2, 8 and 28 days) were placed in 20 mL plastic sample pots containing approximately 10 mL of 10% neutral buffered formalin (NBF) for 36 - 48 hours prior to tissue processing and wax embedding according to [REDACTED] standard operating procedures. The liver and small intestine samples were taken to wax blocks in a timely fashion such that the tissues were in hot wax for less than 2 hours. For each animal, 1 wax block containing liver and duodenum was generated.

Small representative samples of liver were cut into chunks, snap frozen in liquid nitrogen and stored at approximately -70°C for possible future use.

The remaining liver was weighed and scissor-minced in ice-cold 1.15% (w/v) KCl prior to processing to microsomes and stored at approximately -70°C until required for liver biochemistry assessment.

Small intestine processing: As a positive immunohistochemical control for BrdU nuclear incorporation, a 1 cm section of the duodenum was removed, formalin fixed, processed and embedded in the same block as the liver of each animal.

Biochemical Measurements

Clinical chemistry: Alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were measured in all available plasma samples.

Liver Biochemistry: Liver samples were separately homogenised and microsomal and cytosolic fractions prepared by differential centrifugation. Aliquots of the whole homogenate, microsomal and cytosolic fractions from each animal were stored at 70 °C or below. Liver microsomes from each animal were assayed for protein content and for activities of 7-pentoxoresorufin O-depentylase (PROD) and 7-benzyloxoresorufin O-debenzylase (BROD).

Histopathology

Haematoxylin and eosin (H&E) staining and analysis: Paraffin-embedded liver samples were sectioned and stained with H&E, with a section thickness of 4-5 µm, and were sent for assessment by Professor [REDACTED], Consultant Pathologist, Regulatory Science Associates and a Phase Report provided. The histopathology was subject to internal and external peer review.

Cell proliferation analysis: Uptake of BrdU was detected using standard immunohistochemistry techniques. Determination of the BrdU labelling index for the liver was performed by manual analysis of 3000 nuclei in the left lateral lobe with quantification index in accordance with the relevant [REDACTED] Standard Operating Procedure. A piece of duodenum was inserted into each block as a positive control tissue for BrdU immunohistochemical evaluation, but no quantitation of labelling index was conducted for duodenum

Data Evaluation

The statistical analyses used in this study compared Group 1 against Groups 2 and 3 (trend- and pair-wise testing) at each time-point.

Statistics: General Approach: All statistical tests were two-sided with minimum significance levels of 5% and 1%. Non-parametric statistics were not routinely conducted. When used, Dunnett's test was conducted regardless of the outcome of the analysis of variance (ANOVA) or analysis of covariance (ANCOVA).

Data were examined for unusually high or low values which could influence the statistical analysis and interpretation (possible outliers). After examining for any outliers, if the variances were clearly heterogeneous, transformations (e.g. log, double arcsine or square root) were used in an attempt to stabilise the variances. If the transformations failed, the data set was examined and a decision taken on further action.

For Quantitative Data: Body weight, cumulative body weight gain from the start of dosing, food intake, clinical chemistry parameters, absolute liver weight and BrdU labelling index for the liver were analysed using a parametric ANOVA. Liver weight was also analysed by ANCOVA on final body weight. This statistical analysis provided an adjusted liver weight value. Summary values of liver to body weight ratios were presented but not analysed statistically.

Dunnett's test: For all of the parameters evaluated initially by ANOVA or ANCOVA, Dunnett's test was used to compare the control and treated groups, based on the error mean square in the ANOVA or ANCOVA. The Dunnett's test was performed for all continuous data parameters, regardless of whether

the initial ANOVA or ANCOVA was statistically significant, and statistical flags are presented in the tables of results in the final report.

Macroscopic and microscopic findings were analysed by Fisher's Exact Test. Findings with individual group incidences of less than three were not analysed statistically

RESULTS

Clinical Observations: No clinical abnormalities of individual animals were recorded during the study.

BodyWeights: There were no changes in bodyweights at termination when comparing those mice receiving SYN545974-containing diet with those receiving control diet.

Food Consumption and Compound Intake: The food consumption of those mice receiving SYN545974-containing diet was similar to that of mice receiving control diet. The average intake during the 28-day feeding period was 10.0 mg/kg/day for the 75 ppm group and 324.0 mg/kg/day for the 2250 ppm group

Blood Clinical Chemistry: There were no statistically significant differences in ALT or ALP levels between those mice receiving SYN545974-containing diet, at either dose level, and those mice receiving control diet. AST levels were statistically lower reaching 70% and 60% of control values following 28 days administration of 75 ppm and 2250 ppm SYN545974-containing diet, respectively. A decrease in AST levels is considered to be of no toxicological relevance.

Sacrifice and Pathology:

Organ Weights: There were no changes in the liver weights or liver:bodyweight ratios in mice receiving SYN545974-containing diet at 75 ppm. However, there were statistical increases of 24% and 22% in the absolute liver weights of those mice receiving SYN545974-containing diet at 2250 ppm for 7 and 28 days respectively compared to the relevant controls. Consequently, the liver:bodyweight ratios of those mice also increased by 21% and 28% after receiving the SYN545974-containing diet at 2250 ppm for 7 and 28 days respectively, again compared to the relevant controls.

Macroscopic Findings: Necropsy examinations did not reveal any macroscopic abnormalities of the liver.

Microscopic Findings: No changes in liver morphology were observed after treatment with 75ppm SYN545974. Exposure to SYN545974 at 2250 ppm for 2 days resulted in centrilobular hepatocellular hypertrophy and an increased incidence of mitotic figures in the liver. Increased mitosis was not observed following 7 and 28 days of dietary administration. Centrilobular hypertrophy was observed throughout all treatment durations and showed a time-related increase in severity which was greatest after 28 days of exposure to SYN545974.

Cell Proliferation Analysis: Hepatocellular proliferation in the liver, as measured by BrdU incorporation during DNA-synthesis, was increased in the mice receiving SYN545974-containing diet at both dose levels. At 75 ppm, the labelling index was increased by 3.1, 2.3- and 5.6-fold relative to time-matched controls after 2, 7 and 28 days, respectively, and these differences were statistically significant after 7 and 28 days. At the 2250 ppm dose level, the labelling indices were statistically significantly increased 13.6-, 5.4- and 5.6-fold relative to time-matched controls following 2, 7 and 28 days of dietary administration, respectively.

Liver Biochemistry: Hepatic microsomal total cytochrome P450 content and microsomal pentoxoresorufin-O-depentylation (PROD) activity was unchanged between mice receiving control and 75 ppm SYN545974-containing diet. However, mice receiving 2250 ppm SYN545974 in the diet had

CONCLUSION:

(██████████, 2015)

Guidelines: This was an investigative study with no applicable guidelines.

GLP: This study has not been subjected to any study specific Quality Assurance procedures. Study activities at the Test Facility were conducted according to relevant SOPs. There were no deviations from the protocol, or from company procedures, to affect the quality, integrity or achievement of the study objectives. This study was not conducted according to Good Laboratory Practice standards as defined by 40 CFR part 160. No claim of GLP compliance was made for this study.

HSE comment: as a mechanistic study, this study was considered supportive

EXECUTIVE SUMMARY

This study investigated the effects of SYN545974 on cytotoxicity, PROD and BROD enzyme activities and hepatocyte proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) in isolated male CD-1 mouse hepatocyte cultures. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as positive controls.

Primary monolayer cultures of hepatocytes were prepared in 25 cm² flasks, 96- and 6-well plastic tissue culture plates in Leibowitz CL15 medium.

Hepatocytes were exposed to SYN545974 at 4 concentrations (5, 10, 25 and 35 μM), PB at 2 concentrations (100 and 1000 μM) or vehicle (0.5% [v/v] dimethyl sulfoxide [DMSO]) alone for 96 h. There were 3 replicates for each concentration in 25 cm^2 flasks for CYP2B/3A activity measurements (pentoxyresorufin-*O*-depentylation [PROD] and benzyloxyresorufin-*O*-debenzylation [BROD]), 5 replicates for each concentration in 6-well plates for replicative DNA synthesis (proliferation) analysis

Treatment of cultured male CD-1 mouse hepatocytes with SYN545974 resulted in increased cell proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]) and increased CYP2B/3A activities (measured as PROD activity).

Hepatocytes were cultured in Leibowitz CL15 medium for 4 h (to allow for adhesion) before the medium was removed and replaced with medium containing either SYN545974 at one of 4 concentrations (5, 10, 25 and 35 μ M), PB at one of 2 concentrations (100 and 1000 μ M) or (DMSO) alone. The concentrations of SYN545974 used were selected with reference to a preliminary range finding study. The total duration of exposure to SYN545974, PB or DMSO, was 96 h, with daily medium changes. There were 3 replicates for each concentration in 25 cm² flasks for PROD and BROD activities, 5 replicates for each concentration in 6-well plates for replicative DNA synthesis (proliferation) analysis and 6 replicates for each concentration in 96-well plates for ATP measurements.

After 96 h in culture, hepatocytes were either fixed using methanol at approximately -20°C (for assessment of replicative DNA synthesis) or harvested (for assessment of PROD and BROD activities) by scraping them into SET buffer (0.25 M sucrose, 5 mM EDTA, 20 mM Tris-HCl, pH7.4), sonicating the mixture and storing it at approximately -70°C until analysis. Protein in hepatocytes scraped into SET buffer was determined by the method of ██████████ *et al.* (1951).

Assays:

Adenosine 5'-triphosphate (ATP): The bioluminescent determination of ATP released from viable somatic cells was carried out using an assay kit supplied by Promega (CellTitre-Glo luminescent cell viability assay) according to manufacturers' instructions.

Replicative DNA synthesis (S-phase): The number of hepatocytes undergoing replicative DNA synthesis (S-phase of the cell cycle) was determined immunocytochemically following the incorporation of 5-bromo-2-deoxyuridine (BrdU, 10 μ M) into hepatocyte nuclei over the last 72 h of culture. Immunostaining was performed after fixation at 96 h. Data are expressed as a labelling index (% of total hepatocytes that have incorporated BrdU). Epidermal growth factor (EGF; 25 ng/mL, n=5) was included as a positive control. Approximately 1100 hepatocytes were counted per replicate for each concentration.

Pentoxoresorufin-O-depentylation (PROD): The activity of CYP2B/3A in cultured hepatocytes was determined spectrofluorometrically by the formation of resorufin from pentoxoresorufin.

Benzyloxyresorufin-O-debenzylation (BROD): The activity of CYP2B/3A in cultured hepatocytes was determined spectrofluorometrically by the formation of resorufin from benzyloxyresorufin.

Statistics: Statistical comparisons between treated and control groups were undertaken for all numerical data sets using ANOVA and Dunnett's post test.

RESULTS

Cytotoxicity assessment: Treatment of hepatocytes with all SYN545974 and PB concentrations resulted in similar statistically significant reductions in intracellular ATP to approximately 80% of vehicle control.

Pentoxoresorufin-O-depentylation (PROD): Exposure of hepatocytes to 5 and 10 μ M SYN545974 statistically significantly increased PROD activity 1.85- and 1.71- fold respectively when compared to control. However, PROD activity was reduced to 54% and 16% of vehicle control when hepatocytes were treated with 25 and 35 μ M SYN545974 respectively. The decreased PROD activities observed at higher concentration levels are likely due to substrate competition between residual SYN545974 in the cell lysate preparation and pentoxoresorufin, resulting in decreased enzymatic conversion of these substrates to form resorufin. PB treatment resulted in statistically significant increases in PROD activity.

Benzyloxyresorufin-*O*-debenzylation (BROD): Exposure of hepatocytes to 5 and 10 μM SYN545974 statistically significantly increased BROD activity 1.99- and 1.97- fold respectively when compared to control. However, BROD activity was reduced to 73% and 19% of vehicle control when hepatocytes were treated with 25 and 35 μM SYN545974 respectively. The decreased BROD activities observed at higher concentration levels are likely due to substrate competition between residual SYN545974 in the cell lysate preparation and benzyloxyresorufin, resulting in decreased enzymatic conversion of these substrates to form resorufin. PB treatment resulted in statistically significant increases in BROD activity.

Replicative DNA synthesis (S-phase): Treatment with EGF (25 ng/mL) resulted in a statistically significant increase in replicative DNA synthesis to 7-fold control, indicating that the hepatocytes could proliferate following exposure to proliferative stimuli and therefore demonstrating their suitability for use in investigations involving assessing induction of proliferation.

Exposure of hepatocytes with SYN545974 caused a similar increase in the labelling index of 1.34- fold for 5 and 10 μM SYN545974. Treatment of hepatocytes with 25 and 35 μM SYN545974 resulted in a statistically significant increase in the labelling index of 1.90- and 1.51- fold respectively when compared to control. PB treatment resulted in statistically significant increases in replicative DNA synthesis.

Table 6.5-31: Biochemical parameters in cultured male mouse hepatocytes

Treatment	ATP (luminescence units released) ^a	S-phase labelling index (%) ^b	PROD (pmol resorufin/min/mg) ^c	BROD (pmol resorufin/min/mg) ^c
Vehicle control (0.5% v/v DMSO)	674253 \pm 68995 (100 \pm 10.2)	2.65 \pm 0.1 (100 \pm 3.77)	21.26 \pm 3.35 (100 \pm 15.8)	86.40 \pm 36.82 (100 \pm 42.6)
PB 100 μM	595367 \pm 16637* (88.0 \pm 2.5)	3.39 \pm 0.27** (127.93 \pm 10.34)	40.36 \pm 4.65** (189.9 \pm 21.9)	160.93 \pm 19.54* (186.3 \pm 22.6)
PB 1000 μM	547991 \pm 27707** (81.0 \pm 4.1)	4.41 \pm 0.43** (166.48 \pm 16.39)	77.13 \pm 8.76** (362.9 \pm 41.2)	274.3 \pm 25.49** (317.5 \pm 29.5)
SYN545974 5 μM	588475 \pm 21538** (87 \pm 3.2)	3.54 \pm 0.81 (133.92 \pm 30.55)	39.25 \pm 10.23** (184.7 \pm 48.1)	171.48 \pm 46.89* (198.5 \pm 54.3)
SYN545974 10 μM	611652 \pm 24815* (91.0 \pm 3.7)	3.54 \pm 0.57 (133.89 \pm 21.64)	36.41 \pm 5.22* (171.3 \pm 24.5)	170.23 \pm 32.23* (197.3 \pm 37.3)
SYN545974 25 μM	567202 \pm 19849** (84.0 \pm 2.9)	5.03 \pm 0.51** (189.86 \pm 19.38)	11.54 \pm 3.23 (54.3 \pm 15.2)	63.12 \pm 7.74 (73.1 \pm 9.0)
SYN545974 35 μM	555771 \pm 23090** (82.0 \pm 3.4)	3.99 \pm 0.50** (150.90 \pm 18.84)	3.34 \pm 2.01 (15.7 \pm 9.5)	16.78 \pm 11.20 (19.4 \pm 13.0)
EGF 25 ng/mL	-	19.69 \pm 3.14** (743.73 \pm 118.49)		
Values are mean \pm SD. Values in parenthesis are mean % control \pm SD. ^a n = 6 per group, ^b n = 5 per group, ^c n = 3 per group Statistically different from control *p<0.05; **p<0.01 (ANOVA with Dunnetts post test).				

CONCLUSION:

In a mechanistic non-GLP study, male CD-1 mouse hepatocyte cultures were treated with 4 different concentrations (5, 10, 25 and 35 μM) of pydiflumetofen for 96 hours. Slight cytotoxicity (ATP depletion) was observed only at the top concentration. Markers of CYP2B/3A enzyme induction, (PROD and BROD enzyme activities) were increased at 5 and 10 μM . PROD and BROD activities were decreased at 25 and 35 μM . The decreased PROD and BROD activities observed at higher concentration levels were likely due to substrate competition. Similar increases in PROD and BROD activities were seen with the positive control substance phenopbarbital (PB). Treatment with 25 and 35

µM resulted in a significant increase in hepatocyte proliferation as measured by replicative DNA synthesis (maximal at 25 µM resulting in a 1.90-fold increase relative to control).

(████████, 2015)

Report:	K-CA 5.5/07 ██████████ (2015a). SYN545974: <i>In Vitro</i> Hepatocyte Proliferation Indexing and Enzyme Activity Measurements in Male Human Hepatocyte Cultures. CXR Biosciences, 2, James Lindsay Place, Dundee Technopole, Dundee, DD1 5JJ, Scotland, UK. Laboratory Report No.CXR1504, 21 August 2015, Unpublished. Syngenta File No. SYN545974_10270.
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Guidelines: This was an investigative study with no applicable guidelines.

GLP: This study has not been subjected to any study specific Quality Assurance procedures. Study activities at the Test Facility were conducted according to relevant SOPs. There were no deviations from the protocol, or from company procedures, to affect the quality, integrity or achievement of the study objectives. This study was not conducted according to Good Laboratory Practice standards as defined by 40 CFR part 160. No claim of GLP compliance was made for this study.

HSE comment: as a mechanistic study, this study was considered supportive

EXECUTIVE SUMMARY

This study investigated the effects of SYN545974 on cytotoxicity, pentoxoresorufin-O-depentylation (PROD) and benzyloxyresorufin-O-debenzylation (BROD) activities and replicative DNA synthesis (S-phase of the cell cycle) in cryopreserved male human hepatocyte cultures. Phenobarbital sodium salt (PB) and epidermal growth factor (EGF) were included as positive controls.

Primary monolayer cultures of hepatocytes were prepared in 25 cm² flasks, 96- and 6-well plastic tissue culture plates in Leibowitz HCL15 medium.

Hepatocytes were exposed to SYN545974 at 4 concentrations (5, 10, 25 and 35 µM), PB at 2 concentrations (100 and 1000 µM) or vehicle (0.5% [v/v] dimethyl sulfoxide [DMSO]) alone for 96 h. There were 3 replicates for each concentration in 25 cm² flasks for CYP2B/3A activity measurements (pentoxoresorufin-O-depentylation [PROD] and benzyloxyresorufin-O-debenzylation [BROD]), 5 replicates for each concentration in 6-well plates for replicative DNA synthesis (proliferation) analysis and 6 replicates for each concentration in 96-well plates for cell toxicity measurements (measured as the change in cellular adenosine-5'-triphosphate [ATP]).

Treatment with SYN545974 at concentrations greater than 25 µM resulted in cytotoxicity, with intracellular ATP levels being statistically significantly reduced to 58.5% of control.

Treatment of hepatocytes with 5, 10, 25 and 35 µM SYN545974 statistically increased PROD activities by 2.9-, 3.3-, 2.5- and 2.7-fold, respectively when compared to vehicle control. PB treatment resulted in statistically significant increases in PROD activity

Treatment of hepatocytes to 5, 10 and 25 µM SYN545974 statistically increased BROD activities by 3.4-, 5.9-, and 4.3-fold, respectively when compared to vehicle control. PB 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 [S-phase of the cell cycle]) following treatment with SYN545974 at any concentration.

The expected effects were observed for both positive control compounds indicating that the experimental system responded as expected.

Treatment of cultured male human hepatocytes with SYN545974 had no effect on cell proliferation (measured as the change in replicative DNA synthesis [S-phase of the cell cycle]). CYP2B/3A activities (measured as PROD and BROD activities) were elevated by SYN545974 treatment.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974
Description:	Off white powder
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	Not reported
Stability of test compound:	Expiry date end June 2016 (stored at <30°C)

Vehicle / positive controls: 0.5% v/v dimethyl sulfoxide (DMSO) / Phenobarbital sodium salt (PB) and epidermal growth factor (EGF).

Study Design and Methods:

Experimental dates: Start: 4 June 2015

End: 16 July 2015

Preparation and treatment of hepatocyte cultures: Cryopreserved primary male human hepatocytes, were sourced from Invitrogen, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR. Viabilities of the hepatocyte preparation, determined by trypan blue exclusion, were in excess of 70%. Hepatocytes from one donor were used.

Primary monolayer cultures of hepatocytes were prepared in collagen coated 25 cm² flasks, 96- and 6-well plastic tissue culture plates, using Leibowitz HCL15 as the medium. In all 96-well plate cultures the outside wells were not used, but filled with sterile phosphate buffered saline.

Hepatocytes were resuscitated in Cryopreserved Hepatocyte Recovery Medium (CHRM®) then cultured in Cryopreserved Hepatocyte Plating Medium (CHPM®) for 4-6 h to allow adherence. The medium was then removed and replaced with medium containing either SYN545974 at one of 4 concentrations (5, 10, 25 and 35 µM), PB at one of 2 concentrations (100 and 1000 µM) or vehicle (DMSO) alone. The concentrations of SYN545974 used were selected with reference to a preliminary range finding study. The total duration of exposure to SYN545974, PB or DMSO, was 96 h, with daily medium changes. There were 3 replicates for each concentration in 25 cm² flasks for PROD and BROD activities, 5 replicates for each concentration in 6-well plates for replicative DNA synthesis (proliferation) analysis and 6 replicates for each concentration in 96-well plates for ATP s.

After 96 h in culture, hepatocytes were either fixed using methanol at approximately -20°C (for assessment of replicative DNA synthesis) or harvested (for assessment of PROD and BROD activities) by scraping them into SET buffer (0.25 M sucrose, 5 mM EDTA, 20 mM Tris-HCl, pH7.4), sonicating the mixture and storing it at approximately -70°C until analysis. Protein in hepatocytes scraped into SET buffer was determined.

Assays:

Adenosine 5'-triphosphate (ATP): The bioluminescent determination of ATP released from viable somatic cells was carried out using an assay kit supplied by Promega (CellTitre-Glo luminescent cell viability assay) according to manufacturers' instructions.

Replicative DNA synthesis (S-phase): The number of hepatocytes undergoing replicative DNA synthesis (S-phase of the cell cycle) was determined immunocytochemically following the incorporation of 5-bromo-2-deoxyuridine (BrdU, 10 μ M) into hepatocyte nuclei over the last 72 h of culture. Immunostaining was performed after fixation at 96 h. Data are expressed as a labelling index (% of total hepatocytes that have incorporated BrdU). Epidermal growth factor (EGF; 25 ng/mL, n=5) was included as a positive control. Approximately 1100 hepatocytes were counted per replicate for each concentration.

Pentoxoresorufin-O-depentylation (PROD): The activity of CYP2B/3A in cultured hepatocytes was determined spectrofluorometrically by the formation of resorufin from pentoxoresorufin.

Benzyloxyresorufin-O-debenzylation (BROD): The activity of CYP2B/3A in cultured hepatocytes was determined spectrofluorometrically by the formation of resorufin from benzyloxyresorufin.

Statistics: Statistical comparisons between treated and control groups were undertaken for all numerical data sets using ANOVA and Dunnett's post test.

RESULTS

Cytotoxicity assessment: Treatment with 25 and 35 μ M SYN545974 resulted in cytotoxicity, with intracellular ATP levels being biologically and statistically significantly reduced to 57% and 57% of the vehicle control in culture A, and to 76% and 67 % of the DMSO control respectively in culture B. A small, but statistically significant decrease (12%) in ATP levels was observed at 10 μ M SYN545974 in culture A, but no evidence of cytotoxicity was observed at 10 μ M SYN545974 in culture B. There was no effect on intracellular ATP content in hepatocytes treated with 5 μ M SYN545974 and 100 and 1000 μ M PB.

Pentoxoresorufin-O-depentylation (PROD): Exposure of hepatocytes (culture A only) to 5, 10, 25 and 35 μ M SYN545974 statistically increased PROD activities by 2.9-, 3.3-, 2.5- and 2.7-fold, respectively when compared to vehicle control. Hepatocytes treated with 100 and 1000 μ M PB also increased PROD activity by 1.9- and 3.3-fold, respectively.

Benzyloxyresorufin-O-debenzylation (BROD): Exposure of hepatocytes (culture A only) to 5, 10 and 25 μ M SYN545974 increased BROD activities by 3.4-, 5.9-, and 4.3-fold, respectively when compared to vehicle control, and these changes were statistically significant. Exposure of hepatocytes to 35 μ M SYN545974 elevated BROD activity 3.2-fold, but this concentration did not reach statistical significance. Treatment of hepatocytes with 100 and 1000 μ M PB also resulted in increases in BROD activity by 2.7- and 7.4-fold, respectively.

Replicative DNA synthesis (S-phase): Exposure of hepatocytes (culture B only) to SYN545974 caused no change to the labelling index up to 10 μ M. Correlating with the decreases in ATP at 25 μ M and 35 μ M, the labelling index was statistically reduced to 36% and 26% of the DMSO control value, respectively, at these cytotoxic concentrations. PB caused no significant changes to the labelling index in those hepatocytes exposed to either 100 or 1000 μ M PB. The positive control EGF increased the labelling index 6.5-fold relative to the DMSO control.

Table 6.5-32: Replicative DNA synthesis (S-phase) in cultured human hepatocytes

Treatment	Culture B	
	ATP (luminescence units released) ^a	S-phase labelling index (%) ^b
Vehicle control (0.5% v/v DMSO)	268057 ± 19089 (100 ± 7.1)	0.27 ± 0.06 (100 ± 23.08)
PB 100 µM	352620 ± 47400* (132 ± 17.7)	0.30 ± 0.09 (109.38 ± 33.37)
PB 1000 µM	291060 ± 70476 (109 ± 26.3)	0.31 ± 0.06 (116.01 ± 24.01)
SYN545974 5 µM	329623 ± 44433** (123 ± 16.6)	0.26 ± 0.06 (97.90 ± 21.65)
SYN545974 10 µM	284562 ± 29279 (106 ± 10.9)	0.32 ± 0.14 (120.27 ± 50.20)
SYN545974 25 µM	204833 ± 25385** (76 ± 9.5)	0.10 ± 0.06** (36.14 ± 23.31)
SYN545974 35 µM	178281 ± 18062** (67 ± 6.7)	0.07 ± 0.05** (25.58 ± 17.88)
EGF 25 ng/mL		1.76 ± 0.24** (650.01 ± 89.84)

Values are mean ± SD. Values in parenthesis are mean % control ± SD. ^a n = 6 per group, ^b n = 5 per group, ^c n = 3 per group
Statistically different from control *p<0.05; **p<0.01 (ANOVA with Dunnetts post test).

Table 6.5-33: Biochemical parameters in cultured human hepatocytes

Treatment	Culture A		
	ATP (luminescence units released) ^a	PROD (pmol resorufin/min/mg) ^c	BROD (pmol resorufin/min/mg) ^c
Vehicle control (0.5% v/v DMSO)	382773 ± 11244 (100 ± 2.9)	0.11 ± 0.017 (100.0 ± 15.3)	1.15 ± 0.062 (100 ± 5.4)
PB 100 µM	417225.5 ± 46087 (109 ± 12.0)	0.21 ± 0.025** (189.9 ± 22.8)	3.05 ± 0.16** (265.5 ± 14.2)
PB 1000 µM	351707.5 ± 36856 (92 ± 9.6)	0.37 ± 0.045** (332.4 ± 41.4)	8.53 ± 0.65** (743.7 ± 56.4)
SYN545974 5 µM	362620 ± 52838 (95 ± 13.8)	0.32 ± 0.086* (293.6 ± 78.0)	3.87 ± 1.05* (336.9 ± 91.7)
SYN545974 10 µM	335691 ± 29780* (88 ± 7.8)	0.37 ± 0.054** (332.3 ± 48.9)	6.80 ± 0.79** (593.1 ± 69.0)
SYN545974 25 µM	216786 ± 12929** (57 ± 3.4)	0.28 0 ± 0.055** (254.8 ± 49.9)	4.90 ± 0.67** (427.4 ± 57.9)
SYN545974 35 µM	216493 ± 16797** (57 ± 4.4)	0.29 ± 0.018** (267.2 ± 16.1)	3.66 ± 1.70 (318.9 ± 148.0)

Values are mean ± SD. Values in parenthesis are mean % control ± SD. ^a n = 6 per group, ^b n = 5 per group, ^c n = 3 per group
Statistically different from control *p<0.05; **p<0.01 (ANOVA with Dunnetts post test).

CONCLUSION:

In the human-equivalent study of [REDACTED] (2015) above, male human hepatocyte cultures (from one donor) were treated with 4 different concentrations (5, 10, 25 and 35 µM) of pydiflumetofen for 96 hours. Significant cytotoxicity (ATP depletion) was observed at the top two concentrations. Markers of

CYP2B/3A enzyme induction, (PROD and BROD enzyme activities) were significantly increased at all concentrations, although a clear dose-response was not apparent. Similar increases in PROD and BROD activities were seen with the positive control substance phenobarbital (PB). No increase in hepatocyte proliferation as measured by replicative DNA synthesis was observed at any concentration. The positive control substance (EGF) produced a significant elevation in hepatocyte proliferation.

However, the study suffers from significant shortcomings. Only one human donor was used, significantly affecting the ability to address intraspecies variability in the observed response. In addition, significant cytotoxicity (ATP depletion) was observed at the two highest concentrations (25 and 35 μ M) in the human cultures, raising a question on whether the lack of proliferation could have been the consequence of the cytotoxicity. In the mouse culture (██████, 2015), where proliferation did occur, pydiflumetofen was not cytotoxic, except at the top concentration of 35 μ M, at which an associated decrease in proliferation was noted.

(██████, 2015a)

Report:	K-CA 5.5/08 ████████ (2014). SYN545974: CAR3 Transactivation Assay with Mouse, Rat and Human CAR. Department of Veterinary & Biomedical Sciences, 101 Life Sciences Building, Penn State University, University Park, PA 16802, USA. Laboratory Report No. TK0219831, 03 October 2014. Unpublished. Syngenta File No.SYN545974_50044.
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Guidelines: This is an investigative study with no applicable guidelines.

GLP: This study has not been subjected to any study specific Quality Assurance procedures. This study was not conducted in accordance with EPA Good Laboratory Practices, 40 CFR Part 160.

HSE comment: as a mechanistic study, this study was considered supportive

EXECUTIVE SUMMARY

SYN545974 was tested for its ability to directly activate the constitutive androstane receptor (CAR, NR1I3) in a reporter assay that was developed at Penn State University. Briefly, cDNA expression vectors for 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 SYN545974 at concentrations of 1, 3, 10, and 30 μ M. The direct CAR activator artemisinin was also incubated at these same concentrations, and model direct-acting substrates for mouse, rat or human CAR were each incubated at a single concentration. Light emission from the luciferase reporter was quantified to indicate the extent of CAR activation upon incubation with suspected ligands, including SYN545974. Results were reported as normalised luciferase activity and fold change compared to a DMSO solvent control.

SYN545974 was tested 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, SYN545974 produced up to 42-fold activation of rat CAR3 and up to 34-fold activation of mouse CAR3. The response in mouse CAR3 was approaching a maximal response at the lower test concentrations of 1 μ M (24-fold) to 3 μ M (34-fold), and it showed a slightly lower response at the highest mouse test concentration of 30 μ M (20-fold) that correlated with decreased viability in the COS-1 cells at SYN545974 concentrations of 10-30 μ M. SYN545974 activated human CAR3 in a concentration-responsive manner, and the maximal response (15-fold) at 30 μ M was higher than that of

the human CAR positive control compound, CITCO, (10-fold), which was tested at a single concentration of 5 μ M.

The model activators CITCO, TCPOBOP and clotrimazole produced robust responses in human, mouse and rat CAR3 constructs, respectively. Artemisinin also was tested and produced a concentration-dependent response that was much more marked with rat CAR3 than with human or mouse CAR3.

SYN545974 is a direct activator of CAR from mouse, rat and human, and has high efficacy (i.e. maximal fold change compared to model activators) in all three species.

MATERIALS AND METHODS

Materials:

Test Material:	SYN545974 technical
Description:	Off-white powder
Supplied by:	Syngenta Crop Protection, LLC (Greensboro, NC, USA)
Lot/Batch number:	SMU2EP12007
Purity:	98.5% w/w
CAS#:	1228284-64-7
Stability of test compound:	Stable until end June 2016 (stored at <30°C)

Vehicle: Dimethyl sulfoxide (DMSO).

Positive controls: Artemisinin, 6-(4-Chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde-*O*-(3,4-dichlorobenzyl)oxime (CITCO), 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) and clotrimazole.

Study Design and Methods:

Experimental dates: Start: 11 April 2014, End: 20 June 2014

General Principles of Reporter Gene Assays: The details of the luciferase reporter assays have been described in (██████ *et al*, 2005; ██████ *et al*, 2007). cDNA plasmids were synthesized by PCR, verified by DNA sequencing and used in transfection assays conducted with the mammalian COS-1 cell line. COS -1 cells are ideal for these transactivation assays as they are primate-derived, do not express endogenous CAR and thus exhibit negligible background activities, can be transfected with extremely high efficiencies, grow rapidly in culture and are transcriptionally competent. The plasmids used in the transfection/reporter assays encoded the production of:

- CAR3 from a particular species
- An exogenous source of the RXRa dimerization protein
- The CYP2B6 gene's transcriptional response elements fused to a luciferase reporter
- An exogenous *Renilla* gene which is used to normalize for transfection efficiency only, as it is possible that cells growing independently in different culture wells may take up the DNA 'cocktail' with slightly differing efficiencies.

After the various cDNA encoding plasmids were allowed to be expressed in COS-1 cells for a sufficient length of time, approximately 16 to 24 h, the cells were incubated with test chemical or DMSO vehicle for an additional 24 h. Direct activation of the CAR3 construct by ligand binding leads to dimerization with RXR and binding of the dimer to the CYP2B6 response elements that are linked to the luciferase reporter within the plasmid construct. That interaction in turn activates transcription of the luciferase protein whose level of expression can be monitored directly by measure of the corresponding increase in luminescent light output. The light emissions occur in direct proportion to the strength of the CAR3 promoter activation. The output from the COS-1 cells is measured with a luminometer for each

experimental condition using the Dual Luciferase Reporter Assay (Promega, Madison, WI, USA). As described by the manufacturer, in the assay the activities of firefly (*Photinus pyralis*) and *Renilla* (*Renilla reniformis*) luciferases are measured sequentially from a single sample. The firefly luciferase reporter is measured first by adding a reagent to generate a “glow-type” luminescent signal. After quantifying the firefly luminescence, this reaction is quenched, and the *Renilla* luciferase reaction is initiated by simultaneously adding a second reagent to the same tube. This reagent produces a “glow-type” signal from the *Renilla* luciferase, which decays slowly over the course of the measurement. In the Dual Luciferase Assay System, both reporters yield linear assays with subattomole (<10⁻¹⁸) sensitivities and no endogenous activity of either reporter in the experimental host cells. Final outputs are then expressed as normalized luciferase activity (i.e., firefly luciferase/*Renilla* activities) and as a fold-change compared to solvent (DMSO) control.

This CAR3 reporter assay is a sensitive method for detecting direct activators of CAR; it does not respond to indirect activators of CAR (such as PB), because the second messenger systems that are believed to be responsible for indirect activation are not present in COS-1 cells.

CAR3 Reporter Assay Methodology: All transfections using COS-1 cells for luciferase reporter assays were performed in a 48-well format and each condition performed in quadruplicate. In the afternoon of day 1, cells were plated to approximately 50,000 cells per well. While the cells were attaching, DNA transfection mixtures were assembled using Eugene6 transfection reagent (Roche Applied Science, Indianapolis, IN, USA). In general, for assays involving standard reporters, 25 ng of pTracer CMV2 or CMV2-CAR expression plasmid, 25 ng of pcDNA3.1-RXR α expression plasmid, 100 ng of 2B6XREM luciferase reporter, and 10 ng of pRL-CMV (*Renilla* for transfection normalization; Promega) were used for transfection. In all transfections, the transfection reagent was used at a ratio of 1:3 (micrograms of DNA to microliters of transfection reagent) as recommended in the manufacturer's protocol. Within a given experiment, all transfections contained the same total amount of DNA. At the time of transfection (within 1–6 h after plating), cells were approximately 80% confluent and had initiated cell division (in the case of COS-1 cells). The following day (16–18 h after transfection, cells were treated with chemical agents dissolved in DMSO. The test material (SYN545974) was evaluated at 1, 3, 10, and 30 μ M concentrations for each construct, including the negative empty vector control. Artemisinin (1, 3, 10 and 30 μ M) was also evaluated as a substrate that had been tested for concentration-response in prior experiments. DMSO was used as a solvent control. Positive control assays with model direct CAR activators for each construct were used. These consisted of:

- CITCO at a concentration of 5 μ M (model substrate for human CAR3)
- TCPOBOP at a concentration of 0.5 μ M (model substrate for mouse CAR3)
- Clotrimazole at a concentration of 10 μ M (model substrate for rat CAR3)

In addition, phenobarbital (PB) was evaluated at a single concentration of 1 mM, to investigate whether this indirect CAR activator might produce some response in the direct CAR3 reporter assay when tested at a relatively high concentration.

In all treatments, DMSO levels never exceeded 0.1% (v/v). On day 3 (24 h after chemical treatment), cells were washed with PBS and luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega) and measured with a Veritas Microplate Luminometer (Turner Biosystems/Promega). Luciferase assay and stop and glow reagents were diluted with 1 \times Tris-buffered saline, pH 8.0, to a 0.5X final concentration. All other aspects of the assay were performed in accordance to the manufacturer's protocol. Dilution of luciferase reagent had no effect on normalized luciferase values.

Cell Viability Assay Methodology: The CellTiter-Glo® Luminescent Viability Assay (Promega) was used to measure the effect of the chemicals on the viability of the COS-1 cells according to manufacturer's protocol. The assays were performed in a 48-well plate format and each condition was performed in triplicate. In the afternoon of day 1, cells were plated to approximately 50,000 cells per well. The cells were either transfected following the same protocol used for the transactivation assays or remained un-transfected. The following day (16–18 h after transfection), cells were treated with the

respective chemical agents. For the transfected cells, a single concentration of the solvent control, the corresponding positive controls (model CAR activator for each species), phenobarbital, the test compound, or artemisinin were used at their highest concentrations used in the reporter assays. For the un-transfected COS-1 cells, the test chemical and artemisinin were evaluated at 1, 3, 10, and 30 μM concentrations in addition to single concentrations of the solvent control, phenobarbital or the positive control compounds. DMSO was used as a solvent control. On day 3 (24 h after chemical treatment), the cells were incubated at room temperature for 30 min, then washed and the media was replaced with 100 μL of PBS. Following the manufacture's protocol, an equal amount of CellTiter-Glo® Reagent was added to each well (100 μL). The cells were placed on an orbital shaker at room temperature for 2 min to induce lysis and then incubated at room temperature for 10 min to stabilize the luminescent signal. The samples were then transferred to a 96 well white plate (LUMITRAC 200, Greiner Bio-One, Monroe, NC) and the luciferase levels were measured with an Infinite M200 Pro Microplate Luminometer (Tecan, Mannedorf, Switzerland). Results were subsequently normalized to the DMSO solvent control and plotted graphically.

Statistics: Quantitative data were examined by one way analysis of variance followed by the Dunnett's post hoc test to compare both the test compound and artemisinin for statistical difference relative to the DMSO control. A two-tailed unpaired t-test was performed comparing all individual controls and single dose compounds to DMSO. Significance was declared if $p < 0.01$. All statistical analyses were performed using GraphPad Prism v5.03 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

CAR3 Reporter Assay: The results of the cell viability assays indicated that the treatment chemicals were largely well tolerated by the COS-1 cells, typically with viabilities of >85% even at the highest doses of compound tested. An emerging effect on cell viability was observed with SYN545974 at concentrations of 10 – 30 μM , where the cell viability values were 91% and 86%, respectively.

A strong concentration-dependent activation of rodent CAR3 by SYN545974 was observed, with up to ~42-fold activation of rat CAR3 (30 μM). Statistically significant activation of rat CAR3 was achieved at 3, 10 and 30 μM concentrations. SYN545974 treatments resulted in statistically significant activation of mouse CAR3 at all doses tested, even at 1 μM , with >30-fold activation achieved at 3 and 10 μM concentrations. The highest dose (30 μM) of SYN545974 elicited a lower response for mouse CAR3, ~20-fold, likely due to enhanced toxicity of the test agent at the highest dose used in the mouse CAR3 assay. SYN545974 activated human CAR3 in a concentration-responsive manner, with a maximal response (15-fold) at 30 μM and statistically significant increases at 10 and 30 μM .

The model positive control activators, CITCO, TCPOBOP and clotrimazole produced robust responses in human, mouse and rat CAR3 constructs, respectively. Artemisinin was also tested at 1, 3, 10 and 30 μM test concentrations and produced a concentration-dependent response that was much more marked with rat CAR3, followed by mouse CAR3 and human CAR3, respectively. PB at a high concentration (1 mM) produced a statistically significant response in rat CAR3 (6-fold) and mouse CAR3 (2-fold), but it did not produce a response with human CAR3.

Table 6.5-34: CAR3 Report Assay Results with SYN545974 and Model Ligands Summary

Construct	Treatment	Dose level	Normalised luciferase activity	Fold change
Empty vector	DMSO	-	0.011601867	1.0000
	PB	1 mM	0.010391490	0.8957
	CITCO	5 μM	0.010286748	0.8866
	TCPOBOP	0.5 μM	0.008661510	0.7466
	CLOT	10 μM	0.016245838	1.4003
	SYN545974	30 μM	0.008445574	0.7279
	Artemisinin	30 μM	0.010042641	0.8656
Human CAR3	DMSO	-	0.006283901	1.0000
	PB	1 mM	0.005458657	0.8687
	CITCO	5 μM	0.064987884**	10.3420
	SYN545974	1.0 μM	0.009235276	1.4697
		3.0 μM	0.030063022	4.7841
		10 μM	0.079399868**	12.6354
		30 μM	0.093048558**	14.8075
	Artemisinin	1.0 μM	0.007720766	1.2287
		3.0 μM	0.010188791	1.6214
		10 μM	0.017774689	2.8286
		30 μM	0.051286910**	8.1616
Mouse CAR3		DMSO	-	0.011443407
	PB	1 mM	0.023631748**	2.0651
	TCPOBOP	0.5 μM	0.518724797**	45.3296
	SYN545974	1.0 μM	0.274971218**	24.0288
		3.0 μM	0.385683254**	33.7035
		10 μM	0.363857680**	31.7963
		30 μM	0.229388962**	20.0455
	Artemisinin	1.0 μM	0.021702898	1.8965
		3.0 μM	0.045019349**	3.9341
		10 μM	0.125842190**	10.9969
		30 μM	0.147682711**	12.9055
Rat CAR3		DMSO	-	0.005454521
	PB	1 mM	0.032469909**	5.9528
	CLOT	10 μM	0.520540006**	95.4328
	SYN545974	1.0 μM	0.015527670	2.8468
		3.0 μM	0.077953990**	14.2916
		10 μM	0.0200842256**	36.8212
		30 μM	0.228798130**	41.9465
	Artemisinin	1.0 μM	0.015965526	2.9270
		3.0 μM	0.061147666	11.2105
		10 μM	0.206790225**	37.9117
		30 μM	0.286011677**	52.4357
** statistically significant difference from control value p<0.01				

CONCLUSION:

In a mechanistic non-GLP study, pydiflumetofen was tested at concentrations of 1, 3, 10 and 30 µM in COS-1 cells transfected with the human, rat and mouse CAR construct, including a luciferase reporter element. The assay showed that pydiflumetofen was a direct activator in vitro of CAR from mouse, rat and human, and had high efficacy (i.e. maximal fold change compared to model activators) in all three species.

(██████████, 2014)

Report:	K-CA 5.5/09 ██████████ (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. ██████████ ██████████. Laboratory Report No. ██████████, 24 January 2012. Unpublished. Syngenta File No. SYN545974_10209.
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Guidelines: This was a research study with no applicable regulatory guidelines.

GLP: Study activities at the Test Facility (██████████) were conducted according to relevant SOPs and in the spirit of GLP. No Quality Assurance procedures were undertaken for this phase of the study, as no formal claim of Good Laboratory Practice was made for this work.

HSE comment: as a mechanistic study, this study was considered supportive

This study was conducted on 2 Active Ingredients, only data on SYN545974 are presented.

EXECUTIVE SUMMARY

This study was designed to evaluate the effects of SYN545974 on liver peroxisomal and microsomal enzyme expression in male and female mice. Male and female mice were administered diet containing SYN545974 (500-7000 ppm) for 28 days. Satellite study animals were administered SYN545974 (7000 ppm) for 3 or 7 days. At termination, liver tissue was taken, snap frozen and dispatched to ██████████ 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 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), pentoxyresorufin-*O*-deethylation (PROD), benzyloxyquinoline-*O*-debenzylation (BQ) and lauric acid 12-hydroxylation (LAH).

Administration of SYN545974 (500-7000 ppm) to male and female mice did not stimulate hepatic peroxisomal palmitoyl CoA oxidation and only minimal increases (less than 4-fold) in microsomal lauric acid 12-hydroxylation were seen. This indicates that SYN545974 is not a peroxisome proliferator. SYN545974 induced total cytochrome P450, PROD and BQ in a dose-dependent manner. There was a marked induction (up to 20-fold) of PROD, a marker of Cyp2b enzymes. Only minimal increases (up to 1.4-fold) in EROD, a marker for Cyp1a enzymes, were observed. Similar dose-responses were seen in both male and female mice, except in the case of BQ, where much greater fold increases were observed in treated female mice. This is probably due to the considerably lower constitutive Cyp3a activities commonly seen in female mice compared to male mice. SYN545974 showed a time dependent increase in P450, EROD, PROD and BQ activity in both male and female mice. These data suggest that SYN545974 exhibits the properties of a “phenobarbital-like” inducing agent.

Following the administration to male and female mice, SYN545974 did not demonstrate the prototypical properties of peroxisome proliferators. SYN545974 exhibited characteristics in common with “phenobarbital-like” inducing agents.

MATERIALS AND METHODS

Materials:

Liver samples: Groups of mice (6/sex/group) were treated under the [REDACTED] study protocol, Study Number 519644. Following 3, 7 or 28 days exposure, the animals were euthanized and the livers harvested. Liver samples were flash frozen and then stored at approximately -70 °C, prior to dispatch to [REDACTED], for analysis.

The frozen liver chunks were processed to heavy pellets and microsomes. The heavy pellet samples were assayed for total protein content and the activity of CN⁻-insensitive palmitoyl 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), pentoxyresorufin-*O*-depentylation (PROD), benzyloxyquinoline-*O*-debenzylation (BQ) and lauric acid 12-hydroxylation (LAH).

Study Design and Methods:

Experimental dates: Not reported

Male and female mice were administered diet containing SYN545974 (500-7000 ppm for 28 days. Satellite study animals were administered SYN545974 (7000 ppm) for 3 or 7 days.

Treatment summary

Group	Treatment				Number of animals		
					Toxicity study	Interim kill animlas (satellite study)	
	Test item	Description	ppm	Sex	28 days	3 days	7 days
1	Blank diet	control	0	male	6	6	6
				female	6	6	6
2	SYN545974	low	500	male	6		
				female	6		
3		intermediate 1	1500	male	6		
				female	6		
4		intermediate 2	4000	male	6		
				female	6		
5		high	7000	male	6	6	6
				female	6	6	6

At termination liver tissue was taken, snap frozen and dispatched to [REDACTED] for analysis. [REDACTED] assayed all livers from each sex/dose combination.

Subcellular Fractionation: Liver samples were weighed and scissor-minced in ice-cold 1.15% (w/v) KCl prior to homogenisation in SET (250 mM sucrose, 5 mM EDTA, 50 mM tris-HCl, pH 7.4) buffer. Heavy pellet and microsomal fractions were prepared and stored at approximately -70 °C prior to analysis.

Biochemical assays:

- **Peroxisome proliferation:** CN⁻ -insensitive palmitoyl CoA oxidation was determined spectrophotometrically in liver heavy pellet, using palmitoyl CoA as a substrate.
- **Total cytochrome P450:** The microsomal total P450 content was determined spectrophotometrically.
- **Ethoxyresorufin-O-deethylation (EROD):** The activity of Cyp1a in liver microsomes was determined spectrofluorometrically by the formation of resorufin from ethoxyresorufin.
- **Pentoxyresorufin-O-depentylation (PROD):** The activity of Cyp2b in liver microsomes was determined spectrofluorometrically by the formation of resorufin from pentoxyresorufin.
- **Benzyloxyquinoline-O-debenzylation (BQ):** The activity of Cyp3a in liver microsomes was determined spectrofluorometrically by the formation of 7-hydroxyquinoline from benzyloxyquinoline, as described by GENTEST HTS technical bulletin.
- **Lauric acid 12-hydroxylation (LAH):** The activity of Cyp4a in liver microsomes was determined by the formation of 12-hydroxylauric acid from lauric acid using LC/MS/MS detection.
- **Protein assay:** The heavy pellet and microsomal protein concentrations were determined in aqueous solutions and bovine serum albumin standards.

Statistics: A Student's t-test (2-sided) was performed on the results of all the biochemical assays.

RESULTS**28 Day Dose-Response**

Peroxisome proliferation: Administration of SYN545974 to male and female mice resulted in minimal decreases in hepatic peroxisomal β -oxidation as determined by the measurement of CN⁻ insensitive palmitoyl CoA oxidation (PCO). This is considered not to be biologically significant because all values are within the normal day to day variation for this assay.

Table 6.5-35: Hepatic Cyanide-Insensitive Palmitoyl CoA Oxidation (PCO) Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	PCO (nmol NAD ⁺ reduced/minute/mg protein)	
		male	female
Control	0	15.47	14.10
SYN545974	500	9.97**	8.31
	1500	12.95	7.16**
	4000	11.22*	7.97*
	7000	10.69**	6.78*

* Statistically significant difference from control group mean, p<0.05

** Statistically significant difference from control group mean, p<0.01

Total cytochrome P450: Administration of SYN545974 to male and female mice resulted in a dose-related increase in hepatic total P450 content to a maximum of 1.8- and 1.6-fold at 7000 ppm respectively.

Table 6.5-36: Hepatic Total P450 Content Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	Total P450 nmol/mg protein	
		male	female
Control	0	0.51	0.55
SYN545974	500	0.78**	0.65
	1500	0.84**	0.74*
	4000	0.84**	0.95**
	7000	0.90**	0.87**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Ethoxyresorufin-O-deethylation (EROD): Following administration for 28 days SYN545974 had no biologically consistent or significant effect upon EROD activity.

Table 6.5-37: Ethoxyresorufin-O-Deethylation Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	pmol resorufin formed/min/mg protein	
		male	female
Control	0	25.5	23.79
SYN545974	500	18.02*	26.22
	1500	18.36	19.14
	4000	21.22	32.84*
	7000	34.77	33.06**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Pentoxyresorufin-O-depentylation (PROD): Following 28 day administration of SYN545974 at 500 ppm, 1500 ppm, 4000 ppm and 7000 ppm PROD activity was markedly increased in male mice 11-, 9.2-, 9.0- and 15-fold respectively. Administration of SYN545974 for 28 days to female mice at 500 ppm, 1500 ppm, 4000 ppm and 7000 ppm moderately increased PROD activity 5.2-, 5.4-, 4.2- and 3.1-fold respectively.

Table 6.5-38: Pentoxyresorufin-O-Depentylation Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	pmol resorufin formed/min/mg protein	
		male	female
Control	0	2.77	7.50
SYN545974	500	32.68**	38.77**
	1500	25.55**	40.30**
	4000	24.97**	31.70**
	7000	42.50**	23.57**

** Statistically significant difference from control group mean, $p < 0.01$

Benzyloxyquinoline-O-debenzylation (BQ): 28 Day administration of SYN545974 at 500 ppm, 4000 ppm and 7000 ppm resulted in minimal increases in male mice of 1.5-, 2.7- and 3.5-fold respectively. Administration of SYN545974 for 28 days to female mice at both 4000 ppm and 7000 ppm increased BQ activity 2.5-fold.

Table 6.5-39: Benzyloxyquinoline-O-debenzylation Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	nmol 7-Hydroxyquinoline formed/min/mg protein	
		male	female
Control	0	1.73	4.31
SYN545974	500	2.58**	4.28
	1500	2.05	6.38
	4000	4.63**	10.79**
	7000	6.13**	10.82**

** Statistically significant difference from control group mean, $p < 0.01$

Lauric acid 12-hydroxylation (LAH): 28 day administration of SYN545974 at 1500 ppm, 4000 ppm and 7000 ppm resulted in minimal increases in LAH activity in male mice of 2.5-, 2.5- and 3.5-fold respectively. Administration of SYN545974 for 28 days to female mice had little, if any, effect on LAH.

Table 6.5-40: Lauric Acid 12-Hydroxylation Dose-Response in Male and Female Mice Administered SYN545974 for 28 Days

Treatment	Concentration (ppm)	nmol 12-OH Lauric acid formed/10min/mg protein	
		male	female
Control	0	4.12	4.19
SYN545974	500	5.39	2.91
	1500	10.35**	4.13
	4000	10.16**	2.14**
	7000	14.46**	4.22

** Statistically significant difference from control group mean, $p < 0.01$

Time-Course SYN545974

Peroxisome proliferation

Administration of SYN545974 to male and female mice resulted in minimal decreases in hepatic peroxisomal β -oxidation as determined by the measurement of CN⁻ insensitive palmitoyl CoA oxidation (PCO). This is not considered to be biologically significant because all values are within the normal day to day variation for this assay.

Table 6.5-41: Hepatic Cyanide-Insensitive Palmitoyl CoA Oxidation (PCO) Dose-Response in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	PCO (nmol NAD ⁺ reduced/minute/mg protein)					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	12.54	15.52	15.47	12.18	18.14	14.10
SYN545974	7000	8.85**	10.52**	10.69**	9.25	7.59**	6.78*

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Total cytochrome P450: Male mice administered SYN545974 at 7000 ppm for 3, 7 and 28 days showed an increase in hepatic total P450 content of 2.0-, 2.0- and 1.8-fold respectively. Female mice administered SYN545974 at 7000 ppm for 3, 7 and 28 days showed an increase in hepatic total P450 content of 1.8-, 1.8- and 1.6-fold respectively.

Table 6.5-42: Hepatic Total P450 Content in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	Total P450 nmol/mg protein					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	0.47	0.49	0.51	0.42	0.44	0.55
SYN545974	7000	0.97**	0.99**	0.90**	0.76**	0.77**	0.87**

** Statistically significant difference from control group mean, $p < 0.01$

Ethoxyresorufin-O-deethylation (EROD): Male mice administered SYN545974 at 7000 ppm for 3 and 7 days showed minimal increases in EROD activity of 3.4- and 2.0-fold respectively. Female mice administered SYN545974 at 7000 ppm for 3 and 28 days showed increased EROD activity of 2.0- and 1.4-fold respectively.

Table 6.5-43: Ethoxyresorufin-O-Deethylation in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	pmol resorufin formed/min/mg protein					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	17.55	30.27	25.55	43.84	51.72	23.79
SYN545974	7000	62.96**	59.94*	34.77	85.71**	68.16	33.06**

* Statistically significant difference from control group mean, $p < 0.05$

** Statistically significant difference from control group mean, $p < 0.01$

Pentoxoresorufin-O-depentylation (PROD): Male mice administered SYN545974 at 7000 ppm for 3, 7 and 28 days showed a marked increase in PROD activity of 10-, 21- and 15-fold respectively. Female mice administered SYN545974 at 7000 ppm for 3, 7 and 28 days showed a marked to moderate increase in PROD activity of 13-, 11- and 3.1-fold respectively.

Table 6.5-44: Pentoxoresorufin-O-Depentylation in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	pmol resorufin formed/min/mg protein					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	2.21	1.70	2.77	4.76	5.85	7.50
SYN545974	7000	22.22**	35.29**	42.50**	61.07**	63.75**	23.57**

** Statistically significant difference from control group mean, $p < 0.01$

Benzyloxyquinoline-O-debenzylation (BQ): Administration of SYN545974 to male mice at 7000 ppm showed minimally increased BQ activity up to a maximum of 3.5-fold for 28 days. Minimal increases were also noted following administration of SYN545974 at 7000 ppm to female mice for 3, 7 and 28 days of 3.5-, 2.5- and 2.5-fold respectively.

Table 6.5-45: Benzyloxyquinoline-O-debenzylation in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	nmol 7-Hydroxyquinoline formed/min/mg protein					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	1.70	1.76	1.73	2.46	4.07	4.31
SYN545974	7000	7.55**	9.54**	6.13**	8.67**	10.13**	10.82**

** Statistically significant difference from control group mean, p<0.01

Lauric acid 12-hydroxylation (LAH): Male mice administered SYN545974 at 7000 ppm for 3 and 28 days showed minimal increases in LAH activity of 1.8- and 3.5-fold respectively. No significant change in LAH activity was noted for female mice administered SYN545974 at 7000 ppm.

Table 6.5-46: Lauric Acid 12-Hydroxylation in Male and Female Mice Administered SYN545974 for 3, 7 and 28 Days

Treatment	Conc. (ppm)	nmol 12-OH Lauric acid formed/10min/mg protein					
		male			female		
		3 days	7 days	28 days	3 days	7 days	28 days
Control	0	5.51	6.31	4.12	2.47	5.49	4.19
SYN545974	7000	9.90**	12.39	14.46**	3.20	4.05	4.22

** Statistically significant difference from control group mean, p<0.01

CONCLUSION:

Another mechanistic non-GLP study was conducted to investigate and exclude other potential liver MoAs. Groups of 6 male and 6 female mice were treated with pydiflumetofen for 3, 7 or 28 days at doses ranging from 500 to 7000 ppm. Following treatment, at termination, liver tissue was taken and processed to obtain fractions which were then analysed for peroxisome proliferation (CN⁻ -insensitive palmitoyl CoA oxidation), EROD activity (marker of CYP1A, strongly induced by AhR activators), PROD activity (marker of CYP2B, strongly induced by CAR activators), BQ activity (marker of CYP3A, strongly induced by CAR activators) and LAH activity (marker of CYP4A, an indicator of fibrate-dependent peroxisome proliferation).

Pydiflumetofen did not stimulate hepatic peroxisomal palmitoyl CoA oxidation but increases (less than 4-fold) in microsomal LAH activity were seen. This indicates that fibrate-dependent peroxisome proliferation cannot be completely excluded. Pydiflumetofen induced PROD and BQ activity, indicating strong CAR activation, similar to PB. Minimal increases (up to 1.4-fold) in EROD activity were observed, indicating that pydiflumetofen might not be an AhR activator. However, as no positive controls for AhR activation or LAH expression (e.g. 3-methylcholanthrene, omeprazole) had been used to put into context these small increases, these minor enzyme inductions could not be completely disregarded.

(████████, 2012)

B.6.5.4. MoA and human relevance analysis

Report: K-CA 5.5/04 [REDACTED] (2015). SYN545974 – Human Relevance Framework Assessment of Liver Tumour Induction in CD-1 Mice. [REDACTED], 01 September 2015
Unpublished Report No: TK0258437. Syngenta File No. SYN545974_10290.

Guidelines: Since this is an assessment of data there are no regulatory guidelines.

GLP: Since this volume contains an assessment of data from completed studies and additional scientific information in the published literature and is not a study per se, a Good Laboratory Practice (GLP) statement as defined by OECD is not appropriate.

EXECUTIVE SUMMARY

Following dietary administration for up to 80 weeks, treatment with SYN545974 resulted in an increased incidence of liver tumours in male CD-1 mice. The hypothesized mode of action (MOA) and relevance to humans for SYN545974 induced liver tumours is assessed using established international frameworks (International Programme on Chemical Safety (IPCS) and the International Life Science Institute (ILSI)).

The data for SYN545974 support a proposed MOA involving the following key events:

- Activation of the constitutive androstane receptor (CAR).
- An early, transient, increase in hepatocellular proliferation.
- Increased hepatocellular foci as a result of clonal expansion of spontaneously mutated (initiated) cells.
- Eventual progression to form liver tumours.

And the following associative events:

- Increased expression of genes encoding cytochrome P450s (CYPs), particularly Cyp2b/Cyp3a isoforms.
- Increased incidence of hepatocellular hypertrophy.
- Increased liver weight.

The MOA is considered not relevant for human hazard/risk assessment purposes, due to qualitative differences in response to CAR activation between mice and humans. Experimental data demonstrate that SYN545974 does not produce the key event of cell proliferation in human liver cells *in vitro*, and supports the conclusion that SYN545974 does not pose a hepatocarcinogenic hazard to humans.

MOA Hypothesis for SYN545974-Induced Liver Tumour Formation in the Mouse Carcinogenicity Data for SYN545974

The carcinogenic potential for SYN545974 has been evaluated in 104 and 80 week lifetime bioassays in the rat and mouse, respectively ([REDACTED] 2015a and b see section 5.5.1 and 5.5.3 respectively). In summary, no treatment related neoplastic findings were observed in male or female rats or female mice. The only treatment related neoplastic finding was liver tumours in the male mouse at doses ≥ 375 ppm. A No Observed Effect Level (NOAEL) for liver tumours in the male mouse was 75 ppm (Table 6.5-26).

Table 6.5-26: SYN545974 Liver Carcinogenicity Data in the CD-1 Mouse

Males	Dietary Level (ppm)				[REDACTED] Historical Control Data
	0	75	375	2250	

Animals Examined	50	50	49	50	247
Hepatocellular Adenoma [Multiple]	4 (8%) [0]	6 (12%) [0]	9 (18.4%) [7]**	22**,\$\$\$ (44%) [14]**	45 (18.2%) (Range = 10.0 – 28.0%)
Hepatocellular Carcinoma [Multiple]	2 (4%) [0]	3 (6%) [0]	4 (8.2%) [0]	10* (20%) [2]	19 (7.7%) (Range = 6.0 – 10.0%)

Tumour incidences are expressed as total per group, (percentage incidence) and [number with multiple tumors].

Significantly different from control: * = $p \leq 0.05$, ** = $p \leq 0.01$ (Fisher's exact test)

\$\$\$: Statistically significant positive trend by Peto trend test: ($p \leq 0.001$) for groups 1-4

Statement of Mode of Action Hypothesis

There are a number of experimentally derived time- and dose-related key events that have been identified to characterise the MOA for SYN545974-induced liver tumour formation in male mice. Upon exposure to SYN545974, the proposed non-genotoxic MOA is initiated by activation of the constitutive androstane receptor (CAR).

Activation of CAR in male mice results in altered expression of CAR-responsive genes leading to CAR-mediated stimulation of cell proliferation (and associated 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. Combined with suppression of apoptosis this promotes an environment that would allow a spontaneously mutated cell to clonally expand before it could be removed by normal apoptotic control processes. Over time, transformed cells progress to pre-neoplastic foci, with clonal expansion eventually leading to the development of liver tumours. 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.

In addition, CAR activation in male mice also results in the induction of 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. The effects on cytochrome P450s are considered to be associative events in that while they are a characteristic hallmark of CAR activation, 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.

Relevant Data for the assessment of the Mode of Action hypothesis for SYN545974

The following studies were considered when evaluating the mode of action hypothesis for SYN545974

- An assessment of the ability of SYN545974 to activate CAR (██████████, 2014) – see Section 6.5/07.
- Three studies assessing the dietary toxicity over a period of 28 days with mode of action endpoints (██████████ 2012a; ██████████ 2012 and ██████████ 2015) – see Section 5.5.4/07; 5.5.4/08 and 5.5/04 respectively).
- As assessment of the sub chronic toxicology in the CD-1 mouse (██████████ 2015) see Section 5.3.2/02.
- An 80 week carcinogenicity study in the CD1 mouse (██████████ 2015a) – see Section 5.5/02.
- Investigative studies in primary cultures of mouse and human hepatocytes to assess mechanistic endpoints with respect to human relevance (██████████ 2015 & 2015a) – see Section 6.5/05 and 06 respectively.

IPCS/ILSI Framework for the Evaluation of the Human Health Relevance of a Hypothesized Mode of Action

Is the MOA Established in Animals? After the key events within a postulated MOA have been described, the Hill criteria and the framework outlined by IPCS/ILSI require that they be evaluated by a standardized weight of evidence evaluation where the key events which are causally related to the formation of tumours, are clearly shown to be required steps that lead to the cancer and that the findings are reproducible. A weight of evidence analysis for SYN545974 induced liver tumour formation is described in the following sections.

Dose Concordance of Key Events: For the key events in a proposed MOA to be causally related to the formation of tumours, they must show concordance with the dose levels that produce tumours (dose concordance). Table 6.5-27 summarises the dose concordance of the key and associate events (as defined in the hypothesised MOA) with the tumour outcome. This table considers concordance with the following dose levels.

- 75 ppm as the NOAEL for liver tumours in male CD-1 mice
- 2250 ppm as a carcinogenic dose that induces liver tumours in male CD-1 mice

Table 6.5-27 clearly demonstrates that it is only at the tumourigenic doses level (2250 ppm) that all of the key (and associate) events are observed. Early effects at the non-tumourigenic dose level (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 associate events. This is supported by the lack of any other key or associative events occurring at the non tumourigenic dose level, including no effects at any study duration on liver weight or liver histopathology findings. 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 document, the marginal increase in cell proliferation at 75 ppm is considered not a human-relevant adverse effect. Overall, it can be concluded that there is strong concordance between key (and associative) events and dose levels that produce tumours.

Table 6.5-27: Dose Concordance of Key and Associative Events across the SYN545974 Mouse Database

SYN545974 Dose (ppm)	75 ppm	2250 ppm
CAR Activation (<i>in vivo</i>) ^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

^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 SYN545974 to directly activate mouse CAR also has been demonstrated using an *in vitro* transactivation assay.

^b Effects observed in histopathology examinations of the liver.

Temporal Concordance of Key Events

When a tumourigenic dose level (e.g. 2250 ppm) is considered, 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 Figure 5.5-1). The temporal concordance for SYN545974 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 SYN545974 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 hepatocellular proliferation were evident from day 2 through day 28 (the last timepoint tested). Proliferative indices were maximal after day 2, when it was associated with increased mitosis, and decreased with time. Consistent with the typical pattern of CAR activators the histopathology observation 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 Cyp content and PROD activity, was evident after 2 days of exposure and likely occurred throughout the exposure period (day 28 was the latest timepoint tested).
- Elevation of centrilobular hypertrophy was evident after 2 days of exposure to SYN545974, 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 SYN545974 followed a logical time course, with the earlier key events preceding the ultimate progression to adenomas and carcinomas in the liver (Table 6.5-28)

Table 6.5-28: Temporal Concordance of Key and Associative Events across the SYN545974 Mouse Database

Key/Associative Event	Exposure to SYN545974 (2250 ppm)					
	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	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

Yes: Key/Associative Event Occurring

No: Key/Associative Event Not Occurring

^a presumed to still be occurring due to effects observed at earlier times & other associative events occurring.

-: No Data

Reproducibility and Consistency

When all studies with SYN545974 are considered, there is excellent 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 Cyps 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 Cyp3a11 and other Cyps; therefore, the consistent large increases in PROD activity in multiple mouse studies with SYN545974 helps confirm the sustained activation of CAR in the liver at higher dose levels.

The specificity of the hepatocellular adenomas and carcinomas associated with SYN545974 to male mice has been demonstrated (██████████ 2015 and 2015a). Studies have shown that although certain key and associative events such as CAR activation and increased liver weight do occur in the rat (██████████, 2014), tumours were not observed in the 104 week carcinogenicity study in the rat with SYN545974 (██████████, 2015). 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 tumors than rats.

In the mouse database, a very weak proliferative signal after exposure to 75 ppm SYN545974 was observed in the 28 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 this dose level. 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 tumourigenic response.

Biological Plausibility

The liver is by far the most common target tissue affected in cancer bioassays. When evaluating liver tumour susceptibility, there are two metrics typically used to evaluate: 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 SYN545794 supports the promotor, non genotoxic, mode of action, 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, PPAR α and AhR.

Integrated data from multiple studies with SYN545794 demonstrating the temporal and dose concordance of the key events are summarized in Table 6.5-27 (dose concordance) and Table 6.5-28 (temporal concordance). These data are consistent with the current understanding of the necessary key events in the MOA for CAR activators. Thus, the data for SYN545974 support a biologically plausible MOA by a mechanism that is well understood and supported by extensive published research.

Alternative MOA hypotheses

Several modes of action have been identified for liver carcinogenesis, both in humans and in rodent models (Table 6.5-29). Some of the key events described for SYN545974 are common to other known modes-of-action. However, a full examination of the available database for SYN545974 can be used to exclude the alternative MOAs.

Liver carcinogens can be divided into those that are DNA reactive versus those that are non-DNA reactive and both produce their carcinogenic effect by increasing cell proliferation. DNA reactivity can be excluded as SYN545974 tested negative in a battery of *in vitro* and *in vivo* genetic toxicity studies. Furthermore, the tumour profile observed in SYN545974 carcinogenicity bioassays is typical of a non-genotoxic mechanism (single species, single sex and single organ and lack of decreased latency).

MOAs involving induction of other CYP 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 CYP P450 isoforms or peroxisome proliferation can also produce these findings. However, these other MOAs can be ruled out because the experimental evidence shows that SYN545974 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 (██████, 2012). Hepatocellular cytotoxicity and subsequent regenerative proliferation, such as that caused by chloroform, is another mechanism by which carcinogenesis can occur. This mechanism is characterised by sustained diffuse necrosis and cellular proliferation; however, it can be excluded for SYN545974 due to the experimental evidence demonstrating a lack of hepatic damage and regenerative proliferation at all time points investigated. The exclusion of other MOAs for SYN545974 induction of liver tumours are detailed in Table 6.5-29.

Data Gaps, Uncertainties and Inconsistencies

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 SYN545974, is in one 28 day dietary study in CD-1 mice, where there was the lack of observed centrilobular hypertrophy in the presence of increased CYP activity and hepatomegaly (██████, 2012). This is in contrast to centrilobular hypertrophy being observed, associated with these events, in numerous other studies of similar or longer duration (██████, 2015; ██████, 2015, ██████, 2015a)

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 and temporal consistency with the tumour endpoint. In addition this is a well-established MOA based on prior publications, and the parameters essential for describing the MOA have been presented for SYN545974.

Therefore there is a high level of confidence that the postulated MOA was responsible for the tumour outcome in the male CD1 mouse.

Table 6.5-29: Alternative MOAs for Hepatocarcinogenesis

Alternative MOA	SYN545974: Reason for Exclusion
DNA Reactivity	<p>SYN545974 has been demonstrated negative in <i>in vitro</i> bacterial & mammalian mutagenicity assays.</p> <p>SYN545974 has been demonstrated negative in <i>in vivo</i> assays for clastogenicity and aneugenicity (██████, 2012; ██████, 2014; ██████, 2013; ██████, 2013; ██████, 2012 and ██████, 2014.)</p> <p>Late onset of tumours is not indicative of a DNA reactive mechanism.</p>

Alternative MOA	SYN545974: Reason for Exclusion
Peroxisome Proliferator	SYN545974 does not induce peroxisomal palmitoyl CoA oxidase or lauric acid 12-hydroxylase (marker for Cyp4a) activities in microsomal mouse liver preparations. (██████, 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 PPAR α receptor. A 2- to 3-fold increase in lauric acid 12-hydroxylase activity was observed with SYN545974, 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 PPAR α activators (12-fold).
Enzyme induction (AHR)	SYN545974 does not induce substantial EROD activity (a marker for Cyp1a) in microsomal male mouse liver preparations. (██████, 2012). SYN545974 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 has been demonstrated previously (██████ et al., 2006), and is possibly due to some metabolism via induced Cyp2b isoenzymes; these minor changes are in contrast to the large increases by AhR activators.
Statins	SYN545974 was not designed to inhibit HMG-CoA reductase and SYN545974 treatment does not result in decreased cholesterol (██████, 2015).
Cytotoxicity	SYN545974 is not a hepatic cytotoxicant based on (a) lack of evidence of elevated hepatic damage clinical chemistry markers and (b) lack of histopathological evidence of sustained inflammation, necrosis or regenerative proliferation in the liver (██████, 2015; ██████, 2015, ██████, 2015; ██████, 2015a).
Infectious	Excluded based on lack of evidence of infection, cellular inflammatory response or regenerative proliferation (██████, 2015; ██████, 2015a and 2015b)
Increased Apoptosis	There is no histological evidence that SYN545974 increases hepatocyte apoptosis (██████, 2015; ██████, 2015a and 2015b)
Estrogenic Activity	In the large mammalian toxicological database available for SYN545974, including the studies summarised in this document, as well as studies of the effects of SYN545974 on reproduction and development (██████, 2015c; ██████, 2015; ██████, 2015), there is no evidence for oestrogenic stimulation.

Human Relevance

Are the Key Events in the Animal MOA Plausible in Humans?

Once an MOA in mice has been established, the relevance to humans is assessed by examining each key event. Based on experimental data with SYN545974 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 6.5-30 describes the similarities and differences between mice and humans in relation to each key event.

Table 6.5-30: Comparison of Key Events for SYN545974 Across Species

Key Event	Mouse		Human		Comments
	<i>In Vivo</i>	<i>In Vitro</i>	<i>In Vitro</i>	<i>In Vivo</i>	
Activation of CAR & altered gene expression	Yes	Yes	Yes	Likely	Activation of both human and mouse CAR occurs.
CYP450 Induction (Cyp2b and Cyp3a isoforms)	Yes	Yes	Yes	Likely	Based on large ↑ in PROD activities
Cell Proliferation	Yes	Yes	No	Unlikely ^a	^a Based on <i>in vitro</i> ~ <i>in vivo</i> correlation
Tumours	Yes	N/A	N/A-	Unlikely ^b	^b Based on lack of cell proliferation, and data for human drugs that are CAR activators.

^aN/A^a Not Applicable

PROD activity has been reported to be catalysed primarily by Cyp2b family enzymes in mice or rats, but with some contribution from other CyPs including those in the Cyp3a subfamily. With human primary hepatocytes and purified CYP 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 demonstrates that SYN545794 is a direct activator of mouse and human CAR. Further, the concentrations where maximal CAR activation was attained provide evidence that SYN545974 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 SYN545974 resulted in elevations of PROD and BROD activity, which are surrogate markers of CYP2B / CYP3A induction produced via CAR activation (██████, 2015a – see Section 6.5/06). Similar results were obtained in the mouse (██████, 2015 – see Section 6.5/05). 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 SYN545974 to activate CAR.

Therefore the human is not qualitatively different to the mouse in terms of the initial key event, SYN545974-induced CAR activation.

Hepatocyte Proliferation

The ability of SYN545794 to increase the proliferation of human hepatocytes was evaluated by treating male donor hepatocytes in primary culture with SYN545974 and measuring replicative DNA-synthesis by BrdU labeling index as the marker of increased S-phase and proliferation (██████, 2015a – see Section 6.5/06). Similar experiments were performed using mouse hepatocytes in primary culture (██████, 2015 – see Section 6.5/05). At doses ranging from 5µM – 35µM, SYN545794 did not increase BrdU labeling in male human hepatocytes, whereas a robust increase in labeling index was observed in male mouse hepatocytes (██████, 2015 and 2015a). In addition to this, the positive control CAR activator PB, displayed a similar pattern as SYN545794, causing increased BrdU labeling of mouse hepatocytes, but no effect in male human hepatocytes.

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

Therefore the human is qualitatively different to the mouse in terms of the second key event in the MOA for SYN545974, hepatocellular proliferation induced by CAR activation.

Statement of Confidence, Analysis and Implications.

Based on the above, there is strong evidence to support a MOA for SYN545974-induced hepatocellular tumors 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 tumors. Based on the human evidence presented above using comparative studies in hepatocytes there is strong evidence that a qualitative species difference exists between mouse and human for the pivotal key event of increased hepatocellular proliferation; SYN545974 did not produce cell proliferation in human hepatocytes. Based on this species difference in response, SYN545974 is unlikely to cause cell proliferation in humans *in vivo*, and it is therefore unlikely to cause tumors in humans (Table 6.5-1, Figure 6.5-1 and 6.5-2). This conclusion is supported further by epidemiology studies that show a lack of tumor response in patients treated with known CAR activators, which share the same MOA as SYN545974.

CONCLUSION:

Recognising that pydiflumetofen was associated with a hepatocarcinogenic effect in mice, the applicant sponsored a series of mechanistic studies in male mice and culture of mouse and human hepatocytes to investigate a possible non-genotoxic mode of action involving liver stimulation via constitutive androstane receptor (CAR) induction.

The mechanistic data presented suggested that CAR activation was the most plausible MoA for the observed mouse liver tumours, 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.
- Eventual progression to form liver tumours.

And the following associative events:

- Increased expression of genes encoding cytochrome P450s (CYPs), particularly Cyp2b/Cyp3a isoforms (PROD and BROD), associated with CAR activation.
- Increased incidence of hepatocellular hypertrophy.
- Increased liver weight.

In addition, a proliferation assay in male human hepatocytes showed that pydiflumetofen did not increase cellular proliferation up to the top concentration of 35 μM , despite a significant response by the positive control substance EGF. In contrast, cultures of hepatocytes from male mice (also tested up to 35 μM) showed significant proliferation, although a clear dose-response was not apparent. These data would indicate that the proposed liver tumour MoA would not be relevant to humans. However, HSE notes that this key piece of evidence suffered from many shortcomings. Only one human donor had been used, significantly affecting the ability to address intraspecies variability in the observed response. In addition, significant cytotoxicity (ATP depletion) had been observed at the two highest concentrations (25 and 35 μM) in the human cultures, raising a question on whether the lack of proliferation could have been the consequence of the cytotoxicity. In the mouse culture, where proliferation did occur,

pydiflumetofen was not cytotoxic, except at the top concentration of 35 µM, at which an associated decrease in proliferation was noted.

Other potential MoAs were also investigated. Direct cytotoxicity was excluded as there were no indications of liver necrosis or degeneration from the histopathology examinations. Pydiflumetofen did not increase hepatic peroxisomal β-oxidation, a marker of peroxisome proliferation, but did produce a limited increase in lauric acid hydroxylation - LAH (surrogate for CYP4A and fibrates-dependent peroxisome proliferation). This indicates that fibrates-dependent peroxisome proliferation cannot be completely excluded. Pydiflumetofen had also a small effect on EROD activity (CYP1A), a marker for aryl-hydrocarbon receptor (AhR) activation, indicating that pydiflumetofen might not be an AhR activator. However, as no positive controls for AhR activation or LAH expression (e.g. 3-methylcholanthrene, omeprazole) had been used to put into context these small increases, these minor enzyme inductions could not be completely disregarded.

Overall, in agreement with RAC, HSE concludes that the data to investigate alternative MoAs were limited. In addition, an explanation for the differential sensitivity between male and female mice and between rats and mice with respect to the development of liver tumours, despite liver hypertrophy being present in both sexes and species, was also lacking.

HSE agrees with RAC that pydiflumetofen was clearly carcinogenic in the liver of male mice. Several mechanistic investigations indicated that a CAR-mediated MoA was the most plausible MoA for these liver tumours. However, the lack of relevance to humans of such MoA has not been fully demonstrated. Qualitative differences between humans and rodents, particularly in the ultimate key event of hepatocellular proliferation, have not been fully substantiated. The limited *in vitro* study with human hepatocytes has shown significant cytotoxicity at concentrations > 10 µM, confounding the interpretation of the absence of proliferation. In addition, cells from only one donor have been used and the results cannot be interpreted as being representative from a cross section of the human population. No transgenic knockout animals or humanised receptor models have been employed to provide further support for the lack of human relevance.

In addition, alternative MoAs have not been fully excluded. Furthermore, an explanation for the differential sensitivity between male and female mice and between rats and mice with respect to the development of liver tumours is lacking. Based on these considerations, HSE agrees with RAC that a potential carcinogenic hazard to humans cannot be excluded and that classification for carcinogenicity in category 2 (H351) is warranted. For further details, see the [GB MCL Technical Report](#).
(██████████, 2015)

B.6.5.5. Summary of long-term toxicity and carcinogenicity

Pydiflumetofen has been evaluated for chronic toxicity in the rat and for carcinogenic potential in the rat and mouse in GLP and guideline studies.

In the study in rats given doses of 0, 200, 1000 or 6000 ppm to males (9.9, 51, 319 mg/kg bw/d) and doses of 0, 150, 450 or 1500 ppm to females (10.2, 31 and 102 mg/kg bw/d), no tumours were observed in both sexes up to the top dose tested, at which reductions in body weight gain of 22% in males and 13% in females were observed. Although thyroid follicular adenomas were increased in females at the top dose (3/51 (5.9%) vs 1/51 (2%) in controls), the increase was within the laboratory contemporary (5-years) HCD (0 – 5.8%), they were not statistically significant and there was no association with pre-neoplastic lesions. Therefore the NOAEL for carcinogenicity was 6000 ppm (319 mg/kg bw/d) in males and 1500 ppm (102 mg/kg bw/d) in females.

In the study in mice given doses of 0, 75, 375 or 2250 ppm (0, 9.2/9.7, 45.4/48.4, or 288/306 mg/kg bw/d in males/females), there was a dose-related increase in liver adenomas (18% and 44% at mid- and top-dose vs 8% in controls) and carcinomas (8% and 20% at mid- and top-dose vs 4% in controls) in males from the mid dose, which reached statistical significance at the highest dose. The incidences at the top dose were also above the laboratory historical control ranges from 5 studies conducted between 2007-2009 (10-28% for adenoma; 6-10% for carcinoma) and tumour multiplicity was also noted. In addition, pre-neoplastic lesions (eosinophilic foci of cellular alterations: 12.2% and 20% at the mid and top dose respectively vs 2% in controls; above HCD) occurred in males from the mid dose. Overall, pydiflumetofen was clearly carcinogenic in the liver of male mice up to a dose which was not excessively toxic to the animals (7% and 11.6% reduction in terminal body weight in males and females respectively). Therefore the NOAEL for carcinogenicity was 75 ppm (9.2 mg/kg bw/d) in males and the top dose of 2250 ppm (306 mg/kg bw/d) in females. Overall, the most sensitive **carcinogenic NOAEL is 9.2 mg/kg bw/d** in male mice based on liver tumours at the next dose of 45.4 mg/kg bw/d.

Several mechanistic investigations indicated that a CAR-mediated MoA was the most plausible MoA for these liver tumours in male mice. However, the lack of relevance to humans of such MoA has not been fully demonstrated. Qualitative differences between humans and rodents, particularly in the ultimate key event of hepatocellular proliferation, have not been fully substantiated. The limited *in vitro* study with human hepatocytes has shown significant cytotoxicity at concentrations > 10 µM, confounding the interpretation of the absence of proliferation. In addition, cells from only one donor have been used and the results cannot be interpreted as being representative from a cross section of the human population. No transgenic knockout animals or humanised receptor models have been employed to provide further support for the lack of human relevance.

In addition, alternative MoAs have not been fully excluded. Furthermore, an explanation for the differential sensitivity between male and female mice and between rats and mice with respect to the development of liver tumours is lacking. Based on these considerations, HSE agrees with RAC that a potential carcinogenic hazard to humans cannot be excluded and that classification for carcinogenicity in category 2 (H351) is warranted. For further details, see the [GB MCL Technical Report](#).

With regard to chronic toxicity, in rats, pydiflumetofen caused decreases in body weight (10.8%), body weight gain (13%), food consumption and food utilisation, an increase in liver weight (16%) with associated hypertrophy at the mid dose of 1000 ppm in males. These effects became more severe at the top dose of 6000 ppm (e.g. ↓18.2% in body weight; ↓22% body weight gain; ↑36% liver weight) at which an increase in GGT was also seen. In females, adverse effects were only observed at the top dose of 1500 ppm (↓ 9.1 % body weight; ↓ 13% body weight gain, reduced food consumption, ↑15% liver weight; hepatocyte hypertrophy). Therefore a chronic toxicity NOAEL of 200 ppm (9.9 mg/kg bw/d) was identified in males and a chronic toxicity NOAEL of 450 ppm (31 mg/kg bw/d) was identified in females.

In mice, pydiflumetofen caused decreases in terminal body weight (7% and 11.6% in males and females), body weight gain (14% and 24% in males and females) and food consumption at the top dose in both sexes. Food utilisation was also decreased (by 12%) in top dose males. A statistically significant increase in liver weight (by 52% and 17% in males and females respectively) was observed at the top dose in both sexes. In addition, in males, liver weight was increased by 12% at the mid dose. In males, increased liver weight was associated with hepatocyte hypertrophy from the mid dose (6/49 and 18/50 at 375 and 2250 ppm respectively vs 0/50 in controls). Therefore a chronic toxicity NOAEL of 75 ppm (9.2 mg/kg bw/d) was identified in males and a chronic toxicity NOAEL of 375 ppm (48.4 mg/kg bw/d) was identified in females. Overall, the most sensitive **chronic toxicity NOAEL is 9.2 mg/kg bw/d** from the mouse study based on increased liver weight and associated hypertrophy in males at the next dose of 45.4 mg/kg bw/d.

The table below summaries the results of the carcinogenicity studies.

Study & Acceptability	Mode of Dosing	Test Material & Dose Levels	NOAEL	LOAEL	Effects at LOAEL
104 week rat carcinogenicity study with a combined 52 week toxicity study (██████████, 2015) <i>Modern, valid guideline study</i>	Dietary	Pydiflumetofen 98.5% <u>Males</u> 0, 200, 1000 & 6000 ppm; <u>Females</u> 0, 150, 450 & 1500 ppm	<i>Chronic toxicity</i> <u>Males</u> 200 ppm (9.9 mg/kg bw/d); <u>Females</u> 450 ppm (31 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males</u> 6000 ppm (319 mg/kg bw/d) <u>Females</u> 1500 ppm (102 mg/kg bw/d)	<i>Chronic toxicity</i> <u>Males</u> 1000 ppm (51 mg/kg bw/d) <u>Females</u> 1500 ppm (102 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males</u> >6000 ppm (>319 mg/kg bw/d) <u>Females</u> >1500 ppm (>102 mg/kg bw/d)	<i>Chronic toxicity</i> <u>1000 ppm</u> (mid-dose males): ↓ bw and bwg, food utilization, hepatocyte hypertrophy and ↑liver weight. <u>1500 ppm</u> (top-dose females): ↓ bw and bwg, food utilization, ↑liver weight associated with minimal hepatocellular hypertrophy <i>Carcinogenicity</i> No treatment related neoplastic findings.
80 week mouse carcinogenicity study (██████████, 2015a) <i>Modern, valid guideline study</i>	Dietary	Pydiflumetofen 98.5% 0, 75, 375 & 2250 ppm	<i>Chronic toxicity</i> <u>Males:</u> 75 ppm (9.2 mg/kg bw/d) <u>Females:</u> 375 ppm (48.4 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males:</u> 75 ppm (9.2 mg/kg bw/d) <u>Females:</u> 2250 ppm (306 mg/kg bw/d)	<i>Chronic toxicity</i> <u>Males:</u> 375 ppm (45.4 mg/kg bw/d) <u>Females:</u> 2250 ppm (306 mg/kg bw/d) <i>Carcinogenicity</i> <u>Males:</u> 375 ppm (45.4 mg/kg bw/d) <u>Females:</u> >2250 ppm (>306 mg/kg bw/d)	<i>Chronic toxicity</i> <u>375 ppm (males):</u> ↑liver weight associated with hepatocellular hypertrophy <u>2250 ppm (females):</u> ↓ bw and bwg, food consumption, ↑liver weight. <i>Carcinogenicity</i> Liver tumours in males from 375 ppm. No tumours in females up to 2250 ppm

B.6.6. REPRODUCTIVE TOXICITY

See B6 Part II

B.6.6.1. Generational studies

See B6 Part II

B.6.6.2. Developmental toxicity studies

See B6 Part II

B.6.7. NEUROTOXICITY

See B6 Part II

B.6.7.1. Neurotoxicity studies in rodents

See B6 Part II

B.6.7.2. Delayed polyneuropathy studies

See B6 Part II

B.6.8. OTHER TOXICOLOGICAL STUDIES

See B6 Part II

B.6.8.1. Toxicity studies on metabolites and relevant impurities

See B6 Part II

B.6.8.2. Supplementary studies on the active substance

See B6 Part II

B.6.8.3. Studies on endocrine disruption

See B6 Part II

B.6.9. MEDICAL DATA AND INFORMATION

See B6 Part II

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

See B6 Part II

B.6.9.2. Data collected on humans

See B6 Part II

B.6.9.3. Direct observation

See B6 Part II

B.6.9.4. Epidemiological studies

See B6 Part II

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

See B6 Part II

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

See B6 Part II

B.6.10. REFERENCES RELIED ON

See B6 Part II

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation