Committee for Risk Assessment
RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

N-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide; pydiflumetofen

EC Number: -
CAS Number: 1228284-64-7

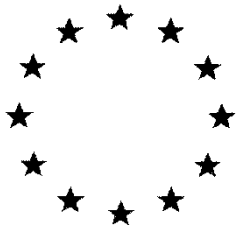
CLH-O-0000001412-86-271/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 March 2019

European Commission



Combined Draft Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

PYDIFLUMETOFEN

Volume 1

Rapporteur Member State: France
Co-Rapporteur Member State: Austria

Version History

When	What
2017-07	Initial DAR-CLH report
2018-02	DAR-CLH report revised in line with requirements of ECHA following the accordance check

Table of contents

Level 1

PYDIFLUMETOFEN

1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 CONTEXT IN WHICH THIS DRAFT ASSESSMENT REPORT WAS PREPARED

1.1.1 Purpose for which the draft assessment report was prepared

This Draft Assessment Report (DAR) has been prepared to evaluate the dossier submitted by Syngenta Crop Protection AG for the first approval of the new active substance Pydiflumetofen (SYN545974) under Regulation (EC) N° 1107/2009.

Pydiflumetofen (SYN545974) is a new fungicide and the dossier contains data and information to support a limited range of representative uses of the active substance for which it is intended to demonstrate that, for one preparation, the requirements of Article 4 of Regulation (EC) No 1107/2009, can be met.

Alongside this application, Syngenta Crop Protection AG has submitted an application to set specific Maximum Residue Levels (MRLS) for the representative and other uses and for import tolerance uses as the new active substance is not mentioned in Annex II/III/IV of Regulation (EC) No 396/2005).

Syngenta Crop Protection AG has also made a proposal for Harmonised Classification and Labelling based on Regulation (EC) No 1272/2008 (CLP Regulation). This has also been evaluated by France and presented in this report using the new common DAR/CLH template to be submitted to ECHA in parallel to the submission to EFSA in order to follow the aligned evaluation process

1.1.2 Arrangements between rapporteur Member State and co-rapporteur Member State

France, acting as the Rapporteur Member State (RMS), evaluated the dossier submitted and wrote the DAR. The DAR was then peer review by Austria (Co-RMS).

1.1.3 EU Regulatory history for use in Plant Protection Products

Not applicable. Pydiflumetofen (SYN545974) is a new active substance and products containing it have not previously authorised in the EU.

1.1.4 Evaluations carried out under other regulatory contexts

According to the applicant, Pydiflumetofen (SYN545974) dossiers have been submitted in the following countries, only within the context of crop protection and as a fungicide: Brazil (24.09.2015), NAFTA (USA, Canada, Mexico 30.09.2015), Argentina (27.10.2015), Australia (01.03.2016) and New Zealand (07.12.2016).
Argentina granted the first authorization for a use on soya on November 2016. The evaluations in the other countries are still pending on April 2017.

1.2 APPLICANT INFORMATION

1.2.1 Name and address of applicant(s) for approval of the active substance

Syngenta Crop Protection AG
Schwarzwaldalle 215
P.O. Box
CH-4002 Basel
Switzerland

For further information relating to the location of plants, please refer to Volume 4 of the DAR.

1.2.2 Producer or producers of the active substance

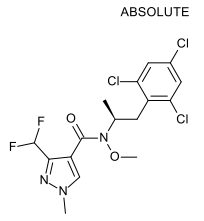
Syngenta Crop Protection AG
Schwarzwaldalle 215
P.O. Box
CH-4002 Basel
Switzerland

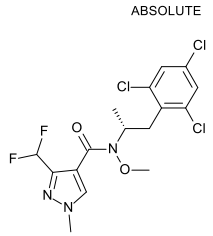
For further information relating to the location of plants, please refer to Volume 4 of the DAR.

1.2.3 Information relating to the collective provision of dossiers

Not applicable, Syngenta Crop Protection AG is the sole notifier.

1.3 IDENTITY OF THE ACTIVE SUBSTANCE

1.3.1 Common name proposed or ISO-accepted and synonyms	Pydiflumetofen (SYN545974)
1.3.2 Chemical name (IUPAC and CA nomenclature)	
IUPAC	*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide
CA	1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-
1.3.3 Producer's development code number	SYN545974
1.3.4 CAS, EEC and CIPAC numbers	
CAS	1228284-64-7
EEC	Not available
CIPAC	Not available
1.3.5 Molecular and structural formula, molecular mass	
Molecular formula	$C_{16}H_{16}O_2N_3Cl_3F_2$
Structural formula	PYDIFLUMETOFEN (SYN545974) consists of two enantiomers as a racemate mixture (50:50) *SYN546968 (S)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide*  *SYN546969 (R)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide*

	ABSOLUTE 
Molecular mass	426.7 g/mol
1.3.6 Method of manufacture (synthesis pathway) of the active substance	Confidential data see vol.4
1.3.7 Specification of purity of the active substance in g/kg	980 g/kg
1.3.8 Identity and content of additives (such as stabilisers) and impurities	
1.3.8.1 Additives	Confidential data see vol.4
1.3.8.2 Significant impurities	Confidential data see vol.4
1.3.8.3 Relevant impurities	No relevant impurity
1.3.9 Analytical profile of batches	Confidential data see vol.4

1.4 INFORMATION ON THE PLANT PROTECTION PRODUCT

1.4.1 Applicant	**Syngenta Crop Protection AG** Schwarzwaldalle 215 P.O. Box CH-4002 Basel Switzerland
1.4.2 Producer of the plant protection product	**Syngenta Crop Protection AG** Schwarzwaldalle 215 P.O. Box CH-4002 Basel Switzerland
1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection product	Trade name: ADEPIDYN™ Code: A19649B
1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection product	
1.4.4.1 Composition of the plant protection product	Confidential data see vol. 4
1.4.4.2 Information on the active substances	200 g/L of pure active substance 204 g/L of technical substance active
1.4.4.3 Information on safeners, synergists and co-formulants	Confidential data see vol. 4
1.4.5 Type and code of the plant protection product	Suspension Concentrate [Code : SC]

1.4.6 Function	Fungicide
1.4.7 Field of use envisaged	Agriculture, horticulture and viticulture
1.4.8 Effects on harmful organisms	PYDIFLUMETOFEN (SYN545974) is a succinate dehydrogenase inhibitor (SDHI). It has curative activity. It targets a broad spectrum of diseases.

1.5 DETAILED USES OF THE PLANT PROTECTION PRODUCT

1.5.1 Details of representative uses

Tradename: A19649B
Active Substances: PYDIFLUMETOFEN (SYN545974) 200 g/L SC formulation

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	**Member state (s)**	**Crop and/or situation (crop destination/ purpose of crop)**		**F G or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Method / Kind**	**Timing/Growth stage of crop & season**	**Max. Number a) per use b) per crop/ season**	**Minimum interval between applications (days)**	**L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season**	**g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min/max**	**PHI (days)**	**Remarks: e.g. safener/synergist per ha**
1	EU	Pome fruit	Apple	F	Powdery mildew *(Podosphaera leucotricha)* + scab *(Venturia inaequalis)*	Foliar	BBCH 56-79	a) 3 b) 3	7	a) 0.25 b) 0.75	a) 50 b) 150	400-1500	65	0.14l/Ha LWA in 18000m² LWA/ha = 0.25 l/ha (17ml/hl)
2	EU		Pear	F	scab *(Venturia pyrina)*	Foliar	BBCH 56-79	a) 3 b) 3	7	a) 0.25 b) 0.75	a) 50 b) 150	400-1500	65	0.14l/Ha LWA in 18000m² LWA/ha = 0.25 l/ha (17ml/hl)
3	EU	Grapes (wine & table)		F	Grey mould *(Botrytis cinerea)*	Foliar	BBCH 67-89	a) 2 b) 2	14	a) 1 b) 2	a) 200 b) 400	500-1400	21	
4	EU	Grapes (wine & table)		F	Powdery mildew (*Uncinula necator*)	Foliar	BBCH 13-77	a) 2 b) 2	10	a) 0.2 b) 0.4	a) 40 b) 80	150-1000	21	
5	EU	Potato		F	Early blight *(Alternaria solani*)	Foliar	BBCH 31-89	a) 3 b) 3	14	a) 0.20 b) 0.60	a) 40 b) 120	200-500	7	

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	**Member state (s)**	**Crop and/or situation (crop destination/ purpose of crop)**		**F G or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Method / Kind**	**Timing/Growth stage of crop & season**	**Max. Number a) per use b) per crop/ season**	**Minimum interval between applications (days)**	**L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season**	**g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min/max**	**PHI (days)**	**Remarks: e.g. safener/synergist per ha**
6	EU	Fruiting vegetables	Tomato	F	Early blight (*Alternaria solani*)	Foliar	BBCH 51-89	a) 2 b) 2	7	a) 0.35 b) 0.70	a) 70 b) 140	300-1000	1	
7	EU	Edible cucurbit	Cucumber	F	Powdery mildew (*Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
8	EU		Courgette/ zucchini	F	Powdery mildew (*Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
9	EU	Inedible cucurbit	Melon	F	Powdery mildew (*Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
10	EU		Watermelon	F	Powdery mildew (*Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
11	EU	Flowering brassica	Broccoli	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
12	EU		Cauliflower	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	**Member state (s)**	**Crop and/or situation (crop destination/ purpose of crop)**		**F G or I**	**Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)**	**Method / Kind**	**Timing/Growth stage of crop & season**	**Max. Number a) per use b) per crop/ season**	**Minimum interval between applications (days)**	**L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season**	**g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season**	**Water L/ha min/max**	**PHI (days)**	**Remarks: e.g. safener/synergist per ha**
13	EU	Leafy brassica	Kale	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
14	EU	Head brassica	Brussels sprouts	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
15	EU		Cabbage	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
16	EU	Kohlrabi		F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	

1.5.2 Further information on representative uses

The maximum number of applications and the minimum interval between applications are provided in columns 8 and 9 of the table of the intended uses.
Considering the activity of the active ingredient, no restrictions need to be applied to avoid phytotoxic effects on succeeding crops.
See Volume 3 CP B.3.8.for more details on proposed instructions for use.

1.5.3 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min - max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
MRL Application (according to Article 8.1(g) of Regulation (EC) No 1107/2009)															
Pome (apple) and other pome fruit including quince, medlar and loquat	eu		NEU/SEU	Powdery mildew (Podosphaera leucotricha) + scab (Venturia inaequalis)	sc	200 g/L	Foliar spray	BBCH 56-79	3	7	-	400-1500	50	65	0.14l/Ha LWA in 18000m² LWA/ha = 0.25 l/ha (17ml/hl)
Potatoes / Sweet Potatoes/ Yams	eu		NEU/SEU	Early blight (Alternaria solani)	sc	200 g/L	Foliar spray	BBCH 31-89	3	14	-	200-500	40	7	
Tomatoes (protected)	EU		G	Leveillula taurica, Oidium lycopersici	SC	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	70	1	
Tomatoes (protected)	EU		G	Botrytis cinerea	SC	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	200	1	
Peppers (protected	EU		G	Leveillula taurica	SC	200 g/L	Foliar	BBCH	2	7	-	300-1000	70	3	

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min - max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
)							spray	51-89							
Peppers/ Sweet peppers/ Bell peppers (field)	EU		NEU/SEU	Leveillula taurica	SC	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	70	3	
Aubergine /Eggplants (field)	eu		NEU/SEU	Early blight (Alternaria solani)	sc	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	70	1	
Aubergine Eggplants (protected)	EU		G	Powdery mildew (Leveillula taurica, Oidium lycopersici)	sc	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	70	1	
Aubergine /Eggplants (protected)	EU		G	Botrytis cinerea	sc	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	200	1	
Okra (protected)	EU		G	Leveillula taurica	sc	200 g/L	Foliar spra	BBCH 51-	2	7	-	300-1000	70	3	

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min - max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
							y	89							
Okra (field)	EU		NEU/SEU	Leveillula taurica	sc	200 g/L	Foliar spray	BBCH 51-89	2	7	-	300-1000	70	3	
Cucurbits Edible peel : cucumber , courgette/ zucchini , Gherkins and others (field)	eu		NEU/SEU	Powdery mildew (Sphaerotheca fuliginea and Erysiphe sp)	sc	200 g/L	Foliar spray	BBCH 20-89	2	7	-	300-1000	50	1	Equivalent to 25 mL/hL
Cucurbits Edible peel (protected)	EU		G	Sphaerotheca fuliginea	SC	200 g/L	Foliar spray	BBCH 20-89	2	7	-	300-1000	50	1	
Cucurbits Inedible peel: melon, watermelon	eu		NEU/SEU	Powdery mildew (Sphaerotheca fuliginea and Erysiphe sp)	sc	200 g/L	Foliar spray	BBCH 20-89	2	7	-	300-1000	50	1	Equivalent to 25 mL/hL

Crop and/or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Preparation**		**Application**				**Application rate per treatment**			**PHI (days)** (m)	**Remarks**
					Type (d-f)	Conc. a.s. (i)	method kind (f-h)	range of growth stages & season (j)	number min-max (k)	Interval between application (min)	kg a.s /hL min - max (l)	Water L/ha min-max	g a.s./ha min-max (l)		
Pumpkin and others (field)															
Cucurbits Inedible peel (protected)	EU		G	Sphaerotheca fuliginea	SC	200 g/L	Foliar spray	BBCH 20-89	2	7	-	300-1000	50	1	
Kale/ chinese cabbage/pe-tsai	eu		NEU/SEU	Alternaria sp. and Mycosphaerella sp.	sc	200 g/L	Foliar spray	BBCH 21-49	7 2	14	-	200-600	70	14	
Soya bean	EU		G	SeptoriaglycinesCercospora sojina, Cercospora kikuchii	SC	75 g/L	Foliar spray	45 DBH	2	15	-	100-400	160 *	30	*160 g/ha - 60 g a.s./ha for PYDIFLUMETOFEN (SYN545974) and 100 g a.s./ha for difenoconazole

*160 g/ha - 60 g a.s./ha for PYDIFLUMETOFEN (SYN545974) and 100 g a.s./ha for difenoconazole

1.5.4 Overview on authorisations in EU Member States

PYDIFLUMETOFEN (SYN545974) is new active substance.

Level 2

PYDIFLUMETOFEN

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

Summary of methodology proposed by the applicant for literature review and for all sections

A literature review was carried out by the applicant for Pydiflumetofen (SYN545974) and its relevant metabolites according to Article 8(5) of Regulation (EC) No 1107/2009. The review itself is in accordance with the EFSA Guidance document as published in EFSA Journal 2011; 9(2):2092 and covers the last ten years before the submission of the dossier (April 2016).

The exact search strategy is detailed in the document MCA Section 9, submitted by the applicant in its dossier, but a general summary of the methodology employed is given below.

1. A very broad search was conducted in 18 scientific source databases for Pydiflumetofen (SYN545974) and its metabolites (SYN545547, SYN548261, SYN547891, 2,4,6-Trichlorophenol, SYN548264, SYN547897, SYN548263, SYN547948, Hydroxylated SYN545974, NOA449410, SYN508272) according to specific criteria relevant for each section. For more details on the search criteria in each section, please refer to the document MCA Section 9.
2. Duplicates titles from between the data bases were automatically removed from the output.
3. A rapid assessment of the titles was conducted to remove any additional duplicates and any obviously irrelevant titles (where enough information was available from the title alone).
4. A further rapid assessment was conducted using summary abstracts and any clearly irrelevant titles were removed.
5. A detailed assessment of the full-text documents for the remaining titles was conducted using the criteria developed for study relevance in each section.
6. Any relevant papers were highlighted and assessed for reliability according to the criteria described by Klimisch *et al.* (1997).

An overview of the results, section by section, is summarised below.

Physical and chemical properties

Data requirement(s) captured in the search	Number PYDIFLUMETOFEN (SYN545974) Initial Search	Number PYDIFLUMETOFEN (SYN545974)	Number PYDIFLUMETOFEN (SYN545974) Specific	Number Common SDHI Metabolites Search	Total
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	1	4	2904	0	2909
Number of *summary records* excluded from the search results after rapid assessment for relevance**	1	4	2904	0	2909
Total number of *full-text* documents assessed in detail*	0	0	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

** aligned with EFSA Journal 2011; 9(2) 2092: rapid assessment means exclusion of "obviously irrelevant records"

based on titles.

All of the references were excluded from the rapid assessment, as no external research has been published on the parent molecule Pydiflumetofen (SYN545974), the specific environmental metabolites (SYN545547 and SYN548261) and the common SDHI environmental metabolite (NOA449410). The Pydiflumetofen specific metabolite search which returned many thousands of hits did not contain any of the Pydiflumetofen metabolites found in the environment, rather many hits for common classes for chemistry e.g. trichlorophenols. Therefore no full text was assessed and no studies were identified as potentially relevant for this submission.

Toxicology

Data requirement(s) captured in the search	**Number PYDIFLUMETOFEN (SYN545974) (Initial**	**Number PYDIFLUMETOFEN (SYN545974)**	**Number Specific Metabolites Search**	**Number Common SDHI Metabolites Search**	**Total**
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	1	17	4072	1	4091
Number of *summary records* excluded from the search results after rapid assessment for relevance**	1	17	3836	1	3854
Total number of *full-text* documents assessed in detail*	0	0	236	0	236
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	180	0	180
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	56	0	56

*both from bibliographic databases and other sources of peer-reviewed literature

** aligned with EFSA Journal 2011; 9(2) 2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

No external research has been published on the parent molecule Pydiflumetofen (SYN545974) or the common SDHI metabolites (NOA449410 and SYN508272) found as metabolites in livestock. The Pydiflumetofen (SYN545974) specific metabolite search which returned many thousands of hits did not contain any of the Pydiflumetofen metabolites, with the exception of 2,4,6-trichlorophenol (2,4,6-TCP) (a metabolite of PYDIFLUMETOFEN (SYN545974) identified in rats, mice and livestock commodities). However, rather many of the hits were for the common class of chemistry e.g. trichlorophenols and the research often included trichlorophenols other than 2,4,6-TCP. Only trichlorophenol research specifically on 2,4,6-TCP was considered potentially relevant for this submission and other trichlorophenol data was not assessed. Following exclusion of references from the rapid assessment, the full text was assessed from the remaining 236 titles which were identified as potentially relevant or unclear on the basis of their titles and/or abstracts identified 59 of the studies as potentially relevant for this submission of Pydiflumetofen. The following paper was not identified in the literature search, but was included as it is an important and reliable publication: *Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K, Tsuda S. The Comet Assay with Multiple Mouse Organs: Comparison of Comet Assay Results and Carcinogenicity with 208 Chemicals Selected from the IARC Monographs and U.S. NTP Carcinogenicity Database"Critical Reviews in Toxicology. (2000 Nov) 30:629-799. Journal code: CRT. ISSN: 1040-8444*. Full details of this literature review is presented in Document MCA Section 9 and reviewed in Volume 3 CA-B6.

Metabolism and Residu

Data requirement(s) captured in the search	Number PYDIFLUMETOFEN (SYN54597	Number PYDIFLUMETOFEN (SYN54597	Number PYDIFLUMETOFEN (SYN54597	Number Common SDHI Metabolites Search	Total
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	1	51	5844	3	5899
Number of *summary records* excluded from the search results after rapid assessment for relevance**	1	51	5844	3	5899
Total number of *full-text* documents assessed in detail*	0	0	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

** aligned with EFSA Journal 2011; 9(2) 2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

All of the references were excluded from the rapid assessment, as no external research has been published on the parent molecule Pydiflumetofen, the Pydiflumetofen specific metabolites and the Pydiflumetofen common SDHI metabolites. The Pydiflumetofen specific metabolite search which returned many thousands of hits did not contain any of the Pydiflumetofen metabolites relevant to metabolism and residues, rather many hits for common classes for chemistry e.g. trichlorophenols. Therefore no full text was assessed and no studies were identified as potentially relevant for this submission of PYDIFLUMETOFEN (SYN545974).

Environmental Fate

Data requirement(s) captured in the search	Number PYDIFLUMETOFEN (SYN54597	Number PYDIFLUMETOFEN (SYN54597	Number PYDIFLUMETOFEN (SYN54597	Number Common SDHI Metabolites Search	Total
Total number of *summary records* retrieved after *all** searches of peer-reviewed literature (excluding duplicates)	3	125	9796	7	9931
Number of *summary records* excluded from the search results after rapid assessment for relevance**	3	125	9796	7	9931
Total number of *full-text* documents assessed in detail*	0	0	0	0	0
Number of *studies* excluded from further consideration after detailed assessment for relevance	0	0	0	0	0
Number of *studies* not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	0	0	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

** aligned with EFSA Journal 2011; 9(2) 2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

All of the references were excluded from the rapid assessment, as no external research has been published on the parent molecule Pydiflumetofen (SYN545974), the Pydiflumetofen specific environmental metabolites (SYN545547 and SYN548261) and the Pydiflumetofen common SDHI environmental metabolite (NOA449410). The Pydiflumetofen specific metabolite search which returned many thousands of hits did not contain any of the PYDIFLUMETOFEN (SYN545974) metabolites found in the environment, rather many hits for common classes for chemistry e.g.

trichlorophenols. Therefore no full text was assessed and no studies were identified as potentially relevant for this submission of Pydiflumetofen.

Ecotoxicology

Data requirement(s) captured in the search	**Number PYDIFLUMETOFEN (SYN545974) Initial Search**	**Number PYDIFLUMETOFEN (SYN545974) Top-Up Search**	**Number PYDIFLUMETOFEN (SYN545974) Specific Metabolites Search**	**Number Common SDHI Metabolites Search**	**Total**
Total number of *summary records*	2	31	3465	4	3502
Number of *summary*	2	31	3465	4	3502
Total number of	0	0	0	0	0
Number of *studies*	0	0	0	0	0
Number of *studies* not excluded for	0	0	0	0	0

*both from bibliographic databases and other sources of peer-reviewed literature

** aligned with EFSA Journal 2011; 9(2) 2092: rapid assessment means exclusion of "obviously irrelevant records" based on titles.

All of the references were excluded from the rapid assessment, as no external research has been published on the parent molecule Pydiflumetofen (SYN545974), the Pydiflumetofen specific environmental metabolites (SYN545547 and SYN548261) and the Pydiflumetofen common SDHI environmental metabolite (NOA449410). The Pydiflumetofen specific metabolite search which returned many thousands of hits did not contain any of the Pydiflumetofen metabolites found in the environment, rather many hits for common classes for chemistry e.g. trichlorophenols. Therefore no full text was assessed and no studies were identified as potentially relevant for this submission of Pydiflumetofen.

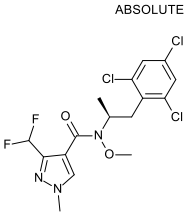
The outcomes of the review of scientific open literature and these scientific papers are discussed by the RMS in Volumes 3 of the DAR for each section.

Identity

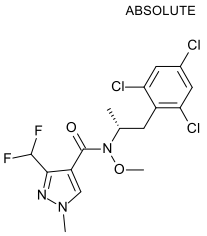
2.1.1 Summary or identity

PYDIFLUMETOFEN (SYN545974) consists of two enantiomers as a racemate mixture (50:50)

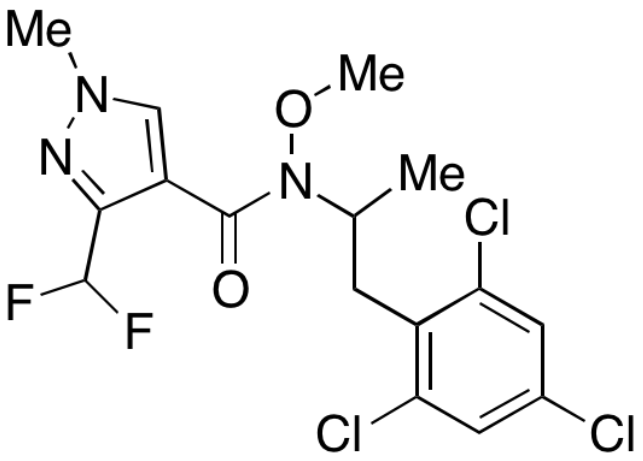
SYN546968 (S)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide



SYN546969 (R)-3-Difluoromethyl-1-methyl-1H-pyrazole-4-carboxylic acid methoxy-[1-methyl-2-(2,4,6-trichloro-phenyl)-ethyl]-amide



RAC general comment
Pydiflumetofen is a new pesticidal active substance in the scope of Regulation 1107/2009. It is a broad-spectrum fungicide for use on various field crops to control for powdery mildew (*Uncinula necator*); Septoria (*Septoria tritici*); Target spot/early blight (*Alternaria solani*); Scab (*Venturia pyrina*) & Grey mould (*Botrytis cinerea*). The active substance is composed of two enantiomers (the S-isomer and R-isomer, present in a 1:1 ratio) marketed as a racemate mixture. It is a pyrazole-carboxamide fungicide that stunts fungus growth by inhibiting succinate dehydrogenase, complex II in the mitochondrial respiration chain, which in turn interferes with the tricarboxylic cycle and mitochondrial electron transport (it therefore belongs to the class of succinate dehydrogenase inhibitors or SDHI fungicides). It interferes with several key fungal life functions, including mycelial growth and conidium germination. It has no current entry in Annex VI of the CLP regulation and all hazard classes are open for assessment in this opinion document.



The toxicological database for pydiflumetofen consists of toxicity studies currently required for hazard assessment purposes, as well as several mechanistic studies to support a proposed mode of action (MOA) for liver tumour formation in mice. The studies were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practice. The available information is considered adequate for characterising the potential health hazards associated with this active ingredient.

The DS presented the CLH report according to the new combined DAR/CLH report template to align the process of substance evaluation under Regulation 1107/2009 through EFSA while simultaneously producing an integrated CLH report to satisfy the process for CLP.

2.2 PHYSICAL AND CHEMICAL PROPERTIES [EQUIVALENT TO SECTION 7 OF THE CLH REPORT TEMPLATE]

2.2.1 Summary of physical and chemical properties of the active substance

Most of tests were performed on the pure active substance (99.5%). The technical material contains 98.5% of active substance (*see table 64*).

Table 1: Summary of physicochemical properties of the active substance

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Pure substance: White powder Technical substance: off-white powder	***O'Connor B. 2012b SYN545974_10025*** ***O'Connor B. 2012c SYN545974_10026***	Visual assessment
Melting/freezing point	112.7 °C	***O'Connor B. 2012 SYN545974_10023*** ***O'Connor B. 2012a***	Measured OECD 102

Property	Value	Reference	Comment (e.g. measured or estimated)
		SYN545974_10024	
Boiling point	NA (decompose from approximately 283°C before boiling)	***O'Connor B. 2012 SYN545974_10023*** ***O'Connor B. 2012a SYN545974_10024***	NA
Relative density	Not applicable for a solid	-	NA
Vapour pressure	1.84 x 10^{-7} Pa at 20 °C 5.30 x 10^{-7} Pa at 25 °C	***Vijayakumar C. 2012 SYN545974_10038***	Measured OECD 104
Surface tension	71.5 mN/m at 21.5 ± 0.5 °C in 90% aqueous saturated solution	***O'Connor B 2014 SYN545974_10382***	Measured OECD 115
Water solubility	1.5 mg/l at 25°C, pH +/- 6.5.	***Halarnakar R. 2012 SYN545974_10031***	Measured EC Test A.6
Partition coefficient n-octanol/water	P_{ow} = 7000 (± 220) log P_{ow} = 3.8 at 25°C	***Halarnakar R., 2012b, SYN545974_10032***	Measured OECD 107
Henry's law constant	H = 5.30.10-10 * 298.15 / 1.5 = 1.05. 10-07 kPa.m3/mol	***Vijayakumar C. 2012 SYN545974_10038***	Calculation
Flash point	Not applicable for a solid	***O'Connor B. 2012a SYN545974_10024***	NA
Flammability	Technical material is not classified as flammable solid. The test substance melted, ignited and charred but extinguished rapidly on removal of the ignition source. Combustion did not propagate along the train and the full burning time over 200mm could not be determined. Heat of decomposition of the test substance : 639 J/g	***Jackson W.A. 2016 SYN545974_10488***	Measured ASTM E537 UN Test N.1
Explosive properties	Technical material is not classified as an explosive substance. No explosions occurred and each test was suspended after a total heating period of 5 minutes. Overall, the highest pressure achieved was 456 kPa. The test substance shows no deflagration when ignited under confinement. The substance did not explode when exposed to heat, mechanical shock or friction.	***Jackson W.A. 2016 SYN545974_10488***	Measured UN Test.2 (b) & (c)
Self-ignition temperature	The substance has a melting point well below 160°C. UN Test N.4 cannot apply to this material and no conclusion can be drawn.	***Jackson W.A. 2012 SYN545974_10036*** ***Jackson W.A. 2016 SYN545974_10488***	Measured EC Test A.16
Oxidising properties	Technical material is not classified as an oxidising substance. The mean burning times of the test substance/cellulose mixtures (261s and 241s) are both greater than the mean burning time of the potassium bromate/cellulose mixtures (75s).	***Jackson W.A. 2016 SYN545974_10488***	Measured UN Test O.1

<table>
<tr><th>Property</th><th>Value</th><th>Reference</th><th>Comment (e.g. measured or estimated)</th></tr>
<tr><td>Granulometry</td><td>-</td><td>-</td><td></td></tr>
<tr><td>Solubility in organic solvents and identity of relevant degradation products</td><td>For the technical material, at 25 °C:
Acetone 220 g/l
Dichloromethane >500 g/l
Ethyl acetate 130 g/l
Hexane 0.270 g/l
Methanol 26 g/l
Octanol 7.2 g/l
Toluene 67 g/l</td><td>Halarnakar R. 2012a SYN545974_10030</td><td>Measured
Similar to CIPAC MT 157.3</td></tr>
<tr><td>Dissociation constant</td><td>There is no pK_a value within the range 2.0 to 12.0 at 25°C</td><td>O'Connor B. 2013 SYN545974_10050</td><td>NA</td></tr>
<tr><td>Viscosity</td><td>Not applicable for a solid</td><td>-</td><td>NA</td></tr>
<tr><td>Spectra (UV/VIS, IR, NMR, MS), molar extinction at relevant wavelengths, optical purity</td><td>UV:
<u>List of characteristic bands:</u>
<table>
<tr><th></th><th>Wavelength [nm]</th><th>Absorbance</th><th>Molar extinction coefficient [l / mol * cm]</th></tr>
<tr><td>neutral solution[1)]</td><td>230</td><td>0.6081</td><td>17736</td></tr>
<tr><td>neutral solution concentrated [1)]</td><td>295</td><td>0.1381</td><td>31.3</td></tr>
<tr><td>acidic solution [1)]</td><td>230
295</td><td>0.6263
0.0019</td><td>18267
59.5</td></tr>
<tr><td>basic solution [1)]</td><td>230
295</td><td>0.6120
0.0017</td><td>17850
53.2</td></tr>
</table>
IR:
<u>Table of absorption peaks:</u>
<table>
<tr><th>Wavenumber [cm^{-1}]</th><th>Assigned to</th></tr>
<tr><td>ca. 3300</td><td>N-H stretch</td></tr>
<tr><td>1625</td><td>N-C=O stretch (amide I)</td></tr>
<tr><td>1544</td><td>N-C=O stretch (amide II)</td></tr>
<tr><td>1089</td><td>C-Cl</td></tr>
</table>
^{1}H-MRN:</td><td>Heintz K. 2016 SYN545974_10172</td><td>Measured
OECD 101</td></tr>
</table>

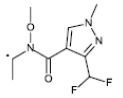
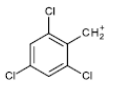
Property	Value	Reference	Comment (e.g. measured or estimated)

Table of chemical shifts:

Chemical shift [ppm]	Assignment	Number of protons
1.32 – 1.34	E	3
2.50 – 2.52	from solvent DMSO	
3.14 – 3.30	C	2
3.32	H_2O from solvent	
3.71	F	3
3.94	I	3
4.73 – 4.82	D	1
7.02 7.15 7.29	G (triplet)	1
7.61	A, B	2 (1 each)
8.27	H	1

MS:

Mass spectrum interpretation:

m/z	Fragment ion
425	$M^{+\cdot}$ (molecular ion), not visible
232	$M^{+\cdot} - 193$ 
193	
159	

Pydiflumetofen is an opaque solid in the form of a fine, non-free flowing powder. Full spectra (UV/Vis, IR, NMR, and MS) were provided. The molar extinction coefficient at λmax= 230 nm is 17736 L.mol-1.cm-1 (in neutral solution), 18267 L.mol-1.cm-1 (in acid solution) and 17850 L.mol-1.cm-1 (in basic solution). Pydiflumetofen melted at 112.7°C. No decomposition occurred below melting point. The mean vapour pressure was 1.84 x 10-7 Pa at 20°C (very low volatility). Henry's law constant (1.05 . 10-7 kPa.m3/mol) indicating a very low probability for volatilisation from water to air.

Pydiflumetofen is not soluble in water (pH6.5: > 1.5 mg/L at 20 °C). Pydiflumetofen is soluble in most of the organic solvents (ranged from 0.27 g.L-1 in hexane to >500 g.L-1 in dichloromethane). Log Po/w is 3.8 at 25°C.

According to CLP regulation, Pydiflumetofen is not flammable, not auto-flammable, not explosive and has no oxidising properties indicating that it does not create problems during transport and storage.

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

There is no classification for the physico-chemical properties (see table 1 above).

2.2.2 Summary of physical and chemical properties of the plant protection product

A19649B is an Suspension Concentrate (SC) formulation. All studies have been performed in accordance with the current requirements and the results are deemed to be acceptable. The appearance of the product is that of off-white liquid, with no particular odor. It is not explosive and has no oxidizing properties. The product is not flammable and has no flash point below 101°C. It has no self-ignition temperature below 650°C. In 1% aqueous solution, it has a pH value of 7.5 at 25°C. There is no effect of low and high temperature on the stability of the

formulation, since after 7 days at 0°C and 14 days at 54°C, neither the active ingredient content nor the technical properties were changed.

The stability data indicate a shelf life of at least 18months at ambient temperature when stored in PET and HDPE packaging. The final study should be provided when it will be finished.

Its technical characteristics are acceptable for a SC formulation.

The formulation is not classified for the physical-chemical aspect.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) does not propose classification of pydiflumetofen for physical hazards on the basis of the following results:

- Negative results in a UN Test.2 (b) & (c) study (*Jackson, 2016*) for testing the capability of pydiflumetofen to be explosive;
- Negative results in a measured ASTM E537/ UN Test N.1 study (*Jackson, 2016*) for testing the flammability of pydiflumetofen;
- Measured EC A.16 test, no self-ignition was detected;
- Measured UN Test O.1 (*Jackson, 2016*) indicated that pydiflumetofen is not oxidising.

Pydiflumetofen is an opaque solid in the form of a fine, non-free flowing powder. Pydiflumetofen melted at 112.7°C. No decomposition occurred below the melting point. The DS considered pydiflumetofen was not flammable, not auto-flammable, nor explosive and had no oxidising properties.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

Criteria for classification of physical hazards have <u>not</u> been satisfied based on the data obtained from several key studies. RAC agrees with the DS proposal for no classification for physical hazards.

2.3 DATA ON APPLICATION AND EFFICACY

2.3.1 Summary of effectiveness

A greenhouse trial on whole plants was conducted to evaluate biological activity of both enantiomers of PYDIFLUMETOFEN (SYN545974) (CSCD746374 and CSCD746375) in comparison to the racemate. The results show that CSCD746375 is slightly less active than CSCD746374 at the lowest rates tested against some targets,

but both enantiomers show relevant biological activity, contributing to the performance of the racemate PYDIFLUMETOFEN (SYN545974).

The field trials data supporting effectiveness of A19649B against these targets comprise 173 trials conducted over 2 years. The trials were undertaken by Official and/or Officially Recognized Organizations., all of which follow EPPO guidelines. Trials were conducted in the following Member States: AT, BE, BG, CZ, DE, ES, FR, GB, GR, HR, HU, IT, LT, LV, NL, PL, PT, RO, SI and SK in 2014 and 2015. These are representative of the following EPPO climatic zones according to EPPO Standard PP1/241 (1).

The results demonstrate that A19649B, containing 200 g/l PYDIFLUMETOFEN (SYN545974) as a suspension concentrate, has a good efficacy on a broad range of crops against a broad ranges of diseases which are all representative across Europe. Detailed consideration of efficacy will occur in the subsequent product authorization process when a full biological assessment dossier will be required.

2.3.2 Summary of information on the development of resistance

PYDIFLUMETOFEN (SYN545974) is a Succinate DeHydrogenase Inhibitor (SDHI). SDHI fungicides are currently classified as bearing medium to high risk by FRAC (Fungicide Resistance Action Committee). Cross-resistance within the same group is to be expected.
For the representative uses, the combined risk can be considered as moderate to high depending on the disease and the level of agronomic risk.

Considering the current knowledge about the resistance to SDHI, the following recommendations should be taken into account in the context of subsequent applications for products authorization:
- the number of applications of SDHI fungicide based products within a total disease management program must be limited and reasoned in function of the claimed use and the resistance situation to SDHI in the Member State,
- When mixtures are used for SDHI fungicide resistance management (in case of pathogen with medium to high resistance risk), applied as tank mix or as a co-formulated mixture, the mixture partner:
  - ✓ should provide satisfactory disease control when used alone on the target disease,
  - ✓ must have a different mode of action (mixture with other SDHI is not considered as appropriate for management of resistance to SDHI.

Monitoring of resistance to PYDIFLUMETOFEN (SYN545974) should be put in place from the marketing of products, in particular in case of moderate to high risk of resistance.

2.3.3 Summary of adverse effects on treated crops

Crop safety evaluations have been carried out in all efficacy trials. In addition, 13 specific crop safety trials which included N and 2N doses were performed in grapes, pome fruits (apple and pear) and tomatoes.
Taking account the results of the trials, A19649B, containing 200 g/L PYDIFLUMETOFEN (SYN545974), is a safe product for all these representative crops, even after multiple applications.

More detailed consideration on adverse effects on treated crops will be fully assessed in the context of subsequent applications for products authorization.

2.3.4 Summary of observations on other undesirable or unintended side-effects

Considering the activity of PYDIFLUMETOFEN (SYN545974) (as fungicide) and the results of crop safety trials, no negative effect is intended on succeeding crops, adjacent crops and beneficials.

However more detailed consideration on side-effects will be fully assessed in the context of subsequent applications for products authorization.

2.4 FURTHER INFORMATION

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

See Volumes 3 B-4 for the active substance and the plant protection product.

2.4.2 Summary of procedures for destruction or decontamination

See Volumes 3 B-4 for the active substance and the plant protection product.

2.4.3 Summary of emergency measures in case of an accident

See Volumes 3 B-4 for the active substance and the plant protection product.

2.5 METHODS OF ANALYSIS

2.5.1 Methods used for the generation of pre-authorisation data

Analytical methods for the determination of the active substance and by-products in the technical material

Analytical method SA-97/1 (Mink C. 2015a&b) for the determination of pydiflumetofen in technical active substance has been provided and validated according to guidance SANCO3030/99/rev.4.

Analytical method SB-97/1 (Mink C. 2015c&d) for the determination of pydiflumetofen by-products (NOA449410 (CA4312), SYN545748, NOA449410 (CA5204), SYN545547, SYN547892, SYN547893, SYN548380, SYN548385) in technical active substance has been provided and validated according to guidance SANCO3030/99/rev.4, but reagent blank solvent chromatogram should be provided to validated the specificity of the analytical method.

2.5.2 Methods for post control and monitoring purposes

Analytical methods for the determination of the active substance in the plant production product

Analytical method SF-636/1 (Voellmin S. 2013) for the determination of pydiflumetofen in plant production product has been provided and validated according to guidance SANCO3030/99/rev.4.

Analytical methods for the determination of Pydiflumetofen residues in foodstuff of plant and animal origin

Plant matrices
A QuEChERS multi-residue analytical method (Meseguer C, 2015 Syngenta File No. SYN545974_10174) and its ILV (Khan A, Merdian H, 2015 study S14-05402) using LC/MS/MS for the determination of PYDIFLUMETOFEN (SYN545974) in crops (high wet, dry, acidic, oily and coffee) were provided and fully validated with a limit of quantification of 0.01 mg/kg. Confirmatory data were provided on a second mass transition according to SANCO825/00 rev8.1.
However, a cross validation with acetonitrile:water 50:50 should be provided to validated the extraction efficiency with this solvent ratio in all type of matrices (acidic, high wet, oily and dry content commodities).

Animal matrices
An analytical multi-residue QuEChERS method and its ILV (Richter S, 2015 Syngenta File No. SYN545974_10169 and Bradford W. 2015 Syngenta File No.SYN545974_10195) were provided and fully validated for the determination of PYDIFLUMETOFEN (SYN545974) in animal matrices (fat, liver, milk, eggs and blood) with a limit of quantification of 0.01 mg/kg. Confirmatory data were provided on a second mass transition according to SANCO825/00 rev8.1.
However, an analytical method fully validated (with confirmatory data) should be provided for the determination of Pydiflumetofen (SYN545974) in muscle.

An analytical method GRM061.07A and its ILV (Mayer L, 2015c; Senciuc M, Asekunowo J, 2015 and Schlewitz P, 2015) using LC/MS/MS for the determination of 2,4,6-trichlorophenol (free and conjugates) in animal commodities (muscle, fat, kidney, milk, eggs and blood) were provided and fully validated with a limit of quantification of 0.01 mg/kg. Confirmatory data were provided on a second mass transition according to SANCO825/00 rev8.1.

Analytical methods for the determination of Pydiflumetofen residues in soil, water and air
An analytical method GRM061.04A (Lin, K., 2013 and Marin, J. E., 2013) using LC/MS/MS for the determination

of pydiflumetofen (SYN545974) in soil was provided and fully validated with a limit of quantification of 0.5 µg/kg. Confirmatory data were provided on a second mass transition according to SANCO/825/00 rev. 8.1. No other data is required.

Analytical methods GRM061.01A and its ILV (Huang S, 2013; Mayer L, 2016 and Marin J, 2013a) using LC/MS/MS were provided and fully validated for the determination of pydiflumetofen (SYN545974) in ground and surface water with a limit of quantification of 0.05 µg/L. Confirmatory data were provided on a second mass transition according to SANCO/825/00 rev.8. No other data is required.

An analytical method GRM061.11A (Göcer M, 2016; Göcer M, 2016a) using LC/MS/MS for the determination of pydiflumetofen (SYN545974) in air was provided and fully validated with a LOQ of 5.4 µg/tube (equivalent to 30 µg/m^3). Confirmatory data were provided on a second mass transition according to SANCO/825/00 rev 8.1. No other data is required.

Analytical methods for the determination of Pydiflumetofen residues in biological fluids and tissues

See animal matrices above.

2.6 EFFECTS ON HUMAN AND ANIMAL HEALTH

More detailed results of the studies are presented in Volume 3, section B.6.

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals *[equivalent to section 9 of the CLH report template]*

Table 2: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Male and female rats administered single oral dose of two radiolabelled forms of [^{14}C]-SYN545974 (5 or 1000 mg/kg bw) or a single intravenous dose of 1 mg/kg bw. PYDIFLUMETOFEN (SYN545974) (purity 99.5%). **Vehicle:** 5 mg/kg oral doses: 5% (v/v) DMSO and 0.5% (v/v) Tween 80 in 0.5% (w/v) aqueous CMC. 1000 mg/kg oral doses: 20% (v/v) DMSO and 0.5% (v/v) Tween 80 in 0.5% (w/v) CMC. The intravenous dose vehicle was 5% (w/v) DMSO in 40% (w/v) aqueous hydroxypropyl-β-cyclodextrin. **Guidelines:** OECD 417 GLP Acceptable	Following oral and intravenous administration, 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 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 PYDIFLUMETOFEN (SYN545974) to form 2, 4, 6-trichlorophenol related metabolites.	Deviations – not applicable.	***Anonymous*** (2015)
Male and female rats administered oral dose of 5, 100 (female only) or 300 (male only) mg/kg bw. PYDIFLUMETOFEN (SYN545974) (purity 98.5%) **Vehicle:** 0.5% (w/v) CMC containing 0.5% Tween 80 **Guidelines:** OECD 417 GLP Acceptable	Irrespective of radiolabel, dose or sex, following a single oral administration, the majority of dose related radioactivity was eliminated by 48 hours post dose and excretion was essentially complete by 168 hours. Absorption was limited by dose from approximately 85-90% of the 5 mg/kg bw oral dose to 19-24% at 300 mg/kg bw in males and 50-55% at 100 mg/kg bw in females. The majority of the absorbed dose was excreted in feces via bile elimination. Seven days after administration, 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 [^{14}C]-SYN545974.	Deviations – not applicable.	***Anonymous*** (2015a)
Male and female rats administered oral dose of 5, 100 (female only) or 300 (male only) mg/kg bw. PYDIFLUMETOFEN (SYN545974) (purity 98.5%) **Vehicle:** 0.5% (w/v) CMC containing 0.5% Tween 80 **Guidelines:** OECD 417 GLP Acceptable	Following a single oral dose, 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 ≤ 3.0% of the administered dose.	Deviations – not applicable.	***Anonymous*** (2015b)
Male and female rats administered oral dose of 5, 100 (female only) or 300 (male only) mg/kg bw. PYDIFLUMETOFEN	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	Deviations – not applicable.	***Anonymous*** (2015)

Method	Results	Remarks	Reference
(SYN545974) (purity 98.5%) **Vehicle:** 0.5% (w/v) CMC containing 0.5% Tween 80 **Guidelines:** OECD 417 GLP Acceptable	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 bw 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.		
Male and female rats administered oral dose of 5, 100 (female only) or 300 (male only) mg/kg bw (See ***Anonymous*** 2015a, b). PYDIFLUMETOFEN (SYN545974) (purity 98.5%) **Vehicle:** 0.5% (w/v) CMC containing 0.5% Tween 80 **Guidelines:** OECD 417 GLP Acceptable	Following a single oral administration, 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 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 bw) 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.	Deviations – not applicable.	***Anonymous*** (2015)
Male and female rats administered daily oral doses for 7 days of 3, 10, 30, 100, 300, 500 or 1000 (males only) mg/kg or a single intravenous dose at 1 mg/kg. PYDIFLUMETOFEN (SYN545974) (purity 99.5%) **Vehicle:** 0.5% (w/v) CMC containing 0.5% Tween 80; intravenous dose DMSO: 10% (w/v) aqueous hydroxypropyl-β-cyclodextrin (5:95, v/v) **Guidelines:** Not applicable GLP Acceptable	Systemic exposure increased sub-proportionally to dose in males (30-1000 mg/kg) and females (3-500 mg/kg). In males, a 33-fold increase in dose from 30-1000 mg/kg bw resulted in a 7.6-fold increase in exposure, but linearity could not be assessed below 30 mg/kg bw. In females, a 167-fold increase in dose from 3 - 500 mg/kg bw resulted in a 12-fold increase in exposure. There was negligible accumulation of PYDIFLUMETOFEN (SYN545974) observed between Days 1 and 7 at the 3 and 10 mg/kg/day doses in females, with systemic exposure to PYDIFLUMETOFEN (SYN545974) being appreciably reduced at doses greater than 10 mg/kg/day on repeat oral administration in both sexes. Systemic exposure was consistently greater in females compared to males throughout the study. No other consistent sex-related trends were observed.	Deviations – not applicable.	***Anonymous*** (2014a)
Male and female mice administered daily oral doses for 7 days of 10, 30, 100, 200, 300, 500, 750 or 1000 mg/kg bw or a single intravenous dose at 1 mg/kg bw. PYDIFLUMETOFEN (SYN545974) (purity 99.5%) **Vehicle:** 0.5% (w/v) (CMC containing 0.5% Tween 80; intravenous dose DMSO: 10% (w/v) aqueous hydroxypropyl-β-cyclodextrin (5:95, v/v) **Guidelines:** Not applicable GLP acceptable	Overall, total systemic exposure increased in a generally proportional manner on Day 1 and in a sub-proportional manner on Day 7 in males and females. However, this increase in $AUC_{(0-t)}$ was characterised by supra-proportional increase between 10 and 100-300 mg/kg bw in males and female after which a sub proportional increase in $AUC_{(0-t)}$ estimates was evident across the subsequent increasing doses. C_{max} increased sub-proportionally across the dose range on Days 1 and 7 in both sexes. Absolute oral bioavailability was very low. Systemic exposure was appreciably reduced at doses greater than 10 mg/kg bw on repeat oral administration. Clearance was less than the known combined hepatic and renal blood flow rates in mice, with PYDIFLUMETOFEN (SYN545974) indicating extensive distribution beyond the central circulation. Systemic exposure was generally comparable between sexes on Day 1. Where a trend was observed, systemic exposure was greater in female	Deviations – not applicable.	***Anonymous*** (2014b)

Method	Results	Remarks	Reference
	than in males at doses 200 to 1000 mg/kg bw. No other consistent sex-related trends were observed.		
Male and female mice administered single oral dose of 10 or 300 mg/kg bw. PYDIFLUMETOFEN (SYN545974) (purity 98.5%) **Vehicle:** CMC 0.5% (w/v) containing 0.5% Tween 80. **Guidelines:** OECD 417 GLP Acceptable	Irrespective of radiolabel, dose or sex, the majority of dose related radioactivity was eliminated by 24 hours post dose and excretion was essentially complete by 168 hours. 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 bw compared to the 10 mg/kg bw dose, suggests that nearly up to 50% of the 300 mg/kg bw dose is unabsorbed. 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. PYDIFLUMETOFEN (SYN545974) was extensively metabolised 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.	Deviations – not applicable.	***Anonymous*** (2015)

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The mammalian fate of PYDIFLUMETOFEN (SYN545974) has been assessed in studies investigating the absorption, distribution, metabolism (qualitative and quantitative) and excretion (ADME) in rats. The excretion and biotransformation of PYDIFLUMETOFEN (SYN545974) was also investigated in mice. The toxicokinetic profile of PYDIFLUMETOFEN (SYN545974) following repeat gavage, dietary or capsule dosing in rats, mice, rabbits and dogs was determined. Toxicokinetic data was used to support dose level selection for 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-^{14}C]- and [phenyl-U-^{14}C]- radiolabelled PYDIFLUMETOFEN (SYN545974) indicated that PYDIFLUMETOFEN (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.

Absorption

The oral absorption of total radioactivity from a 5 mg/kg oral gavage dose of [^{14}C]-SYN545974 was 85-90%, in male and female rats. This was estimated from the percentage of dose recovered in urine, bile and carcass (without gastrointestinal tract) from bile duct cannulated male and female rats, following single oral gavage administration of ^{14}C-SYN545974. The majority of a 5 mg/kg oral dose was systemically available, based on the urinary excretion ratio following oral and intravenous administration and the excretion profile. The oral intravenous urinary excretion ratio was 1:1, calculated from 19-28% of a 5 mg/kg bw oral dose (preliminary and main study) and 19-26% of a 1 mg/kg intravenous dose (preliminary study) excreted in urine. The excretion profile showed that less than 7.9% of a 5 mg/kg oral dose was excreted unchanged in feces and PYDIFLUMETOFEN (SYN545974) was not present in bile.

Absorption became limited as the dose increased, where following oral doses of 100 mg/kg to females and 300 mg/kg to males, absorption was 50-55% and 19-24%, respectively. At these doses, unchanged PYDIFLUMETOFEN (SYN545974) was the major component in feces, at up to 63% of the dose, but with less than 0.2% in bile. This confirms that as the oral dose increased, the absorption decreased limiting systemic exposure. In the mouse, dose limited absorption following oral administration was also evident, based on the percent of dose in excreta. Following 10 mg/kg oral administration, unchanged PYDIFLUMETOFEN

(SYN545974) was only detected in feces accounting for less than 4.4% of the dose; however, after a 300 mg/kg oral dose, parent PYDIFLUMETOFEN (SYN545974) was the major component which accounted for up to 49% of the administered dose.

The kinetic studies show that systemic exposure to PYDIFLUMETOFEN (SYN545974) increased in an approximately proportional manner after both single and repeat dose ranging from 30 to 100 mg/kg. Therefore, oral absorption is constant (85-90%) below 30 mg/kg; however, between 30 and 100 mg/kg, systemic exposure starts to increase less than proportionally to the dose, indicating the fraction of dose absorbed decreases as the dose is increased above 30 mg/kg.

Distribution

The tissue distribution of dose-related radioactivity over time was similar, irrespective of dose, label or sex, following a single oral dose of [14C]-SYN545974 to male (5 and 300 mg/kg) and female rats (5 and 100 mg/kg). 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. The depletion profile of radioactivity from all tissues appeared to mirror that observed in blood/plasma. At termination (96 or 120 h post dose), total tissue and carcass residues accounted for ≤ 3.0% of the administered dose. In a preliminary study, residues continued to decline and at seven days after a single oral dose (5-1000 mg/kg), residues of radioactivity remaining in the carcass of both males and females were ≤ 0.1% of the administered dose. At seven days, radioactivity was detected in the blood, but not reliably detected in the plasma. The highest tissue concentrations were observed in liver and to a lesser extent the kidneys. Concentrations of radioactivity in the remaining tissues were either below that observed in blood or not reliably detected.

In the mouse, excretion was essentially complete in all animals seven days post dose with 0.3% or less remaining in the carcass and gastrointestinal tract, following single oral administration of phenyl or pyrazole labelled [14C]-SYN545974 at 5 and 300 mg/kg.

Excretion

Following oral or intravenous administration of [^{14}C]-SYN545974, greater than 91% of radioactivity was eliminated by 48 hours post dose and excretion was essentially complete by 168 h, irrespective of radiolabel position, dose or sex.

The predominant route of excretion was in the feces with the majority of the absorbed dose eliminated via biliary excretion. The remainder of the dose was recovered from urine, with <0.1% of dose recovered in expired air or in the carcass. After a 5 mg/kg oral dose, up to 81% of the administered dose was excreted in bile with less than 15% recovered in feces. However, at higher doses the percentage of dose recovered in bile decreased to less than 41% in females (100 mg/kg) and 18% in males (300 mg/kg), which is consistent with limited absorption being evident. This decreased biliary excretion was associated with a concomitant increased radioactivity recovered in feces. There is also evidence of enterohepatic recirculation, with lower recovery in the urine in bile duct cannulated animals (10-15%) compared to non-cannulated animals (18-26%) administered 5 mg/kg [^{14}C]-SYN545974.

In the mouse, excretion of the administered dose was essentially complete after seven days, irrespective of dose (10 and 300 mg/kg) or radiolabel following a single oral administration of [^{14}C]-SYN545974. The majority of administered radioactivity (>87%) was excreted in the first 24 hours. The routes and rates were similar for both radiolabels and for males and females, with the majority of the dose excreted in the feces (63-79% at 10 mg/kg and 76-94% at 300 mg/kg). Urinary excretion accounted for the remainder of the dose.

Toxicokinetics

Total radioactivity

Following intravenous administration of phenyl or pyrazole labelled [^{14}C]-SYN545974 (1 mg/kg), blood concentrations steadily declined to 48 h post dose with systemic exposure comparable between radiolabels. Following a single oral administration of 5 mg/kg phenyl or pyrazole labelled [^{14}C]-SYN545974 peak whole blood and plasma concentrations (C_{max}) were observed at 0.5- 2 hours. At the higher doses (100 mg/kg in females or 300 mg/kg in males) maximum concentrations were observed at 8 hours post dose. Overall total systemic exposure was comparable between whole blood and plasma within the same dose levels and radiolabel position. Systemic exposure to total radioactivity (based on $AUC_{(0-t)}$ estimates) increased in a sub proportional manner between the 5 mg/kg and higher dose levels in whole blood and plasma for both males and females. Poor definition of a reliable terminal phase from the concentration-time profiles complicated the determination of the area under the curve; therefore, an accurate assessment of the dose fraction systemically available could not be reliably determined from the kinetics of total radioactivity in blood. The complexity of the kinetic profile of total radioactivity following oral and intravenous administration of labelled [^{14}C]-SYN545974 may be influenced by several factors, such as the extensive first pass metabolism after oral administration, the high number of metabolites produced and enterohepatic recirculation. As outlined under “Absorption” systemic availability was

determined from urinary excretion data.

Parent PYDIFLUMETOFEN (SYN545974)

Rat

The pharmacokinetics of non-radiolabelled PYDIFLUMETOFEN (SYN545974) was investigated in the rat following repeated oral or single intravenous administration of PYDIFLUMETOFEN (SYN545974). PYDIFLUMETOFEN (SYN545974) was rapidly cleared with whole blood concentrations rapidly declining to below the level of quantitation (5 ng/mL) *ca* 6-8 h in males and 8-12 h females, following intravenous administration of PYDIFLUMETOFEN (SYN545974) (1 mg/kg). Clearance was slightly higher in the male but in both sexes was characterised by a half-life of less than 2 h. The volume of distribution indicated extensive distribution of PYDIFLUMETOFEN (SYN545974).

Male rats were dosed orally by gavage with doses ranging from 3 to 1000 mg/kg. However, at 3 and 10 mg/kg, too many measured concentrations of PYDIFLUMETOFEN (SYN545974) were below the limit of quantification to reliably estimate meaningful kinetic parameters. Therefore, the dose range analysed in males for linearity of kinetics was 30-1000 mg/kg. In male rats a 33 fold increase in dose from 30 to 1000 mg/kg resulted in a 7.6 fold increase in exposure. The increase in exposure with dose was non-linear above 300 mg/kg.

In female rats, systemic exposure to PYDIFLUMETOFEN (SYN545974) was higher than male, which enabled the kinetic assessment from 3 mg/kg. As the AUC did not increase much beyond 100 mg/kg, the females were dosed only to 500 mg/kg. After a single and repeat oral administration of PYDIFLUMETOFEN (SYN545974), the increase in AUC was clearly sub-proportional from 100 mg/kg. A 167 fold increase in dose from 3 to 500 mg/kg resulted in a 12-fold increase in exposure. The increase in exposure with dose was non-linear above 100 mg/kg.

Based on the oral data, it is clearly demonstrated that the kinetics of PYDIFLUMETOFEN (SYN545974) are non-linear from 300 mg/kg in male rat and 100 mg/kg in female rat. This non-linearity arises from absorption limiting exposure as the dose increases. This pattern is also seen with the blood concentrations following dietary administration, where there is little difference between the blood concentrations from the top two dose groups (8000 or 16000 ppm) tested in the 90 day study. Therefore, the doses chosen for some repeat dose toxicology studies (2 year carcinogenicity, multi-generation reproductive and developmental toxicity studies) were 300 mg/kg for male rats and 100 mg/kg for female rats.

Mouse

The pharmacokinetics of non-radiolabelled PYDIFLUMETOFEN (SYN545974) in the mouse were also investigated, following multiple oral and single intravenous administration of PYDIFLUMETOFEN (SYN545974). In mice, following intravenous administration of PYDIFLUMETOFEN (SYN545974) (1 mg/kg), PYDIFLUMETOFEN (SYN545974) was rapidly cleared with blood concentrations rapidly declining to below the level of quantitation (5 ng/mL) at *ca* 4-6 h in males and females. Clearance was characterised by a half-life of less than 2 h. The volume of distribution indicated extensive distribution of PYDIFLUMETOFEN (SYN545974). The increase in systemic exposure was non-linear with respect to dose in males and females beyond *ca*. 100 mg/kg *i.e.* for a 10 fold increase in dose (100-1000 mg/kg) mean AUC increased between 2.1 and 3.6 fold. C_{max} increased sub-proportionally across the dose range on Days 1 and 7 in both sexes. The metabolism of PYDIFLUMETOFEN (SYN545974) was induced after repeat dosing at greater than 10 mg/kg/day.

Based on the non-proportionality of the kinetics with respect to dose, the highest dose chosen for the 80 week carcinogenicity study in mice was 300 mg/kg.

Rabbit

The toxicokinetics of non-radiolabelled PYDIFLUMETOFEN (SYN545974) were investigated from pregnant and non-pregnant[1] rabbits and an oral gavage toxicokinetic study in the pregnant rabbit. In the pregnant and non-pregnant rabbit, inter-individual variability in systemic exposure was high following oral administration. However, consistent with rat and mouse, the increase in systemic exposure with respect to dose was non-linear. In the pregnant rabbit from Days 6 to 27 of gestation, systemic exposure was characterised by a sub-proportional increase with respect to dose with no apparent increase in systemic exposure between 750 and 1000 mg/kg/day. On day 27, for a ten-fold increase in dose (100-1000 mg/kg) mean exposure increased less than two-fold. This also correlated with the systemic exposure observed on day 27 of the prenatal developmental toxicity study, where the systemic exposure resulting from a 500 mg/kg dose was also shown to be of a similar magnitude to the 750 and 1000 mg/kg data from the earlier study. There was no evidence of induction with time observed in rabbit.

Based on the non-linear kinetics of PYDIFLUMETOFEN (SYN545974) at doses >300 mg/kg, the highest dose

[1] The study in the non-pregnant rabbit was not included in this submission as it was conducted to provide preliminary data on tolerability and toxicokinetics

chosen for the prenatal developmental toxicity study was 500 mg/kg to allow for inter-individual variability.

Dog

In dogs, from the 90 day study, PYDIFLUMETOFEN (SYN545974) was rapidly cleared with mean elimination half-lives less than 5 hours. Inter-individual variability in exposure was high; however, exposure appeared to increase approximately proportionally to dose across the individuals. There was no evidence of induction or accumulation with time observed in dog. Therefore, the use of toxicokinetic data was not used to set dose levels in the 52 week dog study.

RMS consideration regarding the dose selection to be applied for toxicity studies:

The RMS had some reservations regarding the dose level selection which has been proposed by the applicant on the basis of pharmacokinetic data. First, all of the additional pharmacokinetic studies achieved to determine the TK profile of PYDIFLUMETOFEN (SYN545974) following single or repeated dose in rat, mouse, rabbit or dog 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 PYDIFLUMETOFEN (SYN545974). It is highlighted that as PYDIFLUMETOFEN (SYN545974) is extensively metabolized in rat and mouse, measured blood concentrations of parent PYDIFLUMETOFEN (SYN545974) are extremely low compared to those of metabolites and especially 2,4,6 TCP[2]. Indeed, 2,4,6 TCP is the major circulating metabolite after administration of PYDIFLUMETOFEN (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 PYDIFLUMETOFEN (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 PYDIFLUMETOFEN (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 PYDIFLUMETOFEN (SYN545974) kinetics means that systemic exposure (based on AUC(0-t) estimates) 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 highest dose levels selected by the applicant for the long-term and reproductive toxicity studies. This is confirmed both by the available toxicokinetic and toxicity studies performed with PYDIFLUMETOFEN (SYN545974). Indeed, comparison between plasma AUCs determined after administration in rat of phenyl radiolabeled PYDIFLUMETOFEN (SYN545974) (which permits a follow-up of PYDIFLUMETOFEN (SYN545974) and all its phenyl metabolites including 2,4,6 TCP) at dose levels up to 1000 mg/kg/day, showed that systemic exposure still increases beyond 100 or 300 mg/kg/day: 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 (see details in Volume 3 B.6.1). This is also confirmed by the short-term repeated studies where an increase in toxicity (liver and body weight effects) was observed with increasing doses beyond the maximal doses selected by the applicant for the long-term or reproductive toxicity studies. Thus, the RMS is of opinion that the doses of PYDIFLUMETOFEN (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, high systemic exposures of PYDIFLUMETOFEN (SYN545974) (and consequently 2,4,6 TCP) resulting of dose administration higher than 300 mg/kg have not been tested in the rat long-term study in rat where no tumors were observed (***Anonymous*** 2015; section B.6.5). This is of concern as another long-term toxicity study (NCI 1979; see Volume 3 B.6.8.1) showed that 2,4,6 TCP elicited leukemias in male rats from a dose of 250 mg/kg bw/day.

In this context, a rationale was proposed by the RMS in section 2.6.10.1 in order to verify that the ADI based on the toxicological data-package performed on PYDIFLUMETOFEN (SYN545974), can be considered as sufficiently protective regarding the carcinogenic potential of its major circulating metabolite, 2,4,6 TCP.

Biotransformation

In rat, following a single oral administration of PYDIFLUMETOFEN (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. In both rat and mouse, the major metabolites present were qualitatively and quantitatively similar irrespective of dose and sex. PYDIFLUMETOFEN (SYN545974) was extensively metabolised in rat and mouse via demethylation, hydroxylation, and dechlorination together with glucuronide and sulphate conjugates with the potential for multiple isomers within most types, an overview of the Syngenta codes, structures and species identified in is included in Appendix 1. The molecule also cleaves at the benzylic carbon to yield 2,4,6-trichlorophenol (2,4,6-TCP) and SYN548263, which were further metabolised via direct glucuronidation and

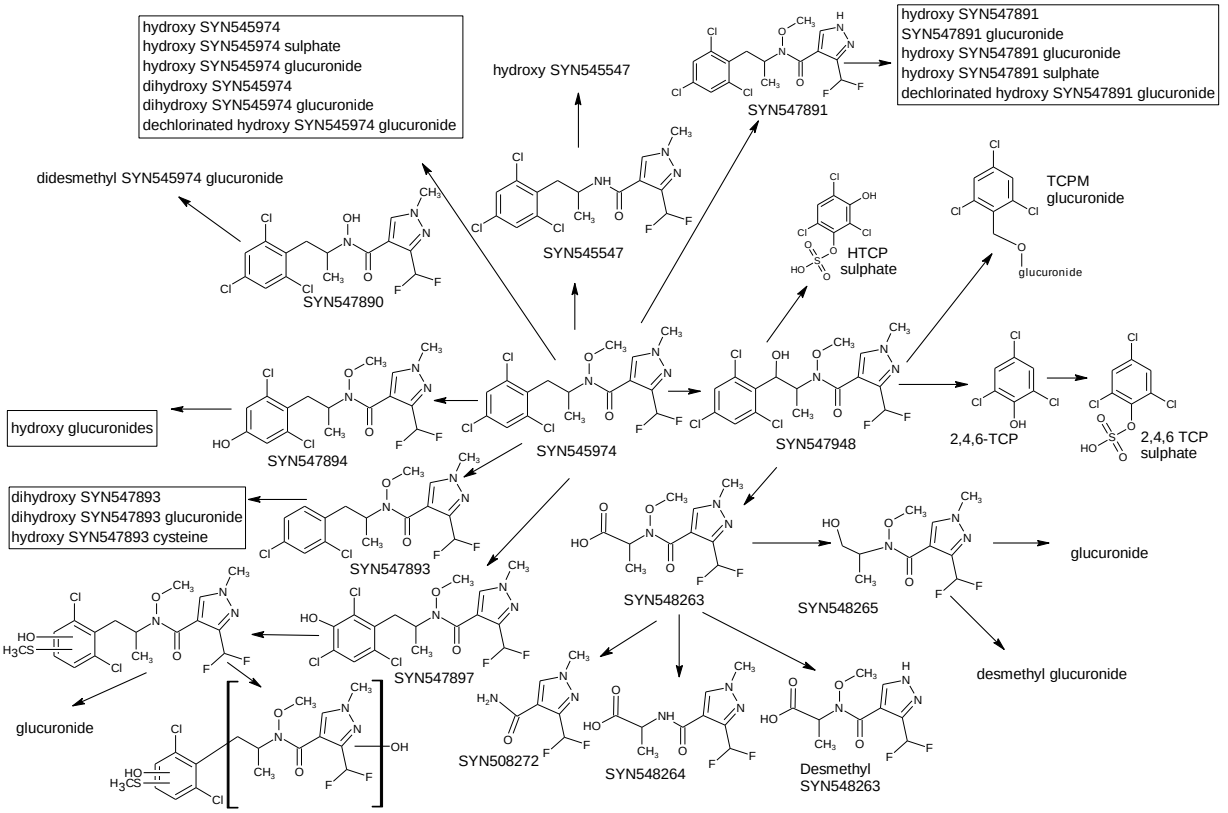
[2] From here on in 2,4,6-TCP refers to "2,4,6-TCP and its related metabolites particularly hydroxyl TCP sulphate and 2,4,6-TCP sulphate", due to the rapid conjugation of 2,4,6-TCP *in vivo*.

sulphation and also following hydroxylation and sulphation to 3-hydroxy-TCP sulphate. In rat, of the absorbed dose, only 2,4,6-TCP sulphate and SYN548263, individually accounted for >10% of the administered dose in excreta.

The biotransformation proceeded by:

- Hydroxylation to SYN547897, SYN547948 and other hydroxylated and dihydroxylated isomers
- Demethylation to SYN547890 and N-desmethyl SYN547890.
- Demethoxylation to SYN545547, followed by subsequent hydroxylation to hydroxy SYN545547.
- Hydroxylation and demethylation to hydroxy SYN547891 and other desmethyl hydroxy metabolites.
- Cleavage of PYDIFLUMETOFEN (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

Figure 1: Biotransformation Pathway Based on Identified Metabolites of PYDIFLUMETOFEN (SYN545974) in Rat



HTCP = hydroxyl 2,4,6-TCP; TCPM – TCP methanol

2.6.2 Summary of acute toxicity

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 3: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral OECD 425 GLP Acceptable	Rat, CRL:(WI) Wistar Female 3/group	PYDIFLUMETOFEN (SYN545974) (purity: 98.5% w/w) Vehicle: 0.5% CMC (w/v)	5000 mg/kg bw Single oral dose (gavage) 14 day post dose observation	> 5000 mg/kg No deaths, minor clinical signs of toxicity (slight decreased activity in one animal)	***Anonymous*** (2012)

Table 4: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 5: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Acute oral neurotoxicity study OECD Guideline 424 GLP Acceptable Rat Han-Wistar (RccHan™:WIST) 10/ sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100 (females only), 300 (males only), 1000 or 2000 mg/kg Single oral (gavage) dose Vehicle: 1% CMC (w/v)	1/10 females at 1000 mg/kg bw showed marked clinical signs and was euthanized ~3.25 hours post dose	LD_{50} > 2000 mg/kg bw	***Anonymous*** (2015a)
Acute oral neurotoxicity study (modified females only) OECD Guideline 424 GLP Acceptable Rat Han Wistar (RccHan™:WIST) 10/females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100, 300 or 1000 mg/kg Single oral (gavage) dose Vehicle: 1% (w/v) CMC	No mortality at any dose level	LD_{50} > 1000 mg/kg bw (highest dose tested)	***Anonymous*** (2015b)

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

In an acute oral toxicity study (***Anonymous***, 2012), no deaths occurred and minor clinical signs of toxicity (slight decreased activity in one animal) were reported at a dose of 5000 mg/kg bw. All animals were symptom free from 4 hours after the treatment. There were no treatment related body weight changes or macroscopic observations at necropsy. The acute oral median lethal dose (LD_{50}), was greater than 5000 mg/kg bw (limit dose) in female rats.

In acute neurotoxicity studies (***Anonymous*** 2015a, b) a single female out of 20 dosed at 1000 mg/kg bw was killed due to the severity of clinical signs but there were no deaths at 2000 mg/kg bw. These data are consistent with an LD_{50} > 2000 mg/kg bw.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

The acute oral median lethal dose was in excess of the upper cut-off criterion of 2000 mg/kg bw.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

According to the CLP criteria, no classification is required for acute oral toxicity.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 6: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute dermal OECD 402 GLP Acceptable	Rat, CRL:(WI) Wistar Male & Female 5/sex/group	PYDIFLUMETOFEN (SYN545974) (purity: 98.5% w/w) Vehicle: none	5000 mg/kg bw 24 hour application period followed by 14 days observation	> 5000 mg/kg Decreased activity in 10/10 animals on Day 1	***Anonymous*** (2013)

Table 7: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 8: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

No mortality was observed in an acute dermal toxicity study at 5000 mg/kg bw (***Anonymous***, 2013). Decreased activity was seen in 10/10 animals on Day 1. There were no treatment related effects on body weight and there were no treatment related macroscopic observations at necropsy. The median lethal dose of PYDIFLUMETOFEN (SYN545974) was > 5000 mg/kg bw in male and female rats.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

As no mortality was observed at 5000 mg/kg bw the data do not meet the criteria for classification and labelling.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

According to the CLP criteria, no classification is required for acute dermal toxicity.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 9: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
Acute inhalation OECD 403 GLP Acceptable	Rat CRL: (WI) Wistar 2/sex – prelim 5/sex – main study	PYDIFLUMETOFEN (SYN545974) (purity: 98.5% w/w) Aerosolised powder MMAD 3.54µm $\pm$ 2.32 (GSD)	Achieved atmospheric concentration : 5.11 mg/L Single 4 hour exposure	> 5.11 mg/L	***Anonymous*** (2013)

Table 10: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 11: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation study (***Anonymous***, 2013) 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 is therefore considered to be greater than 5.11 mg/L.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

As the acute LC_{50} was > 5.11 mg/L (dust) the data do not meet the criteria for classification and labelling.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

According to CLP criteria, no classification id required for acute inhalation toxicity

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of pydiflumetofen with acute oral toxicity based on one negative study performed with Wistar female rats according to GLP and OECD TG 425 (*Anon., 2012*). LD_{50} > 5000 mg/kg bw. Two acute neurotoxicity studies performed according to GLP and OECD TG 424 (*Anon. 2015a, b*) were also assessed: a single female out of 10 dosed at 1000 mg/kg bw was killed due to the severity of clinical signs (no deaths amongst males, *Anon. 2015a*), with no deaths at the high dose of 2000 mg/kg bw. In the second acute neurotoxicity study (*Anon. 2015b*) there were no deaths up to the maximum tested dose of 1000 mg/kg/day. These data are consistent with an LD_{50} > 2000 mg/kg bw and > 1000 mg/kg/day respectively.

The DS proposed no classification of pydiflumetofen for acute dermal toxicity on the basis of no lethalities at the limit dose (5000 mg/kg bw) in a GLP and OECD guideline 402 study (*Anon. 2013*; semi occlusive, 24 hour exposure to 5 male and 5 female CRL:(WI) rats, followed by a 14-day observation period).

The DS proposed no classification for acute inhalation toxicity. In an OECD 403 acute inhalation study (*Anon, 2013*), groups of 5 CRL: (WI) Wistar strain rats/sex/dose were nose-only exposed for 4 h to a dust aerosol of pydiflumetofen at a concentration of 5.11 ± 0.18 mg/L (gravimetrically determined). One female was found dead after exposure. Diffuse, dark red discoloration of the non collapsed lungs were seen in this rat at necropsy, the relationship of these findings to treatment was uncertain. The particle size of the test atmosphere was 3.54 µm MMAD.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

(1) Acute oral toxicity: The oral LD_{50} of > 2000 mg/kg bw for rats is above the value for classification according to CLP guidance.

(2) Acute dermal toxicity: The dermal LD_{50} of > 5000 mg/kg bw for rats is above the value for classification according to CLP guidance.

(3) Acute inhalation toxicity: The 4 h inhalation LC_{50} of > 5.11 mg/L for rats is above the value for classification in the CLP Regulation (i.e. 5 mg/L dust).

RAC considers that the substance **does not warrant classification for acute toxicity** via the oral, dermal and inhalation routes.

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 12: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Acute skin irritation OECD 404 GLP acceptable	Rabbit New Zealand White 3 males	PYDIFLUMETOFEN (SYN545974) (purity: 98.5% w/w)	0.5g applied to shorn flank, moistened with water. 4 hour application (semi occlusive)	No skin reaction in 3/3 animals. No clinical signs in 3/3 animals up to 72 hours post patch removal. **Mean scores / animal** (24, 48 and 72 hours) Erythema: 0, 0, 0; Oedema: 0, 0, 0 Non –irritating to skin.: (P.I.I = 0.00)	***Anonymous*** (2012a).

Table 13: Summary table of human data on skin corrosion/irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 14: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In a primary dermal irritation study in rabbits (***Anonymous***, 2012a) no local dermal signs were observed in the treated animals throughout the study. The primary irritation index was 0.00.

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

As there was no evidence of skin reaction (mean scores for erythema and oedema 0) the data do not meet the criteria for classification and labelling.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

According to CLP criteria, no classification is required for skin corrosion/irritation

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS described a primary dermal irritation study (GLP, OECD TG 404, *Anon, 2012*) where young adult New Zealand white rabbits (3 males) were exposed to 0.5 g pydiflumetofen, 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. No clinical signs were observed in the animals during the study and no mortality occurred. No local dermal clinical signs were observed in the treated animals throughout the study. The primary irritation index (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) was 0.00 and no corrosive effects were noted on the treated skin of any animal at any of the observation intervals.

Mean scores / animal (24, 48 and 72 hours)

Erythema: 0, 0, 0;

Oedema: 0, 0, 0

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There was no evidence of a skin reaction in any of the treated animals (mean scores for erythema and oedema 0), therefore the data **do not meet the criteria for classification and labelling for skin corrosion/irritation**.

Supplemental information - In depth analyses by RAC

Table 6.2.4-1 from the latest (Sept 2018) peer review of the PPP DAR provides individual and mean skin irritation scores for pydiflumetofen.

***Table 1**: Individual and mean skin irritation scores according to the Draize scheme (DAR table 6.2.4-1)*

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**

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 15: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
Acute eye irritation OECD 405 GLP	Rabbit New Zealand White 3 male	PYDIFLUMETOFEN (SYN545974) (purity: 98.5% w/w)	0.1 g instilled in left eye. Control – untreated right eye. Single exposure	An Initial Pain Reaction (IPR) score of 2 was observed in all animals. At 1 hour, discharge was observed in one rabbit and conjunctival redness was seen in all rabbits. Conjunctival redness was seen in one rabbit at 24 and 48 hours after treatment. **Mean Scores / animal** (24, 48 and 72 hours) Cornea:- 0, 0, 0 Iris - 0, 0, 0 Conjunctiva: redness - 0, 0, 0.67 Conjunctiva: chemosis - 0, 0, 0 All symptoms had fully reversed by 72 hours	***Anonymous*** (2012b)

Table 16: Summary table of human data on serious eye damage/eye irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 17: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

In a primary eye irritation study (***Anonymous***, 2012b) minor, transient signs of ocular irritation were observed. At the 1 hour 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. No clinical signs of systemic toxicity were observed in the animals during the study.

Mean scores for corneal opacity, iritis and chemosis were 0 in all animals. Mean score for conjunctival redness (after 24 to 72 hours) was 0 in two rabbits and 0.67 in one rabbit.

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

As mean scores in all animals were considered negative (corneal opacity < 1; iritis < 1; conjunctival redness < 2; chemosis < 2) the data do not meet the criteria for classification and labelling.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

According to CLP criteria, no classification is required for serious eye damage/eye irritation

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

In a GLP, OECD TG 405 (2002) compliant primary eye irritation study (*Anon., 2012b*), minor, transient signs of ocular irritation were observed. At 1 hour after treatment, 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. No clinical signs of systemic toxicity were observed in the animals during the study.

Mean scores for corneal opacity, iritis and chemosis were 0 in all animals. The mean score for conjunctival redness (after 24 to 72 hours) was 0 in two rabbits and 0.67 in one rabbit.

The DS did not propose classification.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

The mean scores in all animals were negative (corneal opacity < 1; iritis < 1; conjunctival redness < 2; chemosis < 2). The data **do not meet the criteria for classification of serious eye damage/irritation**.

Supplemental information - In depth analyses by RAC

Table 6.2.5-1 from the latest (Sept 2018) peer review of the PPP DAR provides individual and mean eye irritation scores for pydiflumetofen.

***Table 2**: Eye irritation scores according to the Draize scheme (DAR table 6.2.5-1)*

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**

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 18: Summary table of animal studies on respiratory sensitisation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results**	**Reference**
No relevant studies					

Table 19: Summary table of human data on respiratory sensitisation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 20: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

There is no evidence from single or repeated dose animal studies or from occupational monitoring that PYDIFLUMETOFEN (SYN545974) has any potential to cause respiratory sensitisation.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

No evidence of specific hypersensitivity in workers. The Occupational Health group of Syngenta has maintained a data base of incidents involving chemical exposure of workers since 1983. From 1994 data has been collected from all our manufacturing, formulation and packing sites around the world. A query of the Syngenta internal database in June 2015 for PYDIFLUMETOFEN (SYN545974) produced zero records of adverse health effects reported during active ingredient manufacture, subsequent formulation and field trials.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There were no specific studies performed with pydiflumetofen. The DS commented that there was no evidence from single or repeated dose animal studies or from occupational monitoring (during active ingredient manufacture, subsequent formulation and field trials) that pydiflumetofen had any potential to cause respiratory sensitisation.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

There is no evidence or data on respiratory sensitisation. **RAC concludes on no classification due to lack of data.**

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 21: Summary table of animal studies on skin sensitisation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Local lymph node assay OECD 429 GLP Acceptable	Mouse CBA/J Rj Female 5/group	PYDIFLUMETOFEN (SYN545974) (purity: 98.5%) Vehicle: AOO (acetone:olive oil 4:1 v/v)	50, 25 or 10% (w/v) Negative control (AOO) Positive control : 25 % (w/v) α-Hexylcinnamaldehyde (HCA) in acetone:olive oil (AOO) 4:1 (v/v). Dermal application on days 1, 2 & 3. Day 6, lymph nodes removed at termination for	No skin sensitisation potential. No irritancy at application site. Test item precipitate observed on ears after treatment at 50% (w/v) on days 1-3 and after treatment at 25% on days 2-3. **Stimulation index values** : 50% (w/v) - 1.0, 25% (w/v) - 1.1 10% (w/v) - 1.1	***Anonymous*** (2013).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
			measurement of cell proliferation.		

Table 22: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 23: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

Skin sensitisation potential was assessed in a mouse Local Lymph Node Assay (***Anonymous*** 2013). No mortality or signs of systemic toxicity was observed during the study. There were no indications of any irritancy at the site of application. 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 indicating that PYDIFLUMETOFEN (SYN545974), when tested in a suitable vehicle, was shown to have no skin sensitisation potential (non-sensitizer) in the Local Lymph Node Assay.

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

As no evidence of skin sensitisation (stimulation index < 3) was observed in an appropriate Local Lymph Node Assay in the mouse the data do not meet the criteria for classification and labelling.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

According to CLP criteria, no classification is required for skin sensitization

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Skin sensitisation potential was assessed in a GLP and OECD TG 429 (2010) compliant, mouse Local Lymph Node Assay (*Anon., 2013*) using groups of five female CBA/J Rj mice. The criterion for a positive response is 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) pydiflumetofen preparations in acetone:olive oil 4:1 (v:v). A positive control group received 25% α-Hexylcinnamaldehyde (HCA) in the same vehicle mixture.

No mortality or signs of systemic toxicity were observed during the study. There were no indications of any irritancy at the site of application. 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, indicating that pydiflumetofen was shown to be a non-sensitiser in the Local Lymph Node Assay. In the positive control group, α-Hexylcinnamaldehyde induced a positive response

with a stimulation index of 7.4, confirming the validity of the protocol used in this study.

The DS did not propose classification for skin sensitisation.

Comments received during public consultation

No comments received.

Assessment and comparison with the classification criteria

As no evidence of skin sensitisation was observed in the Local Lymph Node Assay in the mouse (i.e. the data gave a stimulation index < 3), the criteria for classification according to CLP were not met. RAC concludes that **no classification for skin sensitization is warranted**.

2.6.2.8 Phototoxicity

Table 24: Summary table of studies on phototoxicity

Method, guideline, deviations[1] if any	Test substance	Dose levels duration of exposure	Results	Reference
In vitro 3T3 NRU phototoxicity test OECD 432 GLP Acceptable	PYDIFLUMETOFEN (SYN545974) (purity: 98.5%) Vehicle: DMSO/EBSS 1:100 ratio	125.00; 39.53; 12.50; 3.95, 1.25; 0.40; 0.13, 0.04 µg/mL Negative control : 1% DMSO in EBSS 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).	Cytotoxic effect with and without irradiation. EC50 (-UVA) = 41.66 µg/mL EC50 (+UVA) = 24.56 µg/mL PIF = 1.7	***Anonymous*** 2015

Table 25: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 26: Summary table of other studies relevant for phototoxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

The phototoxicity potential of PYDIFLUMETOFEN (SYN545974) was analysed using an *in vitro* 3T3 NRU test in a GLP study, conducted to current OECD Test Guideline No. 432 (Gehrke 2015). In this study, PYDIFLUMETOFEN (SYN545974) showed a cytotoxic effect with and without irradiation. As the photo-

irritation-factor (PIF) is < 2, it is concluded that PYDIFLUMETOFEN (SYN545974) has no phototoxic potential.

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 27: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

PYDIFLUMETOFEN (SYN545974) is a solid and no studies have been conducted to assess aspiration hazard.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

As PYDIFLUMETOFEN (SYN545974) is a solid there is no risk of aspiration.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

No classification

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

It should be noted that there is no specific section corresponding to the hazard class STOT SE in Volume 3. For more detailed data on toxicity after single exposure, please refer to Volume 3, section B.6.2 and/or B.6.7. For neurotoxicity studies, please refer also to Volume 1 Level 2, section 2.6.7.

Table 28: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference

Acute oral neurotoxicity study OECD Guideline 424 GLP Acceptable Rat Han-Wistar (RccHan™ WIST) 10/ sex/group (see also section 2.6.7)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100 (females only), 300 (males only), 1000 or 2000 mg/kg Single oral (gavage) dose Vehicle: 1% CMC (w/v)	**100 mg/kg bw (females)** No differences from control. **1000 mg/kg bw (females)** 1/10 females at 1000 mg/kg showed marked clinical signs and was euthanized ~3.25 hours post dose *Clinical signs at 6 hours post dose:* recumbency 1/9, piloerection 4/9, reduced activity 2/9, abnormal gait 1/9, skin cold to touch 1/9; pupillary reflex impaired 1/9 and mydriasis1/9; ↓ 2.6% body temperature; ↓ decrease in locomotor activity (48%, 66% in mean distance travelled and number of rearings) *Clinical signs after 1 day:* No differences from control. **2000 mg/kg bw (females)** *Clinical signs at 6 hours post dose only:* Hunched posture 2/10, piloerection 4/10, reduced activity 1/10, abnormal gait 1/10; ↓ 3.1% body temperature; ↓ decrease in locomotor activity (59%, 81% in mean distance travelled and number of rearings, respectively) *Clinical signs after 1 day:* No differences from control. No treatment–related histopathological findings. No treatment-related effects observed in males. **NOAEL General toxicity and neurotoxicity: 2000 mg/kg (males) / 100 mg/kg (females)**	***Anonymous*** (2015a)
Acute oral neurotoxicity study (modified females only) OECD Guideline 424 Acceptable Rat Han Wistar (RccHan™: WIST) 10/ females/group (see also section 2.6.7)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100, 300 or 1000 mg/kg Single oral gavage dose Vehicle: 1% CMC (w/v)	**100 mg/kg bw** *Clinical signs* ~ 2-5 hours post dose: in 2/10 animals the following were observed; ruffled fur, eyes half closed and ventral recumbency. *Clinical signs* 6 hours post dose*:* piloerection 1/10, skin cold to touch 2/10; impaired extensor thrust reflex 2/10; ↓ 0.8% body temperature; ↓ decrease in locomotor activity 4%, in mean distance travelled (no difference in number of rearings) **300 mg/kg bw** *Clinical signs* 6 hours post dose*:* ↓ 1.1% body temperature; ↓ decrease in locomotor activity (26%, 37% in mean distance travelled and number of rearings) **1000 mg/kg bw** *Clinical signs* ~ 3 hours post dose: in 1/10 animals the following were observed; ruffled fur, eyes half closed and ventral recumbency. *Clinical signs* 6 hours post dose: piloerection 1/10, skin cold to touch 1/10, tremor 1/10, impaired extensor thrust reflex 1/10; ↓ 1.1% body temperature; ↓ decrease in locomotor activity (28%, 41.0% in mean distance travelled and number of rearings) **NOAEL General toxicity and neurotoxicity (females):100 mg/kg**	***Anonymous*** (2015b)

Table 29: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 30: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference

No relevant studies

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

The acute neurotoxicity of PYDIFLUMETOFEN (SYN545974) has been evaluated in the rat (***Anonymous***, 2015a, 2015b). In the acute studies, single gavage doses of 0, 300 (males) or 100 (females), 1000 and 2000 mg/kg produce some clinical signs, effect on body temperature and locomotor activity (LMA) at dose levels ≥ 1000 mg/kg only in females. No effects were observed in males and no gross or histopathological findings in the central or peripheral nervous system were seen. A subsequent modified acute neurotoxicity study in female rats only, with single oral gavage doses of 0, 100, 300 and 1000 mg/kg, produce the same effects from the dose level of 300 mg/kg. All signs of toxicity were resolved by day 2. In the acute oral toxicity study, slight decreased activity in one animal were reported at a dose of 5000 mg/kg bw.

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed effects are considered.

According to CLP regulation and section 3.8 of the CLP guidance, STOT-SE should be considered where there is a clear evidence for specific organ toxicity especially when it is observed in absence of lethality. In single dose neurotoxicity studies in the rat clinical signs were seen within 2-6 hours of dosing at 300 mg/kg and above in females only (recumbency, piloerection, reduced activity, abnormal gait, skin cold to touch; pupillary reflex impaired and mydriasis, reduced body temperature; decrease locomotor activity). Effects observed in these studies were transient and rapidly reversible. No gross or histopathological findings in the central or peripheral nervous system were seen.

Regarding STOT SE 1 or 2, decrease in locomotor activity was observed at a dose (300 mg/kg bw) which is within the guidance value range for STOT SE 1 (C ≤ 300 mg/kg bw). However, this effect was observed without any further impact on health and was not considered by the RMS to be "more than transient in nature" (CLP guidance 3.8.2.1.7.3 (b)). In addition, evaluation of FOB information in the 90-day and 2-year toxicity studies in rats indicates an absence of such effects after test item repeated administration. Indeed, no such effect was observed in the 90-day toxicity study in rat (***Anonymous*** 2015), were functional observation battery (FOB) parameters including detailed clinical observations or on motor activity were analyzed up to 16000ppm (equivalent to 1322-1174 mg/kg bw/d in males and females; respectively). In addition, there were no treatment effects in the FOB parameters or on motor activity following administration of PYDIFLUMETOFEN (SYN545974) at doses levels up to 6000ppm in males (319 mg/kg/day) and 1500 ppm in females (102 mg/kg/day) in the 104-week toxicity study in rat (***Anonymous*** 2015a) (see section 2.6.7). Overall, it was concluded that classification of PYDILUMETOFEN (SYN545974) for STOT SE 1 or 2 is not warranted.

The hazard class STOT SE 3 should cover 'transient' narcotic effects occurring after single exposure. Although classification in Category 3 is primarily based on human data, if available, animal data can be included in the evaluation. In the CLP guidance (November 2013), it is indicated (Chapter 3.8.2.2.2) that 'narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia'. In the oral single dose studies, some symptoms were observed in rats (decrease motor activity). These symptoms occurred quickly after dosing, appeared to be unspecific and were transient in nature. Overall, it was concluded that the results from the standard acute and acute neurotoxicity studies do not indicate that there is specific organ toxicity following a single exposure: the very transient and slight reported narcotic/neurotoxic signs do not fulfil the criteria for STOT SE 3 and it was not consided that additional classification for STOT SE 1 or 2 is necessary either.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

No classification is required.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

1. Relevance of the provided information on STOT-SE

Acute Neurotoxicity in the rat

The DS described two acute neurotox studies (*Anon., 2015a, 2015b*) followed by an assessment of the evidence from supporting repeat dose studies such as the FOB (functional observation battery) information in the 90-day and 2-year toxicity studies in rats.

In the acute studies, single gavage doses of 0, 100, 300, 1000 and 2000 mg/kg produced some clinical signs at dose levels ≥ 300 mg/kg in females only. Clinical signs of neurotoxicity were seen within 2-6 hours of dosing at 300 mg/kg and above (recumbency, piloerection, reduced activity, abnormal gait, skin cold to touch; pupillary reflex impaired and mydriasis, reduced body temperature; decrease in locomotor activity). These effects were transient and rapidly reversible. No gross or histopathological findings in the central or peripheral nervous system were observed.

Other evidence

The DS found no evidence within the FOB information in the 90-day and 2-year toxicity studies in rats to support effects on locomotor activity that might warrant classification for STOT SE.

In the acute oral toxicity study, 3 female CRL:(WI) rats were given a single oral (gavage) dose of 5000mg/kg/day. Treatment caused a slight decrease in activity in one animal.

In the acute inhalation toxicity study, 10 (5 male and 5 female) CRL: (WI) Wistar strain rats, were exposed to an aerosol concentration of 5.11 mg/L pydiflumetofen. The animals were exposed for 4 hours using a nose-only exposure system. Laboured, gasping and noisy respiration, sneezing, decreased activity, prostration and ataxia were recorded for the exposed animals on the day of exposure. One female was found dead after exposure. Noisy respiration or weakness were recorded in 3 males and 3 females the day after exposure, weakness was noted in 2 males and 1 female 2 days after exposure and no significant clinical signs were observed from Day 3 until scheduled necropsy.

Conclusion of the Dossier Submitter

Overall, the DS concluded that the results from the standard acute and acute neurotoxicity studies did not indicate that there was specific organ toxicity following a single exposure. In addition, very transient and slight clinical signs reported for narcotic/neurotoxic effects did not fulfil the criteria for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Introduction

According to CLP, STOT-SE should be considered where there is a clear evidence for specific organ toxicity, especially when it is observed in absence of lethality. It is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance or mixture. All significant health effects that can impair function, reversible and irreversible, immediate and/or delayed are considered.

STOT SE 1 or 2

A decrease in female locomotor activity (LMA, distance travelled and number of rears), at 6 hrs post-dose on day 1 was observed in one of the acute neurotoxicity studies at a dose (300 mg/kg bw) which was within the guidance value range for STOT SE 1 (C ≤ 300 mg/kg bw). This effect was observed without any further impact on health and was only observed on the day of dosing. The difference in LMA relative to the control group did not attain statistical significance. No gross or histopathological findings in the central or peripheral nervous system were seen. Such effects were not observed in the 90-day toxicity study in female rats at doses up to 1174 mg/kg/day. RAC does not consider this effect sufficient for classification as STOT-SE 1 or 2.

STOT SE 3

The hazard class STOT SE 3 should cover 'transient' narcotic effects occurring after single exposure. Such narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. In the oral single dose studies, some symptoms were observed in rats (decreased locomotor activity). These clinical signs occurred rapidly after dosing, appeared to be unspecific and were transient in nature. RAC is of the opinion that the very transient and slight reported LMA signs **do not fulfil the criteria for STOT SE 3 and does not propose classification**.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE) [equivalent to section 10.12 of the CLH report template]

Table 31: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

For more detailed data on STOT RE effects please refer to Volume 3, sections B.6.3, B.6.5 B.6.6 and B.6.7

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Oral studies			
Rat			
28 day toxicity	PYDIFLUMETOF	**500 ppm (43 and 40 mg/kg/day, males and females respectively)**	***Anonymous*** (2012a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
study OECD 407 Non-GLP Acceptable Rat: Han Wistar (Crl:WI(Han)) 6/sex/group. An additional 6/sex/ group (high dose and control) were killed at days 3 and 7 for cell proliferation investigations	EN (SYN545974) (purity 98.6%) 0, 500, 4000, 8000 and 16000 ppm Actual doses 0, 43, 343, 677 and 1322 mg/kg/day (males) and 0, 40, 322, 619 and 1174 mg/kg/day (females) Continuous in the diet for 4 weeks.	*Clinical chemistry:* ↓ glutamate dehydrogenase for females *Liver weights*: ↑ covariate (13% females). **4000 ppm (343 and 322 mg/kg/day, males and females respectively** *Food consumption* : ↓ females (<42%) for the first 1 to 2 days of treatment *Clinical Chemistry:* ↓ glutamate dehydrogenase for females. *Liver weights*: ↑ absolute (male 19%, females 20%) and covariate (males 22%, females 28%). *Microscopic findings*: minimal centrilobular hepatocellular hypertrophy in 4/6 males **8000 ppm (677 and 619 mg/kg/day, males and females respectively)** *Food consumption* : ↓ females (<75%) for the first 1 to 2 days of treatment *Clinical Chemistry:* in females, ↓ glutamate dehydrogenase and ↓ alanine aminotransferase activity *Liver weights*: ↑ absolute (male 26%, females 22%) and covariate (male 31%, females 34%). *Microscopic findings*: minimal centrilobular hepatocellular hypertrophy in 5/6 males and 3/6 females. **16000 ppm (1322 and 1174 mg/kg/day, males and females respectively):** *Body weight :* ↓ BW (↓ 13% males) and BW gain (↓ 34% males, ↓ 31% females*) *Food consumption*: markedly ↓ day 1 for males (47%), and days 1-2 for females (60%) *Clinical Chemistry:* in females, ↓ glutamate dehydrogenase and ↓ alanine aminotransferase activity *Liver weights*: ↑ absolute (males 19%, females 26%) and covariate (male 34%, females 41%) *Microscopic findings:* minimal to mild centrilobular hepatocellular hypertrophy in 6/6 males and minimal hypertrophy in 5/6 females. **NOAEL 500 ppm (43 mg/kg/day males and 40 mg/kg/day females)**	
13-week dietary study in rats OECD 408, GLP Acceptable Rat:Han wistar (Crl:WI(Han)) 10/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) 0, 250, 1500, 8000 or 16000 ppm Actual doses 0, 18.6, 111, 587 and 1187 mg/kg/day (males) and 0, 21.6, 127, 727 and 1324 mg/kg/day (females) Continuous in the diet for 13 weeks.	**250 ppm (males 18.6 mg/kg/day, females 21.6 mg/kg/day)**: No effect observed. **1500 ppm (males 111 mg/kg/day, females 127 mg/kg/day)**: *Clinical Chemistry:* ↓ alkaline phosphatase activity (ALP) in males (30%) and females (41%) *Liver weights:* ↑ absolute (21% males, 16% females) and covariate (29% males, 18% females). *Histopathology*: ↑ minimal hepatocyte hypertrophy in 5/10 males. Minimal thyroid follicular cell hypertrophy in 4/10 males. **8000ppm (males 587 mg/kg/day, females 727 mg/kg/day)**: *Body weight:* ↓ 12% BW (males at week 13) and ↓ BW gain (27% males and 21% females) *Food consumption*: ↓ < ~ 50% during first 2 or 3 days for males and females respectively *Food Utilisation:* during weeks 1-4, food utilisation was ↓ 27% males and ↓ 25% females. During weeks 1-13, food utilisation was ↓ 23% for both males and females. *Clinical Chemistry:* ↓ alkaline phosphatase activity (ALP) in males (31%) and females (42%); ↑ cholesterol (35%) in females *Liver weights:* ↑ absolute (23% males, 36% females) and covariate (44% males, 41% females). *Histopathology*: ↑ minimal hepatocyte hypertrophy in 7/10 males and 6/10 females. Minimal/mild thyroid follicular cell hypertrophy in 6/10 males and 4/10 females. **16000 ppm (males 1187 mg/kg/day, females 1324 mg/kg/day)**:	***Anonymous*** (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		Body weight: ↓ 15% BW *(*males at week 13) and ↓ BW gain (34% males and 25% females) *Food consumption*: ↓ ~ 50% during first 2 or 3 days for males and females respectively *Food Utilisation:* during weeks 1-4, food utilisation was ↓ 38% males and ↓ 32% females. During weeks 1-13, food utilisation was ↓ 31% males and ↓ 25% females. *Clinical Chemistry:* ↓ alkaline phosphatase activity (ALP) in males (39%) and females (39%); ↑ cholesterol (35%) in females *Liver weights:* ↑ absolute (26% males, 40% females) and covariate (52% males, 43% females). *Histopathology*: ↑ minimal hepatocyte hypertrophy in all males and 9/10 females. Minimal/mild thyroid follicular cell hypertrophy in 7/10 males and 8/10 females. **NOAEL 250 ppm (males 18.6 mg/kg/day, females 21.6 mg/kg/day)**	
Combined chronic toxicity/carcinogenicity study OECD 453 GLP Acceptable Rat: Han Wistar Crl: WI (Han) 64/sex/group (52/sex/group plus 12/sex/group for interim kill at 12 months). (see also section 2.6.5)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) Males 0, 200, 1000 and 6000 ppm; Females 0, 150, 450 and 1500 ppm Actual dose 0, 9.9, 51.0 and 319 mg/kg/day (males) and 0, 10.2, 31.0 and 102 mg/kg/day (females) Continous in the diet for 52 or 104 weeks	Only data from the toxicity phase (52 week exposure) are presented. **200 ppm males (9.9 mg/kg/day); 150 ppm females (10.2 mg/kg/day)** No adverse effects up to week 52. **1000 ppm males (51 mg/kg/day); 450 ppm females (31 mg/kg/day)** *Body weights:* ↓ throughout the 52 week period (6% males and 7% females). *Food consumption:* reduced at times throughout, e.g. ↓ 7% males week 4, and ↓ 10% females week 18. *Food utilisation:* ↓ for males throughout 13 week period (5%). Slight decrease for females. *Organ weights:* ↑ relative liver weights (16%* males, 9%* females). *Histology:* Hepatocellular hypertrophy observed in 5/12 males. **6000 ppm males (319 mg/kg/day); 1500 ppm females (102 mg/kg/day)** *Body weights:* ↓ throughout the 52 week period (13% males, 10% females). *Food consumption:* ↓ at times throughout, e.g.males week 4, 8% females week 18. *Food utilisation:* ↓ for males throughout 13 week period (11%). Slight decrease for females. *Organ weights:* ↑ relative liver weights (38% males, 19% females). *Histology:* prominent liver lobular architecture in 3/12 males. Hepatocellular hypertrophy observed in 11/12 males and 4/10 females. **NOAEL 200 ppm males (9.9 mg/kg/day), 450 ppm females (31 mg/kg/day)**	***Anonymous*** (2015a)
Two generation reproduction OECD 416 GLP acceptable Oral (continuous in diet) Rat, Crl:WI (Han) 24/sex/group (see also sections 2.6.6, 2.6.6.1 and 2.6.6.3)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) Males: 0, 150, 750 & 4500 ppm Females: 0, 150, 450 & 1500 ppm Continuous in the diet	***Parental toxicity - Males*** 150 ppm (9.1 mg/kg/day, F0; 11.9 mg/kg/day, F1) No effects 750 ppm (46 mg/kg/day, F0; 59 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑ 9%(males) ; F1: ↑ 12%) 4500 ppm (277 mg/kg/day, F0; 364 mg/kg/day, F1) F0: ↓ body weight gain (10% weeks 0-17); ↑ liver weight adjusted for bw (↑38% males) and ↑15% females); ↑ incidence of hepatocyte hypertrophy (slight): males 19/24 (control = 0/24 incidence); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24 (control = 1/24) in males. F1: ↓ body weight gain (10% weeks 0-17); ↓ food consumption (8% weeks 0-17); ↑ liver weight adjusted for bw (↑42% males and ↑ 17% females); ↑ incidence of hepatocyte hypertrophy (slight) 18/24 (controls = 0/24); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24(controls = 2/24). ***Parental toxicity - Females*** 150 ppm: (11.9 mg/kg/day, F0; 14.1 mg/kg/day, F1) No effects	***Anonymous*** (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		450 ppm (36 mg/kg/day, F0; 42 mg/kg/day, F1) F0: ↑ liver weight adjusted for bw (↑ 6%) 1500 ppm (116 mg/kg/day, F0; 141 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑15% and F1: 19%) F0: ↑ incidence of hepatocyte hypertrophy (minimal) 8/24 (controls = 0/24) **NOAEL (parental) 750/450 ppm (46/36 mg/kg/day F0 generation pre-pairing) in males and females respectively** ***Reproductive toxicity*** No effects at any dose level **NOAEL (reproductive) 4500/1500 ppm (277/116 mg/kg/day F0 generation pre-pairing) in males and females respectively.** ***Offspring toxicity - Males*** 750 ppm (59 mg/kg/day) No effects 4500 ppm (364 mg/kg/day) F1: delayed sexual maturation (45.9 days versus 43.0 days in controls) considered secondary to ↓ body weight ***Offspring toxicity - Females*** 450 ppm (42.4 mg/kg/day) No effects 1500 ppm (141 mg/kg/day) F1: delayed sexual maturation (33.0 days versus 30.3 days in controls) considered incidental as no effect on subsequent oestrus cycling, mating performance or fertility and no effect on ano-genital distance **NOAEL (offspring) 4500/450 ppm (277/36 mg/kg /day F0 generation pre-pairing) in males and females respectively.**	
Mice			
28 day toxicity study OECD 407 Non-GLP Acceptable Mice: CD-1 (Crl:CD-1) 6/sex/group. An additional 6/sex/ group (high dose and control) were killed at days 3 and 7 for cell proliferation investigations	PYDIFLUMETOFEN (SYN545974) (purity 98.6%) 0, 500, 1500, 4000 and 7000 ppm Actual dose 0, 76, 213, 612 and 1115 mg/kg/day (males) and 0, 96, 266, 701 and 1312 mg/kg/day (females) Continuous in the diet for 4 weeks	**500 ppm (76 and 96 mg/kg/day, males and females respectively)** *Body weight :* ↓ BW (8%*) and BW gain 0-28d (55 %*) in males. *Liver weights*: ↑ absolute (9% male, 14% female) and covariate (17% males, 28% females). **1500 ppm (213 and 266 mg/kg/day, males and females respectively)** *Body weight gain:* ↓ BW (5%*) and BW gain 0-28d (16 %*) in males *Liver weights*: ↑ absolute (25% male, 23% female) and covariate (32% males, 34% females). **4000 ppm (612 and 701 mg/kg/day, males and females respectively)** *Body weight gain:* ↓ BW (6%*) and BW gain 0-28d (45 %*) in males *Liver weights*: ↑ absolute (46% males, 39% females) and covariate (55% males, 48% females). **7000 ppm (1115 and 1312 mg/kg/day, males and females respectively):** *Body weight gain*: ↓ BW (11%*) and BW gain 0-28d (80 %) in males *Clinical chemistry* : ↑ 215% triglycerides in males; ↓ 34% Phosphate *Liver weights*: ↑ absolute (52% males, 51% females) and covariate (66% males, 63% females). NB: There is no dose response for decreased bodyweight gain in males. **No NOAEL was achieved in this study. The LOAEL was 500 ppm (76 and 96 mg/kg/day, males and females respectively) based on lower bw gain in males.**	***Anonymous*** (2012b)
13 week dietary toxicity Study OECD 408	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) 0, 100, 500, 4000	**100ppm (17.5 and 20.4 mg/kg/day, males and females respectively):** No treatment related effects	***Anonymous*** (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
GLP Acceptable Mice: CD-1 (Crl:CD-1) 10/sex/group	and 7000 ppm Actual dose 0, 17.5, 81.6, 630 and 1158 mg/kg/day (males) and 0, 20.4, 106, 846 and 1483 mg/kg/day (females) Continuous in the diet for 13 weeks	**500ppm (81.6 and 106 mg/kg/day, males and females respectively):** *Liver weight:* ↑ absolute (18% males) and covariate (15% males) *Histology:* mild centrilobular hepatocyte hypertrophy in 2/10 males **4000ppm (630 and 846 mg/kg/day, males and females respectively):** *Clinical chemistry:* ↑ cholesterol (26% males, 29%* females); *Liver weight:* ↑ absolute (42% males, 60% females) and covariate (48% males, 62% females). *Histology:* mild centrilobular hepatocyte hypertrophy in 4/10 males and 6/10 females. **7000ppm (1158 and 1483 mg/kg/day, males and females respectively):** *Clinical chemistry:* ↑ cholesterol (51% males, 36% females); ↑ triglycerides (86% males, 57% females). *Liver weight:* ↑ absolute (67% males, 54% females) and covariate (75% males, 64% females). *Histology:* mild centrilobular hepatocyte hypertrophy in 5/10 males and 7/10 females **NOAEL 100/500 ppm (17.5 mg/kg/day for males 106 mg/kg/day for females)**	
Dog			
13-week oral (capsule) toxicity OECD 409, GLP Acceptable Dog: pure-breed Beagles 4/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 30, 300, 1000 mg/kg/day Capsule administration. No vehicle 13-week duration	**30 mg/kg/day:** No adverse effects. **300 mg/kg/day:** *Body weight:* Slight loss for females, weeks 1-2. *Clinical chemistry:* At week 13, ALP ↑ (males 268%, females 204%); triglycerides ↑ (males 68%) *Liver weights:* ↑ absolute (34% males) and covariate weight (31% males, 16% females). **1000 mg/kg/day:** *Body weight*: 2/4 males and all females lost weight during 1st week, resulting in overall (week 1-13) lower body weight gain for females (not statistically significant). *Food consumption:* ↓ 6% males and 17% females over 13 weeks. *Clinical chemistry:* At week 13, ALP ↑ (males 460%, females 321%); triglycerides ↑ (males 144%, females 37%). *Liver weights:* ↑ absolute (44% males, 38% females) and covariate weight (41% males, 46% females). *Histology:* minimal hepatocyte hypertrophy in all animals. **NOAEL 30 mg/ kg bw/day.**	***Anonymous*** (2015a)
52-week oral (capsule) toxicity OECD 452 GLP acceptable Dog: pure-breed Beagles 4/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 30, 100, 300 mg/kg/day Capsule administration. No vehicle 52-week duration	**30 mg/kg/day:** No treatment related effects. **100 mg/kg/day:** No treatment related effects. **300 mg/kg/day:** *Clinical chemistry:* ↑ ALP throughout the study (at week 52, males 282%, females 211%). *Liver weights:* ↑ covariate weight (34% males, 28% females). **NOAEL 100 mg/kg/day**	***Anonymous*** (2015b)
Dermal studies			
28-day dermal OECD 410, GLP	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100, 300 and	**30 mg/kg/day:** No effects **300 mg/kg/day:**	***Anonymous*** (2013)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acceptable Rat:Han wistar (RccHan™: WIST 10/sex/group	1000 mg/kg/day. Vehicle: none, moistened with de-ionised water 20 applications over 28 days	No effects **1000 mg/kg/day:** No toxicologically significant effects. *Clinical chemistry:* Males ↑ globulin (7%), total protein (3%); females ↑ in calcium (4%), phospholipids (25%) and total cholesterol (28%) **NOAEL 1000 mg/kg/day**	

* Statistical significance not reached.

Table 32: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 33: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

The short-term toxicity of PYDIFLUMETOFEN (SYN545974) has been evaluated by the oral route in rats, dogs and mice and by the dermal route in a 28-day study in the rat. In all species, the target organ was the liver (and thyroid in rats only).

Rats

In rats, the target organs were the liver and thyroid, with increase in liver weight and hypertrophy in both organs. Effects also observed after short-term administration of PYDIFLUMETOFEN (SYN545974) were a reduction in body weight gain with some minor changes in food consumption and food utilisation.

In the 28-day rat study, at doses ≥4000 ppm (~343/322 mg/kg/day in males/females respectively), liver weight increase ≥ 20% of controls and hepatocyte hypertrophy were observed. The NOAEL of the 28-day study was considered to be 500 ppm (40-43 mg/kg/day).

In the 90-day rat study, an increase in absolute and group mean covariant liver weight compared to controls of greater than 20% were observed at doses ≥ 1500 ppm (111 mg/kg/day) in males. At these dose levels, liver hepatocyte hypertrophy and thyroid follicular cell hypertrophy were also noted. In females, an increase in absolute and covariant liver weight of greater than 20% associated with hepatocyte hypertrophy and blood clinical chemistry changes were observed at doses ≥ 8000 ppm (727 mg/kg/day). At the lower dose level of 1500 ppm, an increase in liver weight (absolute and covariant) greater than 15% and a reduction in alkaline phosphatase activity were observed in females. Decreased body weight gains were also noted from the dose of 8000 ppm (587-727 mg/kg/day) in males and females. The NOAEL of the 90-day study was considered to be 250 ppm (18.6 mg/kg/day and 21.6 mg/kg/day for males and females, respectively)

After 1 year administration of PYDIFLUMETOFEN (SYN545974) to rats in the chronic toxicity study, an

increase in liver weight compared to controls of greater than 15% at doses of 1000 ppm (51 mg/kg/day) in males and 1500 ppm (102 mg/kg/day) in females, was observed. Hepatocyte hypertrophy was observed in males at ≥1000 ppm (51 mg/kg/day) and females at 1500 ppm (102 mg/kg/day). In males, the increase in liver weight was also associated with prominent liver lobe architecture at the dose of 6000 ppm (319 mg/kg/day). There were no effects on the thyroid after 1 year administration of PYDIFLUMETOFEN (SYN545974). A NOAEL was established at 200 ppm (9.9 mg/kg/day in males) and 450 ppm (31.0 mg/kg/day in females).

In the two-generation reproduction toxicity study, increases in body weight-related liver weight was seen in males given 750 ppm (46 mg/kg/day) and 4500 ppm (277 mg/kg/day), in both generations, for the P generation females given 450 ppm and for the P and F1 generation females given 1500 ppm. The liver weight increases observed at 750 ppm in males in both generations was slight (< 15%) and not associated with histological findings. Indeed, microscopic findings in the liver (diffuse hepatocyte hypertrophy) were only seen at 4500 ppm for P and F1 generation males and 1500 ppm for P generation females only. Microscopic findings in the thyroid (minimal follicular epithelial hypertrophy) were seen in males of both generations given 4500 ppm. The NOAEL for parental toxicity was considered to be 750 ppm for P and F1 generation males (46 mg/kg/day (pre-pairing)) and 450 ppm for females in the P and F1 generations (36 mg/kg/day (pre-pairing)).

In a 28 day dermal toxicity study in the rat conducted up to the limit dose of 1000 mg/kg/day, there were no indications of local effects at the application site nor were there any signs of systemic toxicity.

Mice

In the 28 day mouse study, PYDIFLUMETOFEN (SYN545974) caused a reduction in body weight gain at all doses in males over the initial week of the study, with an overall reduction in body weight gain for the duration of the study at 7000 ppm (1115 mg/kg/day). Increases in liver weight were observed in both sexes at all doses with an increase >15% from the dose of 1500 ppm, but there were no histopathology effects in the liver at any dose. No NOAEL was established in males in the 28 day study based on effects on bodyweight.

In the 90 day study, which was conducted using a similar dose range as the 28 day study, body weight gain was lower in all male groups and in females receiving 4000 ppm (846 mg/kg/day) or 7000 ppm (1483 mg/kg/day). However, in males, group mean body weight and body weight gain did not demonstrate a dose response and, as body weight gain at 500 ppm (81 mg/kg/day) was 95% of controls at day 91, effects on body weight and body weight gain at doses of 500 ppm and lower are considered incidental to treatment. In females, differences in group mean body weight and body weight gain did not achieve statistical significance and did not demonstrate a dose response and are considered incidental to treatment. Adverse effects were limited to greater than 15% increases in liver weight (absolute and covariant) associated to hepatocyte hypertrophy at doses of 500 ppm and higher in males (81.6 mg/kg/day) and at dose of 4000 ppm a higher in females (846 mg/kg/day). Changes in clinical chemistry (cholesterol and/or triglyceride) were also observed from the dose of 4000 ppm (630-846 mg/kg/day) in males and females. The NOAEL for short-term administration (90-day) in the male mouse was 100 ppm (17.5 mg/kg bw/day) and in female mice the NOAEL was 500 ppm (106 mg/kg/day).

Dog

In the 90 day dog study, there were signs of general toxicity at the top dose of 1000 mg/kg/day including slight initial body weight loss and overall lower body weight gain associated with reduced food consumption. Effects were observed in the liver in males and females: blood clinical chemistry changes (ALP and TG increase) and greater than 15% increases in liver weight (absolute and covariant) at doses of 300 and 1000 mg/kg/day and hepatocyte hypertrophy at dose of 1000 mg/kg/day. The NOAEL was established at 30 mg/kg/day. In the 1 year dog study, the same effects (blood clinical chemistry changes and increased liver weight) were observed at the highest dose of 300 mg/kg/day and the NOAEL was established at 100 mg/kg/day.

The liver weight increases observed in rats, mice and dogs have been taking into account for the NOAEL setting when this increase was >15% compared to control and associated with histopathological changes in the liver (hypertrophy) and/or significant liver blood chemical chemistry changes. However, it is noted that these findings reflect the adaptive capacity of the liver being overwhelmed by PYDIFLUMETOFEN (SYN545974).

Table 34: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/day)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Anonymous (2012a)	343/322	28 days	114.3/107.3	No
Anonymous (2015)	111/127	90 days	111/127	No
Anonymous (2015a)	51/102	1 year	204/408	No
Anonymous (2012b)	76/96*	28 days	25.3/32	No
Anonymous (2015)	81.6/846	90 days	81.6/846	No
Anonymous (2015a)	300	90 days	300	No
Anonymous (2015b)	300	1 year	1200	No
Anonymous (2013)	>1000	28 days	> 333	No

* LOAEL based on reduced body weight gains observed at all doses in males. However, no dose response was observed and the body weight gain reduction reached statistical significance only at the highest dose level. In a conservative approach no NOAEL was determined by the applicant and a LOAEL of 76/96 mg/kg bw/d was proposed.

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

According to the CLP criteria, substances should be classified for repeated dose toxicity if significant adverse effects, which indicate functional impairment, occur at dose levels ≤100 mg/kg bw/d in a 90-day oral rodent study. Such effects may include significant consistent and adverse changes in clinical biochemistry, haematology, or urinalysis parameters; significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination; or morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction. In contrast, adaptive responses that are not considered toxicologically relevant do not warrant classification.

Repeated dose toxicity of PYDIFLUMETOFEN (SYN545974) was investigated in several species (rats, mice and dogs). The liver was identified as the target organ with a consistent pattern of increased liver weight associated with histopathological changes and/or modified clinical chemistry. These findings reflect the adaptive capacity of the liver being overwhelmed by PYDIFLUMETOFEN (SYN545974). In addition, lower body weight gains were observed in all species and thyroid hypertrophy in rats only. All of these effects were observed at doses above the guidance value for STOT RE 2 (H373) (100 mg/kg bw).

It can be concluded that repeated dosing with PYDIFLUMETOFEN (SYN545974) produced no effects that were considered to be indicative of organ dysfunction at dose level below the guidance value for classification.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

No classification is required for STOT RE (specific target organ toxicity-repeated exposure)

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS did not propose classification for STOT RE. The repeated dose toxicity of pydiflumetofen was investigated in several species (rats, mice and dogs). The liver was identified as the target organ with a consistent pattern of increased liver weight associated with histopathological changes and/or modified clinical chemistry. In addition, lower body weight gains were observed in all species and thyroid hypertrophy was only observed in rats. All these effects were observed at doses above the critical guidance values for STOT RE 2. The DS concluded that repeated dosing with pydiflumetofen did not produce results that indicated severe or toxicologically relevant effects on organs at dose levels below the guidance values for classification.

Table 31 in the CLH report summarises the repeat dose studies on pydiflumetofen which were conducted in rats (28-day dietary, 28-day dermal, 90-day dietary, 2-year dietary combined chronic toxicity/ carcinogenicity study, 2-generation reproductive dietary study), mice (28-day dietary, 90-day dietary) and dogs (90-day oral (capsule), 1-year oral (capsule)).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Comparison with the criteria

***Table**: Summary of the most relevant effects for consideration of STOT-RE in doses requiring comparison to the trigger dose values.*

Study	Relevant effect level	Cat. 1 mg/kg/day	Cat. 2 mg/kg/day	Significant & Potentially Relevant Effects (dose response? Y/N)	Reference
Rat, 28 day oral dietary **Not sufficient for classification**	4000 ppm **Males**: 343 mg/kg/day **Females**: 322 mg/kg/day	≤ 30	≤ 300	**Males**: (control vs 4000 ppm) ↑ Abs. liver wt. (+19%)** (Y) ↑ Rel. liver wt. (+21%) (Y) Liver: ↑ hypertrophy: 0 vs 4 (min)[1] (Y) **Females**: (control vs 4000 ppm) ↑ Abs. liver wt. (+20%)* (Y) ↑ Rel. liver wt. (+24%) (Y)	DAR B.6.3.1 (K-CA 5.3.1/01)

Rat, 90 day oral dietary **Not sufficient for classification**	1500 ppm **Males**: 111 mg/kg/day **Females**: 127 mg/kg/day	≤ 10	≤ 100	**Males**: (control vs 1500 ppm) ↑ Abs. liver wt. (+21%)* (Y) ↑ Rel. liver wt. (+26%) (Y) Liver: ↑ hypertrophy: 0 vs 5* $(min)^2$ (Y) Thyroid: ↑ follicular hypertrophy: 0 vs 4 $(min)^2$ ↓ serum ALP (-30%)** **Females**: (control vs 1500 ppm) ↑ Abs. liver wt. (+16%)** (Y) ↑ Rel. liver wt. (+18%) (Y) ↓ serum ALP (-41%)**	DAR B.6.3.2.1 (K-CA 5.3.2/01/02)
Mouse, 28 day oral dietary **Not sufficient for classification**	1500 ppm **Males**: 213 mg/kg/day **Females**: 266 mg/kg/day	≤ 30	≤ 300	**Males**: (control vs 1500 ppm) ↓ bw gain (-16%) (N) ↑ Abs. liver wt. (+25%)** (Y) ↑ Rel. liver wt. (+31%)** (Y) No histopathological findings. **Females**: (control vs 1500 ppm) ↓ bw gain (-23%) (N) ↑ Abs. liver wt. (+23%) (Y) ↑ Rel. liver wt. (+30%) (Y) No histopathological findings.	DAR B.6.3.1 (K-CA 5.3.1/02)
Mouse, 90 day oral dietary **Not sufficient for classification**	500 ppm **Males**: 81.6 mg/kg/day	≤ 10	≤ 100	**Males**: (control vs 500 ppm) ↑ Abs. liver wt. (+18%)* (Y) ↑ Rel. liver wt. (+14%) (Y) Liver: ↑ hypertrophy: 0 vs 2/10 (mild) (Y) No other histopathological findings	DAR B.6.3.2.2 (K-CA 5.3.2/03/04)
Dog, 90 day oral (capsule) **Not sufficient for classification**	30 mg/kg/day	≤ 10	≤ 100	**Males**: No effects. **Females**: No effects.	DAR B.6.3.2.3 (IIA 5.3.2/05)
Dog, 12 month oral dietary **Not sufficient for classification**	30 mg/kg/day	≤ 2.5	≤ 25	**Males**: (control vs 30 mg/kg/day) ↑ Rel. liver wt. (+19%) (Y) ↑ Rel. thyroid wt. (+29%) (Y) **Females**: (control vs 30 mg/kg/day) ↑ Rel. liver wt. (+22%) (Y)	DAR B.6.3.2.3 (IIA 5.3.2/06)
Rat, 28 day dermal **Not sufficient for classification**	300 mg/kg/day	≤ 60	≤ 600	**Males**: No effects. **Females**: No effects.	DAR B.6.3.3 (IIA 5.3.3/01)

2 year dietary study in rats **Not sufficient for classification**	200 ppm **Males**: 9.9 mg/kg/day	≤ 1.25	≤ 12.5	**Males**: (control vs 200 ppm) Liver: ↑ prominent lobular architecture: 5 vs 15 (animal incidence) (N)	DAR B.6.5.1 (IIA 5.5/01)
18 month dietary study in mice **Not sufficient for classification**	75 ppm (lowest dose tested) **Males**: 9.2 mg/kg/day **Females**: 9.7 mg/kg/day	≤ 1.67	≤ 16.7	**Males**: No effects. **Females**: No effects.	DAR B.6.5.2 (IIA 5.5/03)
Two generation reproduction Rat, oral dietary **Not sufficient for classification**	750 (M)/ 450 (F) ppm **Males**: F0: 46 mg/kg/day F1: 59 mg/kg/day **Females**: F0: 36 mg/kg/day F1: 42 mg/kg/day	≤ 10	≤ 100	**Males**: (control vs 750 ppm) F0/ F1: No adverse effects. **Females**: (control vs 450 ppm) F0/ F1: No adverse effects.	DAR B.6.6.1 (IIA 5.6.1/01)

* significantly different from control, p ≤ 0.05

** significantly different from control, p ≤ 0.01

[1]animals affected out of 6 (min = minimal grading)

[2]animals affected out of 10 (min = minimal grading)

The table above presents the most pertinent data for consideration of STOT-RE classification. Based on the data neither a category 1 or a category 2 classification is warranted. Severe toxicological effects were not demonstrated at or below the guidance critical values. The target organ in all species is the liver. Table 31 in the CLH report provides a greater level of detail for all effects at all dose levels in those studies most suitable for STOT RE.

RAC concludes that repeated dosing with pydiflumetofen produced no effects that were indicative of organ dysfunction at dose levels below the guidance value for classification as STOT-RE, therefore **no classification is proposed**.

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 35: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
Bacterial reverse mutation OECD 471 GLP Acceptable	PYDIFLU METOFEN (SYN545974) (purity 98.5% w/w) Vehicle: DMSO	*Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) cells. *Escherichia coli* strains WP2uvrApKM101 and WP2pKM101 Concentrations 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate. Plate incorporation test (experiment 1) and the pre-incubation test (experiment 2).	+ S9: Negative - S9: Negative Cytotoxicity observed in strains TA1535 and TA1537 with metabolic activation (+S9) in experiment 1 and in experiment 2 in strains TA1537 and TA98 without metabolic activation (-S9) and in strain TA1535 with metabolic activation (+S9). Positive controls included	Sokolowski (2012)
Bacterial reverse mutation OECD Guideline 471 GLP Acceptable	PYDIFLU METOFEN (SYN545974) (purity 96.7% w/w) Vehicle: DMSO	*Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) cells. *Escherichia coli* strains WP2uvrApKM101 and WP2pKM101 Concentrations 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate. Plate incorporation test (experiment I) and the pre-incubation test (experiment II).	+ S9: Negative - S9: Negative Cytotoxicity observed in strain TA1537 in experiment I with and without metabolic activation (+ and – S9) and in strain TA 98 in experiment II with metabolic activation (+S9). Positive controls included	Sokolowski (2014)
Mammalian cell gene mutation test OECD 476 GLP Acceptable	PYDIFLU METOFEN (SYN545974) (purity 98.5% w/w) Vehicle: DMSO	*L5178Y TK +/-* mouse lymphoma cells Concentrations: <u>In the absence of S9</u> Experiment I: 7.5; 15.0; 22.5; 30.0; 60.0 µg/mL; Experiment II: 7.5; 15.0; 30.0; 45.0; 60.0 µg/mL <u>In the presence of S9</u> Experiment I: 7.5; 15.0; 30.0; 45.0; 60.0 µg/mL; Experiment II 7.5; 15.0; 30.0; 60.0; 90.0 µg/mL; Experiment III 40.0; 80.0; 90.0; 100.0; 110.0 µg/mL	+ S9: Negative - S9: Negative Cytotoxicity occurred without metabolic activation (-S9) in the following experiments: Experiment I at 60 µg/mL Experiment II at 45.0 µg/mL and above Experiment III at 80.0 µg/mL and above Positive controls included	Wollny (2013)
Chromosome aberration test OECD 473 GLP Acceptable	PYDIFLU METOFEN (SYN545974) (purity 98.5% w/w) Vehicle: DMSO	Human lymphocytes Concentrations: <u>Absence of S9</u> Experiment I: 16.1, 28.1, 150.8 µg/mL. Experiment IIA: 5.3, 9.2, 16.1 µg/mL, Experiment IIB: 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 15.0, 20.0, 40.0 µg/mL <u>Presence of S9</u> Experiment I: 16.1, 28.1, 49.2 µg/mL, Experiment IIA: 9.2, 16.1, 2475.4, 4332.0 µg/mL. 4332.0 µg/mL of the test substance, approx. 10 mM	+ S9: Negative - S9: Positive clastogenic Experiment IIA without metabolic activation (-S9) one statistically significant increase in aberrant cells after treatment with 5.3 µg/mL. In confirmatory Experiment IIB statistically significant increases occurred after treatment with 20.0 and 40.0 µg/mL Cytotoxicity observed in Experiments I and IIA without metabolic activation (-S9). No cytotoxicity in Experiment I and IIA in the presence of metabolic activation (+S9) or in the confirmatory Experiment IIB without metabolic activation (-S9). Positive controls included	Bohnenberger (2013)

Table 36: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations/Results**	**Reference**

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
Micronucleus test OECD 474 GLP	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) Vehicle: 1% CMC (w/v)	Mouse NMRI 7 males/dose/sampling time. Negative and positive control consisting of 5 males Single oral gavage administration, bone marrow cells collected after 24 and 48 hours Doses 24 hour preparation interval: 500, 1000, and 2000 mg/kg bw; 48 hour preparation interval: 2000 mg/kg bw. 2000 mg/kg suitable maximum tolerated dose	Negative 2000 polychromatic erythrocytes scored per animal Positive controls included	***Anonymous*** (2012)
Micronucleus test OECD 474 GLP	PYDIFLUMETOFEN (SYN545974) (purity: 96.7% w/w) Vehicle: 1% CMC (w/v)	Mouse NMRI 7 males/dose/sampling time. Negative and positive control consisting of 5 males Single oral gavage administration, bone marrow cells collected after 24 and 48 hours Doses 24 hour preparation interval: 500, 1000, and 2000 mg/kg bw; 48 hour preparation interval: 2000 mg/kg bw. 2000 mg/kg suitable maximum tolerated dose	Negative 2000 polychromatic erythrocytes scored per animal Positive controls included	***Anonymous*** (2014)

Table 37: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

In a mutagenicity study in mammalian cells, testing for forward mutation in mouse lymphoma cells, PYDIFLUMETOFEN (SYN545974) did not increase the mean mutant frequency in the presence or absence of S9-mix. PYDIFLUMETOFEN (SYN545974) is therefore considered non-mutagenic in cultured mammalian cells (Wollny, 2013).

The clastogenic effect of PYDIFLUMETOFEN (SYN545974) was tested in an *in vitro* chromosome aberration study in human lymphocytes (Bohnenberger, 2013). In two of 3 experiments, in the absence of S9 mix, there were statistically significant increases in chromosomal aberrations. In Experiment IIA in the absence of S9 mix, one statistically significant increase in aberrant cells was observed after treatment with 5.3 µg/mL (concentrations tested were 5.3, 9.2, 16.1 µg/mL). In a confirmatory Experiment IIB, statistically significant increases occurred after treatment with 20.0 and 40.0 µg/mL; these were the two highest concentrations tested.

In vivo PYDIFLUMETOFEN (SYN545974) was found negative in two studies designed to detect clastogenicity (mouse bone marrow micronucleus test) (***Anonymous***, 2012; ***Anonymous***, 2014). There was no evidence of chromosome damage at the maximum dose of 2000 mg/kg bw in two separate studies.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

PYDIFLUMETOFEN (SYN545974) was found negative in a reverse mutagenicity test in bacteria with and without metabolic activation and also in a mammalian cell mutation test. PYDIFLUMETOFEN (SYN545974) is therefore considered non-mutagenic in bacteria and in cultured mammalian cells.

The clastogenic effect of PYDIFLUMETOFEN (SYN545974) was investigated both *in vitro* and *in vivo*. In the *in vitro* test in human lymphocytes there was some evidence of chromosome damage in cultures without metabolic activation. *In vivo* however, PYDIFLUMETOFEN (SYN545974) did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice. Although the mean number of polychromatic erythrocytes was not substantially decreased after the treatment compared to controls, systemic exposure to PYDIFLUMETOFEN (SYN545974) has been demonstrated in mice previously (see section 2.6.1). Based on these studies it is concluded that PYDIFLUMETOFEN (SYN545974) is not clastogenic *in vivo*.

The RMS proposes to require a confirmatory *in vivo* test to definitely exclude the clastogenic effect observed *in vitro* on human lymphocytes. Since *in vitro* clastogenic effect in absence of metabolic activation and carcinogenic effect (liver tumors, see 2.6.5) in male mouse were observed after pydiflumetofen exposure, a comet assay should be performed on an organ from the gastrointestinal tract (stomach and/or colon and/or duodenum) and also on liver. The choice of gastrointestinal tract tissue is considered particularly relevant because positive results in the *in vitro* test were observed without metabolic activation. Thus, it is considered valuable to have additional information on the genotoxic potential of pydiflumetofen *in vivo* on a local tissue (before liver metabolism).This additional test would allow to definitely exclude the possibility of a genotoxic mode of action for the liver tumor and ensure of the absence of genotoxicity on a local tissue exposed upstream liver metabolism (e.i. gastrointestinal tract). Anyway, the RMS proposed that a discussion regarding this issue (need of a confirmatory genotoxicity test) could take place during the EFSA peer review process. During the commenting period (December 2017), different position Member Sates/ EFSA positions on the need for a confirmatory genotoxicity assay (and if yes, which one) have emerged, which supports the RMS request for an Expert consultation on this point.
Pending the conclusion of these future discussions, no classification regarding genotoxicity is proposed due to lack of data.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

No classification is required for genotoxicity/germ cell mutagenicity (a confirmatory *in vivo* genotoxicity assay is required)

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS reported that pydiflumetofen was tested in a range of *in vitro* and *in vivo* genotoxicity assays (see also the table below).

In vitro assays included:

- two *in vitro* Ames tests (reverse mutation assay with *Salmonella typhimurium* and *Escherichia coli*) (*Sokolowski*, 2012, 2014),
- an *in vitro* cell gene mutation test (in mouse lymphoma L5178Y cells) (*Wollny*, 2013),
- an *in vitro* chromosome aberration test (in human lymphocytes) (*Bohnenberger*, 2013).

In vivo assays included:

- two *in vivo* micronucleus assays in mouse rat bone marrow (*Anon.*, 2012, 2014),
- an *in vivo* rat bone marrow chromosome aberration test (not reported in the CLH report, but it is reported in the 2018 DAR) (*Anon.*, 2017),

***In vitro* results**

Pydiflumetofen was found to be negative in two reverse mutagenicity tests in bacteria with and without metabolic activation. In a mutagenicity study in mammalian cells, testing for forward mutation in mouse lymphoma cells, pydiflumetofen did not increase the mean mutation frequency in the presence or absence of S9-mix. However, the clastogenic effect of pydiflumetofen was tested in an *in vitro* chromosome aberration study in human lymphocytes (*Bohnenberger, 2013*). In 2 of 3 experiments, in the absence of S9 mix, there were statistically significant increases in chromosomal aberrations.

***In vivo* results**

The clastogenic effect of pydiflumetofen was further investigated *in vivo*. In the *in vitro* test in human lymphocytes there was some evidence of chromosome damage in cultures without metabolic activation (this was considered negative by most of the experts during a recent EFSA expert meeting (PPR 182, September 2018)). *In vivo* pydiflumetofen did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice. The mean number of polychromatic erythrocytes was not decreased after treatment indicating that pydiflumetofen did not have any significant cytotoxic properties in the bone marrow.

Conclusion

According to the DS, when considering all the data, pydiflumetofen did not present a genotoxic hazard. There were no studies in germ cells. The DS did not propose to classify pydiflumetofen as mutagenic.

***Table**: Summary of genotoxicity tests with pydiflumetofen adapted from table 35 and 36 in the CLH report, and table 6.4-1 in the DAR.*

Study	Result	Test System	Reference
***In vitro* studies:**			
Bacterial mutagenicity	negative	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA1535, TA1537, TA98, TA100 *E. coli* strains WP2uvrApKM101 and WP2pKM101	*Sokolowski,* (2012)
Bacterial mutagenicity	negative	GLP, OECD TG 471 (1997) *Salmonella* Strains: TA1535, TA1537, TA98, TA100 *E. coli* strains WP2uvrApKM101 and WP2pKM101	*Sokolowski,* (2014)
Mammalian cell mutagenicity	negative	GLP, OECD TG 476 (1997) Mouse Lymphoma L5178Y Cells (Thymidine Kinase locus)	*Wollny,* (2013)
Clastogenicity	Positive or equivocal?	GLP, OECD TG 473 (1997) cultured human lymphocytes	*Bohnenberger,* (2013)
***In vivo* studies:**			
Micronucleus	negative	GLP, OECD TG 474 (1997) Male NMRI mouse bone marrow (short term)	*Anonymous*, (2012)

Micronucleus	negative	GLP, OECD TG 474 (1997) Male NMRI mouse bone marrow (short term)	*Anonymous*, (2014)
Clastogenicity (chromosome aberration assay)	negative	GLP, OECD TG 475 (2016) Han Wistar rat, male (single oral gavage); bone marrow	*Anonymous,* (2017)

Comments received during public consultation

One MSCA supported the proposal not to classify pydiflumetofen for mutagenic effects. One other comment was made by the same MSCA in relation to a proposed request for an additional study – a comet assay. They did not support the request for a new study.

One Company-Manufacturer provided an additional *in-vivo* rat bone marrow chromosome aberration assay on the batch of material (SMU2EP12007) that gave a positive response in the *in-vitro* chromosome aberration test in human lymphocytes.

Assessment and comparison with the classification criteria

In Vitro Tests

Summary

Pydiflumetofen gave negative responses in the Ames tests and the mouse lymphoma L5178Y test, and a positive result in the *in vitro* chromosome aberration test in human lymphocytes; indicative of an *in vitro* clastogenic effect.

Bacterial and mammalian cell mutagenicity tests

The Ames studies were conducted on the toxicology batch of material (SMU2EP12007; *Sokolowski, 2012*) and on a batch of material spiked with potential impurities (SMU4FL762; *Sokolowski, 2014*) to support the technical specification of pydiflumetofen. No substantial increase in revertant colony numbers of any of the six tester strains was observed following treatment at any dose level (up to 5000 µg/plate), either in the presence or absence of metabolic activation (S9 mix). Appropriate reference mutagens were used as positive controls and showed an increase in induced revertant colonies.

Pydiflumetofen 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 (*Wollny, 2013*). Methyl methanesulphonate (19.5 µg/mL) and cyclophosphamide (3.0 and 4.5 µg/mL) were used as positive controls and showed an increase in induced total mutant colonies within acceptable levels of toxicity.

RAC agrees with the DS, pydiflumetofen shows no potential for *in vitro* mutagenicity.

Mammalian cell chromosome aberration test

A GLP, guideline compliant *in vitro* chromosome aberration test in human lymphocytes (*Bohnenberger, 2013*) in the absence of S9 mix, showed a weak positive response at a low concentration of 5.3 µg/mL in a series of tested concentrations after 22 hours of exposure. Tests at 9.2 and 16.1 µg/mL were negative.

Layout of the experiments in the *Bohnenberger* (2013) study (positive aberrations

associated with concentrations in bold):

- Experiment I:
  - exposure 4 hr: [16.1 - 28.1 - 150.5] µg/mL; without S9 mix
  - exposure 4 hr: [16.1 – 28.1 – 49.2] µg/mL; with S9 mix
- Experiment IIA:
  - exposure 22 hr: [**5.3** – 9.2 – 16.1] µg/mL; without S9 mix
  - exposure 22 hr: [9.2 – 16.1 – 2475 - 4332] µg/mL; with S9 mix
- Experiment IIB: (confirmatory experiment)
  - exposure 22 hr: [3.0 – 4.0 – 5.0 – 6.0 – 7.0 – 10.0 – 15.0 – **20.0** – **40.0**] µg/mL; without S9 mix

Experiment I is a short term (4 hr exposure ± S9 mix) test. Experiment IIA is a 22 hr exposure test (-S9 mix) and a 4 hr exposure test (+S9 mix). Experiment IIB is a 22 hr confirmatory test (-S9 mix) to investigate if the chromosomal aberrations observed with pydiflumetofen in the absence of S9 mix were repeatable.

In Experiment IIA in the absence of S9 mix, one statistically significant increase (6.5 % aberrant cells, excluding gaps) above the range of the laboratory historical solvent control data (0.0 - 3.0% aberrant cells, excluding gaps), was observed after treatment with 5.3 µg/mL (table below). No dose-dependency was observed. Based on this weak positive result, a confirmatory test (Experiment IIB) was conducted. Statistically significant increases occurred after treatment with 20.0 and 40.0 µg/mL (7.5 and 9.5 % aberrant cells, excluding gaps respectively) and the results clearly exceeded the laboratory historical solvent control range of 0.0 - 3.0% (table below).

There was no evidence of an increase in polyploid metaphases after treatment with the test substance relative to the control cultures. Positive controls (ethyl methanesulphonate [-S9 clastogen] and cyclophosphamide [+S9 clastogen]) behaved appropriately and showed clear increases in cells with structural chromosome aberrations. Cytotoxicity was not an issue at the concentrations of test substance employed in the study. Precipitates were visible at the highest concentrations tested.

Conclusion

The *in vitro* mutagenicity tests were all negative. Pydiflumetofen induced structural chromosomal aberrations in human lymphocytes *in vitro* in the absence of metabolic activation. The responses were not linear but were dose related and clearly outside the distribution of the historical negative control data. The study was acceptable from a regulatory point of view and is a recent GLP and guideline compliant investigation. RAC considers the data weakly positive to equivocal regarding evidence for chromosomal aberration and places more weight on the *in vivo* studies for the overall assessment for the potential for clastogenicity. The potential for chromosomal aberrations was further investigated in two *in vivo* mouse micronucleus studies and one *in vivo* rat bone marrow chromosome aberration assay. These are discussed below.

Table: Summary of results of the in vitro chromosomal aberration study with pydiflumetofen

Exp.	Preparation interval	Test substance concentration in µg/mL	Mitotic indices in % of control	Aberrant cells in % incl. gaps*	Aberrant cells in % excl. gaps*	Aberrant cells in % 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

[1]DMSO 1.0% (v/v); [2]EMS (770 µg/ml); [P]Precipitation occurred at the end of treatment; [S]Statistically significant

***In Vivo* Tests**

Summary

As determined by a pre-experiment in male and female mice, 2000 mg pydiflumetofen per kg bw was considered suitable by the applicant as the highest dose. Since no obvious gender-specific differences in the sensitivity to the test substance were observed, the Sponsor performed the main experiment using male animals only. Pydiflumetofen did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice.

In vivo mouse bone marrow micronucleus tests

Two *in vivo* mouse bone marrow micronucleus tests were conducted, one of which was on the batch of pydiflumetofen (SMU2EP12007) that gave a positive response in the *in vitro* chromosome aberration test. The second study was conducted on a batch of material spiked with potential impurities (SMU4FL762) to support the technical specification of the test substance. Both *in vivo* micronucleus tests gave negative results. These studies demonstrated that the positive *in vitro* clastogenicity finding for pydiflumetofen was not corroborated *in vivo*. The *in vivo* mouse bone marrow micronucleus tests did not include an assessment of bone marrow exposure to pydiflumetofen, however in both *in vivo* studies the observed clinical signs at the highest dose were typically indicative of systemic absorption of the test material. No dose dependence was observed in either study. A dose of 40 mg/kg bw cyclophosphamide administered orally was used as the positive control, which showed a statistically significant increase of induced micronuclei.

The plant protection product DAR summarised extensive toxicokinetic investigations into pydiflumetofen in both rats and mice (DAR section B.6.1.1). Following gavage dosing pydiflumetofen and its metabolites were shown to be present in blood and plasma over at least a 24 hour period post-dosing in both species. The bone marrow may be considered to have been sufficiently exposed to pydiflumetofen and its metabolites to reliably assess clastogenicity and aneugenicity. Evaluation of the pharmacokinetics of pydiflumetofen in the mouse following one of the dose regimes with 1000 mg/kg bw (DAR B.6.1.1, *Anon, 2014a*; K-CA 5.1.1/07) revealed pydiflumetofen was quantifiable in blood at all sampling timepoints (0.5, 1, 2, 4, 8, 12 and 24 hours). The increase in systemic exposure was non-linear with respect to dose in male mice beyond *ca.* 100 mg/kg. As all doses in the micronucleus assays were above 100 mg/kg, exposure of the bone marrow to pydiflumetofen was assumed.

In vivo rat bone marrow chromosome aberration assay

During the peer review of pydiflumetofen within the scope of Regulation 1107/2009 for the evaluation of plant protection products, the industry applicant supplied a third *in vivo* study to the Rapporteur Member State (RMS) for inclusion into the DAR (*Anon, 2017*). This study, a rat bone marrow chromosome aberration assay, was performed according to GLP and OECD TG 475 (2016) compliant. Exposure consisted of a single dose administered by oral gavage.

The limit dose of 2000 mg/kg was well tolerated in males and females and was selected as the maximum dose level for the main experiment. Intermediate 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 study was conducted in male Crl:WI(Han) Wistar rats only.

Bone marrow was sampled at two time points: 16 hours and 42 hours after test substance administration. At both time-points the mean percentage of cells with aberrations (excluding gaps) was not substantially changed from controls (table below).

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 (not statistically

significant). 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 historical control range.

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 (not statistically significant). 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.

***Table**: Summary of results of cells with structural aberrations (excluding gaps) following exposure to pydiflumetofen in the Wistar rat.*

16 Hr data:

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

42 Hr data:

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

Cyclophosphamide (30 mg/kg) was used as a positive control. At the 16 hour sample time, the mean percentage of cells with aberrations (excluding gaps) was 29.8%.

Several pharmacokinetic studies in the rat were evaluated in the plant protection DAR. In a preliminary pharmacokinetics study (DAR B.6.1.1; *Anon, 2015*; K-CA 5.1.1/01), at a dose of 1000 mg/kg, pydiflumetofen and its metabolites were observed in blood over a 72 post-dose period. It may thus be concluded that the bone marrow was exposed to pydiflumetofen and its metabolites at doses of 500, 1000 or 2000 mg/kg prior to the 16 hour sampling point and 2000 mg/kg prior to the 42 hour sampling point in the chromosome aberration test.

RAC concludes that pydiflumetofen did not induce chromosome aberrations in the bone marrow cells of male rats treated up to 2000 mg/kg.

Conclusion

The *in vitro* mutagenicity tests were negative. There was limited evidence that pydiflumetofen induced structural chromosomal aberrations in an *in vitro* clastogenicity study in human lymphocytes, RAC considers the study equivocal. The *in vivo* mouse bone marrow micronucleus tests were negative, the test substance did not induce micronuclei and pydiflumetofen is considered to be non-mutagenic in this assay. Furthermore, in an *in vivo* rat bone marrow chromosome aberration assay, pydiflumetofen was negative in male rats treated up to 2000 mg/kg.

Classification Assessment

Muta 1

No human data are available for pydiflumetofen, therefore a classification with Muta. 1A is not supported. Pydiflumetofen is negative in acceptable *in vitro* tests and negative in *in vivo* somatic cell mutagenicity guideline tests in mammals. Data are not available illustrating the induction of mutagenic effects in germ cells (a criterion for Category 1B). RAC does not support classification with Muta. 1A or B.

Muta 2 Assessment and conclusion

The overall weight of evidence for pydiflumetofen supports no potential for genotoxicity in somatic cells from a battery of *in-vivo* and *in-vitro* GLP and guideline compliant studies. Therefore no classification in category 2 is warranted.

RAC agrees with the DS that **no classification of pydiflumetofen for genotoxicity is warranted**.

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 38: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
2 year chronic toxicity/ carcinogenicity OECD 453 GLP Acceptable Rat: Han Wistar Crl: WI (Han) 64/sex (52/sex/group main group, 12/sex/group interim kill after 12 months)	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) Males 0, 200, 1000 & 6000 ppm; Females 0, 150, 450 & 1500 ppm Actual doses 9.9, 51.0 and 319 mg/kg/day (males); 10.2, 31.0 and 102	***Non-neoplastic findings:*** no treatment related increase in mortality **150 ppm (females 10.2 mg/kg/day):** No treatment-related effects. **200 ppm (males 9.9 mg/kg/day):** No treatment-related effects. **450 ppm (females 31.0 mg/kg/day):** Body weight: ↓ BW 7.6% week 104 *Food consumption:* slightly lower and achieved statistical significance on several occasions **1000 ppm (males 51.0 mg/kg/day)**: *Body weight*: ↓ BW (↓ 10.8 %) and BW gain (↓13%) at week 104. *Food consumption:* slightly lower and achieved statistical significance on several occasions *Food utilisation:* ↓ 7.2 % weeks 1 to 4	***Anonymous*** (2015a)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>(see also section 2.6.3)</td><td>mg/kg/day (females).
Continuous in the diet for 104 weeks</td><td>Liver weight : ↑ covariate weight (12%).
Pathology: ↑ hepatocyte hypertrophy 3/52 minimal
<u>1500 ppm (females 102 mg/kg/day)</u>:
Body weight: ↓ BW (↓ 9.1 %) and BW gain (↓ 13%) at week 104
Food consumption: consistently slightly lower and achieved statistical significance on several occasions
Liver weight: ↑ covariate weight (10%).
Pathology: ↑ hepatocyte hypertrophy 3/52 minimal-mild
<u>6000 ppm (males 319 mg/kg/day)</u>:
Body weight: ↓ 4.4% week 4, 18.2% week 104
Food consumption: consistently slightly lower and achieved statistical significance on several occasions (↓ 12% week 4)
Food utilisation: ↓ 15.7 % weeks 1 to 4
Liver weight: ↑ covariate weight (24%).
Pathology: ↑ hepatocyte hypertrophy 39/52 minimal to moderate; ↑ eosinophilic inclusions hepatocytes 19/52
NOAEL for toxicity 200 ppm (equivalent to 9.9 mg/kg/day for males and 450 ppm (equivalent to 31.0 mg/kg/day) in females
Neoplastic findings
No treatment-related changes in neoplastic findings at any dose level.
NOAEL for carcinogenicity 6000 ppm (equivalent to 312 mg/kg/day for males and 1500 ppm (equivalent to 102 mg/kg/day) in females</td><td></td></tr>
<tr><td>Carcinogenicity
OECD 451
GLP
Acceptable
Mice: CD-1 (ICR)
50/sex /group</td><td>PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w)
0, 75, 375 & 2250 ppm
Actual doses 0, 9.2, 45.4 and 287.9 mg/kg/day for males and 0, 9.7, 48.4 and 306.2 mg/kg/day for females
Continuous in the diet for 80 weeks</td><td>Non-neoplastic findings
<u>75 ppm (males 9.2 mg/kg/day, females 9.7 mg/kg/day)</u>:
No toxicologically significant treatment-related effects.
<u>375 ppm (males 45.4 mg/kg/day, females 48.4 mg/kg/day)</u>:
Pathology: ↑ 6/49 hepatocellular hypertrophy in males
<u>2250 ppm (males 287.9 mg/kg/day, females 306.2 mg/kg/day)</u>:
Bodyweight: ↓ 6.9% males; 11.6% females (week 80).
Food consumption: slightly lower and achieved statistical significance on several occasions
Food utilisation: ↓ 11.8% males weeks 1-13 (not statistically significant)
Liver weights: ↑ 52.3% males, 17.1% females covariate values
Pathology: ↑ 18/50 hepatocellular hypertrophy, ↑ 10/50 eosinophilic foci of cellular alteration in males
NOAEL for non-neoplastic change was 75 ppm in males, equating to dose levels of 9.2 mg/kg/day and 375 ppm in females, equating to 48.4 mg/kg/day
Neoplastic findings
No treatment-related neoplastic findings in females at any dose level.
In males only, hepatocellular carcinomas and adenomas were increased at 375 and 2500 ppm, correlating to liver masses observed at necropsy.
<table>
<tr><th>MALES</th><th colspan="4">Dose level (ppm)</th></tr>
<tr><th>Finding</th><th>0</th><th>75</th><th>375</th><th>2250</th></tr>
<tr><td>Liver (no. examined)</td><td>50</td><td>50</td><td>49</td><td>50</td></tr>
<tr><td>hepatocellular carcinoma</td><td>2 (4%)</td><td>3 (6%)</td><td>4 (8.2%)</td><td>10* (20.0%)</td></tr>
<tr><td>hepatocellular adenoma</td><td>4</td><td>6</td><td>9</td><td>22**</td></tr>
</table></td><td>Anonymous (2015b)</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
		(8.0%) (12.0%) (18.4%) (44.0%) Historical control data Hepatocellular Carcinoma 19 (7.7%) range 6-10%, n=250 Hepatocellular adenoma 45 (18.0%) range 10-28%, n=250 **NOAEL for carcinogenicity was 2250 (306.2 mg/kg/day) in females and 75 ppm (9.2 mg kg/day) in males.**	

Table 39: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 40: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
CAR activation assay i*n vitro* Non-guideline investigative study. Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w). Concentrations 1, 3, 10, and 30 µM. Vehicle: DMSO	Expression vectors for CAR3 variants of mouse, rat and human CAR Positive controls CITCO, TCPOBOP and clotrimazole produced robust responses in human, mouse and rat CAR3 constructs, respectively.	**30 µM** rat CAR3 ↑ 42 fold mouse CAR3 ↑ 20 fold human CAR3 ↑ 15 fold **10 µM** rat CAR3 ↑ 37 fold mouse CAR3 ↑ 32 fold human CAR3 ↑ 13 fold **3 µM** rat CAR3 ↑ 14 fold mouse CAR3 ↑ 34 fold human CAR3 ↑ 5 fold **1 µM** rat CAR3 ↑ 2.8 fold mouse CAR3 ↑ 24 fold human CAR3 ↑ 1.5 fold **PYDIFLUMETOFEN (SYN545974) is a direct activator of CAR from mouse, rat and human, and has high efficacy in all three species.**	Omiecinski (2014)
Non-guideline investigative study. Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity: 98.8% w/w). Concentrations 5, 10, 25 and 35 µM. Vehicle: DMSO	*In vitro* mouse hepatocyte study, male CD-1 (CRL) hepatocyte cultures ATP 6 samples per group; S-phase labelling index 5 samples per group; BROD and PROD 3 samples per group Positive controls included: Phenobarbital sodium salt	**All groups** ↓ 9.3-17.6% ATP as an indicator of cytotoxicity **35 µM** ↑ 50.6% hepatocyte proliferation as measured by replicative DNA synthesis ↓ PROD and BROD enzyme activities (not statistically significant) **25 µM** ↑ 89.8% hepatocyte proliferation as	Lowes (2015a)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
		(PB) and epidermal growth factor (EGF)	measured by replicative DNA synthesis ↓ PROD and BROD enzyme activities (not statistically significant) **10 µM** ↑ 71.3 and 97.0% respectively PROD and BROD enzyme activities **5 µM** ↑ 84.6 and 98.5% respectively PROD and BROD enzyme activities **Treatment of cultured male CD-1 mouse hepatocytes resulted in increased cell proliferation (S-phase of the cell cycle) and increased CYP2B/3A activities (measured as PROD activity).**	
In vitro hepatocyte proliferation indexing and enzyme activity measurements Non-guideline investigative study Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity 98.8% w/w). Concentrations 5, 10, 25 and 35 µM. Vehicle: DMSO	Human male hepatocyte cultures ATP 6 samples per group; S-phase labelling index 5 samples per group; BROD and PROD 3 samples per group Positive controls included: Phenobarbital sodium salt (PB) and epidermal growth factor (EGF)	**35 µM** ↓ 34 % ATP as an indicator of cytotoxicity ↑ 164 and 218% respectively PROD and BROD enzyme activities **25 µM** ↓ 35 % ATP as an indicator of cytotoxicity ↑ 155 and 326% respectively PROD and BROD enzyme activities **10 µM** ↑ 236 and 491% respectively PROD and BROD enzyme activities **5 µM** ↑ 191 and 237% respectively PROD and BROD enzyme activities **Treatment of cultured male human hepatocytes had no effect on cell proliferation (S-phase of the cell cycle). CYP2B/3A activities (measured as PROD and BROD activities) were elevated.**	Lowes (2015b)
In vivo 28 day mouse study mode of action study Non-guideline investigative study. Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) 0, 75 & 2250 ppm Actual doses 0, 10.0 or 324.0 mg/kg/day Continuous in the diet for 2, 7 or 28 days	Mouse CD-1 (CRL) 10 males/dose/ termination time after 2, 7 or 28 days	**2250 ppm (324 mg/kg/day)** ↑ absolute liver weight 22% and 24% after 28 and 7 days respectively ↑ liver:body weight ratio 28% and 21% after 28 and 7 days respectively ↓ 40% AST activity 28 days ↑ cytochrome P450 approx. 2-fold after 2, 7 and 28 days ↑ PROD activity 28, 36 and 37-fold after 2, 7 and 28 days respectively (marker of Cyp2b activity) ↑ BrdU incorporation 14-, 5 and 6-fold after 2, 7 and 28 days respectively ↑ Centrilobular hepatic hypertrophy in 9/10, 9/10 and 10/10 animals after 2, 7 and 28 days respectively ↑ Mitotic cells in liver 7/10 after 2 days **75 ppm (10 mg/kg/day)** ↑ PROD activity 1.9. 1.6 and 2.4-fold after 2, 7 and 28 days respectively not	***Anonymous*** (2015)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			statistically significant ↑ BrdU incorporation 3.1, 2.3- and 5.6-fold after 7 and 28 days respectively	
Ex-vivo enzyme analysis of liver samples taken at termination 28 day dietary study in the mouse Non-guideline investigative study. Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity 98.6% w/w) 0, 500, 1500, 4000 or 7000 ppm Continuous in the diet for 3, 7 or 28 days	Mice: CD1 6 males and 6 females/group killed after 28 days. Satellite groups control and high dose killed after 3 and 7 days	No increase in peroxisome palmitoyl CoA oxidation and only minilam increase (<4fold) in microsomal lauric acid 12-hydroxylation, indicating PYDIFLUMETOFEN (SYN545974) is not a peroxisome proliferator.EROD (marker for Cyp1a activity), no biologically significant effects. Dose related increase in hepatic total P450 content to maximum of 1.8 and 1.6 fold (males and females) at 7000 ppm on day 28. **7000 ppm:** ↑ PROD activity 10, 21 and 15 fold in males 13, 11 and 3-fold in females after 3. 7 and 28 days (marker of Cyp2b activity) Minimal↑ Benzyloxyquinoline-O-debenzylation (BQ) (<5 fold) in males and females at all timepoints (marker Cyp3a activity) **4000 ppm- 28 days** ↑ hepatic total P450 1.6 and 1.7-fold in males and females ↑ PROD activity 9.0 and 4.2-fold in in males and females (marker Cyp2b activity) ↑ BQ 2.7 and 2.5-fold in males and females (marker Cyp3a activity) **1500 ppm- 28 days** ↑ hepatic total P450 1.6 and 1.3-fold in males and females ↑ PROD activity 9.2 and 5.4-fold in in males and females (marker Cyp2b activity) ↑ BQ 1.2-fold in males (marker Cyp3a activity) **500 ppm** ↑ hepatic total P450 1.5 and 1.2-fold in males and females ↑ PROD activity 11.8 and 5.2-fold in in males and females (marker Cyp2b activity) ↑ BQ 1.5-fold in males (marker Cyp3a activity) **PYDIFLUMETOFEN (SYN545974) did not demonstrate the prototypical properties of peroxisome proliferators but exhibited characteristics in common with "phenobarbital-like" inducing agents**.	***Anonymous*** (2012)
Effect on rat thyroid peroxidase activity *in vitro* Investigative	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w)	Rat: Pooled thyroid gland microsomal fraction from male Wistar Han	Treatment with PYDIFLUMETOFEN (SYN545974) had no significant effect on rat thyroid peroxidase activity at any concentration tested. Positive control item, 6-propyl-2-	Lake (2014)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
study no relevant guidelines Non-GLP Supportive (mechanistic data)	Concentrations used: 0, 0.007, 0.1, 1.5 and 10 µM Vehicle: DMSO		thiouracil (PTU) resulted in a 99.9% inhibition of thyroid peroxidase activity **PYDIFLUMETOFEN (SYN545974) is not an inhibitor of rat thyroid peroxidase activity *in vitro***	
Effect on hepatic UDP-glucuronosyltransferase activity *in vitro* Investigative study no relevant guidelines Non-GLP Supportive (mechanistic data)	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) Concentrations used: 0, 250, 1500 and 8000 ppm 18.6, 111 and 587 mg/kg/day	Rat male Han Wistar (Crl:WI(Han) hepatic microsomes from 90 day study (***Anonymous***, 2015).	**Values expressed as % of control** **8000 ppm (587 mg/kg/day)** ↑ UDPGT activity 288 % specific activity, ↑ 347% per gram of liver, 421% per liver; ↑ 486% per relative liver weight **1500 ppm (111 mg/kg/day)** ↑ UDPGT activity 171 % specific activity, ↑ 194% per gram of liver, 244% per liver; ↑ 239% per relative liver weight **250 ppm (18.6 mg/kg/day)** ↑ UDPGT activity 152% specific activity; ↑ 162% per gram of liver. Changes in activity based on per liver and per relative liver weight were not statistically significant. **PYDIFLUMETOFEN (SYN545974) is an inducer of hepatic microsomal UDP glucuronosyltransferase activity towards thyroxine as substrate in male rats**	Lake (2015)

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

PYDIFLUMETOFEN (SYN545974) has been evaluated for chronic toxicity in the rat and for carcinogenic potential in the rat and the mouse.

In a 2 year combined chronic toxicity/carcinogenicity study in Wistar rats, PYDIFLUMETOFEN (SYN545974) was tested at dietary inclusion levels of 0, 200, 1000 and 6000 ppm for males and 150, 450 and 1500 ppm for females. Significantly lower body weight, body weight gain and food consumption were observed in both sexes at the mid and high dose (1000 and 6000 ppm in males; 450 and 1500 ppm in females). Food utilisation was also lower at the top dose in males and females. In males at the mid and high dose and females at the high dose liver hepatocyte hypertrophy was observed at 52 and 104 weeks, with a corresponding increase in liver weight in both sexes from the mid dose. In addition, in males at 6000 ppm grossly prominent lobular architecture of the liver was observed at 52 and 104 weeks and hepatocyte cytoplasmic eosinophilic inclusions in males at 6000 ppm at 104 weeks. There were no other treatment related effects on organ weight or histopathology. There were no treatment-related neoplastic findings. A NOAEL was established at 200 ppm (9.9 mg/kg/day in males) and 450 ppm (31.0 mg/kg/day in females).

In a carcinogenicity study in the mouse, groups of 50 male and 50 female CD-1 mice were fed diets containing 0, 75, 375 or 2250 ppm of PYDIFLUMETOFEN (SYN545974) for a period of at least 80 weeks. Statistically significantly lower group mean body weight was observed in males and females treated with 2250 ppm. This was associated with a statistically lower group mean body weight gain compared to the control animals and lower food consumption. Food utilization was only significantly lower in males at the high dose. Liver weights were increased in both sexes at the high dose. There were no treatment-related neoplastic findings in females in this study. In males, neoplastic and non-neoplastic findings were observed in the liver only. Treatment related increased incidence of hepatocellular carcinomas and adenomas (present as multiple adenomas) were observed in males at 375 and 2250 ppm, which were statistically significant at 2250 ppm only. In addition, centrilobular hypertrophy was observed in males only at 375 and 2250 ppm. Although there was a slightly higher incidence of eosinophilic foci of cellular alteration in the liver of male mice at 75 ppm when compared with controls, this

difference was not statistically significant and is considered to be incidental to treatment.

The NOAEL for the 80 week mouse study was established at 75 ppm in males, which is equivalent to 9.2 mg/kg/day and 375 ppm in female, which is equivalent to 48.4 mg/kg/day in females.

Additional studies have been conducted to elucidate the mode of action (MOA) for the liver hepatocellular carcinomas and adenomas observed in male mice and detailed study summaries are presented in Table 53. In addition, a review of the data and an assessment of the relevance of the mouse liver tumours to humans has been conducted following the IPCS and ILSI/HESI framework and is also presented as a detailed review and comparison with criteria (Cowie, 2015; See volume 3 B.6.5).

Based on an evaluation of the MOA studies and the regulatory toxicology database the following key events in the MOA have been demonstrated:

- 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.

Alternative modes of action leading to liver tumourogenesis were evaluated and excluded.
The key events of CAR activation and proliferation were demonstrated to occur in *in vitro* mouse hepatocyte cultures while no increase in proliferation was shown to occur in cultures of human hepatocytes, despite CAR activation occurring and the human hepatocytes being able to respond to proliferative stimuli.
Based on the available data, the MOA for liver tumour formation in male CD-1 mice treated with PYDIFLUMETOFEN (SYN545974) has been established. This MOA involves key events that include an initial activation of CAR, altered CAR-dependent gene transcription, and a critical key event of increased cell proliferation. Based on the qualitative species difference in the hepatocellular proliferation response to PYDIFLUMETOFEN (SYN545974) it has been established that this MOA is not relevant to humans.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

Table 41: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Mice: CD-1 (ICR)	Hepatocellular carcinoma 19 (7.6%) range 6-10%, n=250 Hepatocellular adenoma 45 (18.0%) range 10-28%, n=250	No	No	No	Single	No	Oral, diet	CAR activator not relevant to humans
Rat: Han Wistar Crl: WI (Han)	None	n/a	n/a	n/a	n/a	n/a	Oral, diet	n/a

It is considered that the available data from targeted investigative studies support the conclusion that PYDIFLUMETOFEN (SYN545974) does not pose a hepatocarcinogenic hazard to humans.

There are no human data for PYDIFLUMETOFEN (SYN545974). The available animal studies demonstrate that PYDIFLUMETOFEN (SYN545974) is not carcinogenic in the rat or female mouse, but at high doses in male mice increased incidences of liver adenomas and carcinomas were observed. PYDIFLUMETOFEN (SYN545974) is not genotoxic, but resulted in increased incidences of tumours in a single tissue (liver) of a single species (mice) in one sex (males). Treatment-related inductions of tumours in a single species and one sex with a non-genotoxic mode of action could warrant classification for category 2 carcinogen. However, the evaluation of

mode of action studies against the IPCS and ILSI/HESI framework demonstrates a CAR activation MOA. Data generated in human hepatocytes confirm the human non-relevance due to quantitative differences in responses to proliferative stimuli. Due to the demonstrated human non-relevance of the liver tumours observed in male mice, PYDIFLUMETOFEN (SYN545974) does not meet the criteria for classification.

RMS consideration regarding the carcinogenic potential of 2,4,6 TCP and the dose level selection for PYDIFLUMETOFEN (SYN545974) toxicity studies:
As previously mentioned, the RMS had some reservations regarding the dose level selection which has been proposed by the applicant based on pharmacokinetic data (see section 2.6.1 and B.6.1 in volume 3). The RMS questioned on the possibility that the doses of PYDIFLUMETOFEN (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, 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). In order to verify whether higher doses of PYDIFLUMETOFEN (SYN545974) (>300 and up to 1000mg/kg bw/day) would have actually covered the carcinogenic potential of 2,4,6 TCP, systemic exposure of 2,4,6 TCP (and related compound) have been estimated for these non-experimentally tested doses levels. Taking into consideration the dose-limited oral absorption, the estimated systemic exposure of 2,4,6 TCP following an oral administration of 1000 mg/kg/day PYDIFLUMETOFEN (SYN545974) in rats, would give a value close to the dose of TCP which causes 25% of leukemia in the rat (T25) in the NTP study (see details in volume 3B.6). It may therefore be difficult in this context to conclude on the carcinogenicity classification of PYDIFLUMETOFEN (SYN545974) without long-term studies performed with sufficiently high level of the test substance. However, the maximal tolerable dose (MTD) is often used to decide whether the top dose tested in a long-term toxicity study is adequate to give confidence in a negative result. There is actually broad acceptance that the top dose selected in a long-term study should ideally provide some signs of toxicity (such as slight depression of body weight gain (not more than 10%)), without causing e.g., tissue necrosis or metabolic saturation and without substantially altering normal life span due to effects other than tumors (OECD 2012[3]). Indeed, the MTD was reached in the 2-year study in rat, as the top dose in the male (300 mg/kg/d) and the top dose in the female (100 mg/kg/d) resulted in a 18% and 9% reduction of body weight, respectively. In conclusion, it can be reasonably considered that the carcinogenic potential of PYDIFLUMETOFEN (SYN545974) was appropriately assessed.

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

Based on the available animal studies, PYDIFLUMETOFEN (SYN545974) does not meet the criteria for classification.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two guideline and GLP compliant long-term oral (dietary) toxicity/carcinogenicity studies were available to the DS: a 2-year combined chronic toxicity/carcinogenicity study in the Han Wistar rat (*Anonymous, 2015a*) and an 18-month carcinogenicity study in the CD-1 mouse (*Anonymous, 2015b*). Study details were summarised in Table 38 in the CLH report. Pydiflumetofen had no treatment-related neoplastic findings in rats. However, in mice there was a treatment related increased incidence of hepatocellular carcinomas and adenomas (present as multiple adenomas) observed in males at 45 and 288 mg/kg/day, which were statistically significant at the high dose only. There were no treatment-related neoplastic findings in females at similar doses in this study. Several additional studies were conducted to investigate the Mode of Action (MoA) and human health relevance of the rodent tumours. The DS concluded from this data that pydiflumetofen did not meet the criteria for classification and therefore no classification for carcinogenicity was proposed.

[3] OECD 2012: Guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452 and 453 2nd édition. ENV/JM/MONO(2011)47.

1. In-vivo animal studies

1.1 Rat 2-year dietary toxicity/oncogenicity study

In a rat GLP and OECD TG 453 (2009) compliant carcinogenicity dietary study (*Anonymous, 2015a*), treatment with pydiflumetofen did not reduce the survival of rats up to the highest doses tested. Crl:WI (Han) strain rats were divided into treatment groups and scheduled kills were conducted after 12 months treatment for 12 animals/sex/group and at study termination after 24 months treatment for 52 animals/sex/group.

Table*: Mean dose received (mg/kg/day)*

Dietary concentration of pydiflumetofen (M/F ppm)	0	200/ 150	1000/ 450	6000/ 1500
Males	0	9.9/	51/	319/
Females	0	10.2	31.0	102

Dose level selection was based on pharmacokinetic data. The DS did not explain the rationale behind the dose selection. They only noted the concern raised in the DAR on whether the doses were high enough to account for the presence of the primary metabolite 2,4,6 trichlorophenol (2,4,6-TCP) which has a Carcinogenicity Category 2 classification in Annex VI to CLP.

General toxicity was displayed by significantly lower body weight (9-18%), body weight gain (13-22%) and slight reductions in food consumption which were observed in both sexes at the mid and top dose (51 and 319 mg/kg/day in males; 31 and 102 mg/kg/day in females). The liver was clearly a target organ, with slight effects also observed in the thyroid. The effects observed included increased liver weight and hepatocellular hypertrophy associated with cytoplasmic eosinophilic inclusions, and small increases in incidence of thyroid follicular cell hyperplasia.

Organ weight changes considered to be treatment related were observed in the liver. There were no treatment related differences in the functional observation battery parameters following administration of pydiflumetofen. There were no treatment-related neoplastic findings at the 12-month interim sacrifice.

1.1.1 Neoplastic findings

According to the CLH report there was no evidence of an increase in liver neoplastic lesions or early onset of neoplasms or other potential carcinogenic findings following exposure to pydiflumetofen at doses up to 319 and 102 mg/kg bw/day in males and females, respectively.

In the response to comments document following public consultation of the CLH report, the DS acknowledged that an increase in the incidence of thyroid follicular cell adenomas was observed at the highest tested dose of 102 mg/kg/day in females (3/51 (5.9%) *vs* 1/51 (2%) in the control group). The DS noted a re-evaluation of the historical control data and submission of additional information from the applicant following peer review under Regulation 1107/2009. They concluded that the increase in the incidence of thyroid follicular cell adenomas was not treatment related because they fell within the revised historical control data (7 studies; 2009-2015, background incidence of 0-5.8%; DAR table 6.5-16).

The DS also noted that although it considered the dose levels selected for the long term/carcinogenicity study in rat to be low, it also concluded that the maximum tolerated

dose (MTD) had indeed been reached. This was in line with many published technical guidelines for chronic or lifetime bioassays. Significant treatment-related decreases were observed in body weight gains in high dose males (↓18%) and high dose females (↓13%). Additionally, significant increases were observed in males for absolute and relative liver weights, which were associated with hepatocellular hypertrophy in both sexes and liver eosinophilic inclusions (a non-neoplastic lesion according to the US NTP Non-neoplastic Lesion Atlas).

1.2 Mouse 18-month dietary carcinogenicity study

In a mouse GLP and OECD TG 451 (2009) compliant carcinogenicity dietary study (*Anonymous, 2015b*), treatment with pydiflumetofen did not reduce the survival of mice up to the highest doses tested. Groups of 50 male and 50 female CD-1 mice were fed diets containing 0, 75, 375 or 2250 ppm of pydiflumetofen for a period of at least 80 weeks.

Table: *Mean dose received (mg/kg/day)*

Dietary concentration of pydiflumetofen (ppm)	0	75	375	2250
Males	0	9.2	45.4	287.9
Females	0	9.7	48.4	306.2

General toxicity was limited, but a small effect was observed on mean body weight in males and females treated at the high dose. The magnitude of the differences in body weight compared to controls was moderate after 80 weeks (-7% in males; -12% in females), indicating that there was no exacerbating systemic toxicity. This was associated with a statistically lower group mean body weight gain compared to the control animals and lower food consumption. Food utilization was only significantly lower in males at the high dose.

Liver weights were increased in both sexes at the high dose. Males showed an enhanced sensitivity to treatment with absolute and relative liver weights increased by 37% and 47% respectively over controls, compared with females showing increases of 9% and 26% respectively over controls. Prominent lobular architecture (seen in the rat) was not a feature of treatment in mice. Centrilobular hypertrophy was observed in males only at 45 and 288 mg/kg/day. There was an increasing incidence of eosinophilic foci of cellular alteration in the liver of male mice with indications of a dose response relationship. The incidence was statistically significant at the top dose of 288 mg/kg/day and historical controls were exceeded at ≥ 45 mg/kg/day. The DS concluded that the slightly higher incidence of eosinophilic foci of cellular alteration in the liver of male mice at 45 mg/kg/day was incidental to treatment.

1.2.1 Neoplastic findings

No neoplastic findings were observed in female mice.

A higher incidence of hepatocellular carcinomas and adenomas was observed in males administered pydiflumetofen at 45 and 288 mg/kg/day compared with the control group. These correlated with liver masses observed at necropsy. Hepatocellular adenomas and carcinomas in treated groups were only detected at terminal necropsy, i.e. there was no indication of a reduction in tumour latency. While the incidences of hepatocellular adenomas ($p \leq 0.01$) and carcinomas ($p \leq 0.05$) were statistically significantly increased in the top dose group, the increased incidence at 45 mg/kg/day was also considered to be treatment-related due to the number of animals with multiple hepatocellular adenomas in

the liver. A clear positive dose response relationship was evident for (1) incidences of animals with tumours and (2) incidence of animals with multiple tumours.

2. Mechanism of action and supporting data relevant for findings in the mouse liver

2.1 Description and results from the mechanistic studies

The DS briefly described several mechanistic studies (table 40 of the CLH report) that were conducted to elucidate the mode of action (MOA) for the liver hepatocellular carcinomas and adenomas observed in male mice. However, several alternative possible mechanistic explanations were not investigated in detail. Enzyme analysis of the liver indicated no to low involvement of peroxisome proliferation and some minor increase in Cyp1a activity. The available investigations focused on providing evidence in support of one mode of action, i.e. on a non-genotoxic mode of action involving hepatocyte proliferation, induced via constitutive androstane receptor (CAR) activation.

The DS summarised the available studies in a table which included:

- 1 x *in-vitro* CAR reporter gene assay (*Omiecinski, 2014*).
- 1 x *in-vivo* 28-day mouse dietary studies for evaluation of liver effects (*Elcombe, 2015*).
- 1 x *in-vitro* study with mouse hepatocytes (*Lowes, 2015a*)
- 1 x *in-vitro* study with human hepatocytes (*Lowes, 2015b*).
- 1 x *ex-vivo* 28-day dietary mouse hepatocellular proliferation/enzyme induction study (*Haines, 2012*).

There were no *in-vivo* studies with CAR-knock out rodents for confirmation of CAR mediated effects.

The *in vitro* CAR activation assay confirmed pydiflumetofen was a direct activator of CAR from the mouse, rat and human, and that the CAR constructs from the different species were efficacious in that they were all functionally expressed in COS-1 cells (monkey kidney immortal fibroblast cell line), although the human constructs showed lower activity than those from rodents.

The *in vivo* 28-day male mouse dietary study for investigation of liver effects confirmed (at the highest dose, 324 mg/kg/day), increased liver weights (↑ 22-24%), 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 was accompanied by a histopathology finding of increased mitosis.

The *in vitro* study with male CD-1 mouse hepatocytes resulted in increased cell proliferation (S-phase of the cell cycle) and in initially increased PROD and BROD activities (CYP2B/CYP3A expression).

The *in vitro* study with male human hepatocytes (from a single donor) had no effect on cell proliferation (S-phase of the cell cycle). CYP2B/3A activities (measured as PROD and BROD activities) were elevated.

The *ex vivo* 28-day mouse dietary study focused on enzyme analysis of liver samples. The study was designed to evaluate liver peroxisomal and microsomal enzyme expression in male and female mice on days 3, 7 and 28-days following exposure to pydiflumetofen at

up to 7000 ppm (dose in mg/kg not reported).

- CN^--insensitive palmitoyl CoA oxidase: slight suppression → no evidence for enhanced hepatic peroxisomal β-oxidation.
- total cytochrome P450 content (1.2 – 1.8-fold vs controls, day-28): dose-related increase in hepatic total P450 content
- EROD activity: weak effect (1.3 – 1.4-fold vs controls, 28-days) → no evidence for significant CYP1A expression. There was an initial burst of activity on days 3 and 7 (2-3 fold increase relative to control).
- PROD: substantial and significant enhancement of activity (9 – 15-fold in males, [about 3-5-fold in females] vs controls, 28-days) → evidence of strong CYP2B enhanced expression.
- BQ: small enhancement of enzyme activity (1.5 – 3.5-fold vs controls, day-28) → evidence for limited CYP3A enhanced expression.
- Lauric acid 12-hydroxylation (LAH): weak enhancement of enzyme activity in males only (1.3 – 3.5-fold vs controls, day-28) → evidence for limited CYP4A activity

The study showed liver enzyme effects consistent with a typical CAR/PXR activator; a dose-dependent increase of total cytochrome P450 content (max. 1.8x), PROD (max. 15x) in males, max. 5x in females), and BQ (max. 3.5x in males, max. 2.5x in females) was observed. AhR involvement was shown to be weak (no significant EROD activity). There was limited evidence for a small increase in expression of CYP4A which suggests some limited involvement of PPARα activation though there was no clear evidence for enhanced hepatic peroxisomal β-oxidation.

Inhibition of apoptosis and other associative events in the CAR associated tumour model have not been investigated (e.g. altered epigenetic changes, gap junctional intercellular communication and oxidative stress).

The available mechanistic data indicate that the MoA for liver tumours in mice is supportive of hepatocellular proliferation induced by activation of the CAR.

2.2 Conclusions

The evaluation by the DS of mode of action studies against the IPCS and ILSI/HESI framework demonstrated a CAR activation MOA. Data generated in human hepatocytes indicated human non-relevance due to quantitative differences in responses to proliferative stimuli

3. Mechanism of action and supporting data relevant for neoplastic findings in the female rat thyroid

3.1 Description and results from the mechanistic studies

The DS briefly referenced two mechanistic studies (table 40 of the CLH report) that were conducted to clarify the mode of action (MOA) for the slight numerical increase in the incidence of thyroid follicular cell adenomas (only in the female rat), at the top dose of 102 mg/kg/day. The incidence of adenomas did not reach statistical significance on pairwise comparison or show a dose-related trend. There was no discussion about these studies in the CLH report, only in the DAR:

- 1 x *in vitro* thyroid peroxidase activity in rats (*Lake, 2014*).
- 1 x *ex vivo* hepatic microsomal UDP-GT activity in livers of male rats (*Lake, 2015*). Samples derived from male rats on 90-day dietary study.

The aim of the *in vitro* thyroid peroxidase activity study was to evaluate the effect of pydiflumetofen (0, 0.007, 0.1, 1.5 and 10 µM) on rat thyroid peroxidase activity *in vitro*. A pooled thyroid gland microsomal preparation from five male rats was assayed for thyroid peroxidase activity by determining the monoiodination of L-tyrosine. The positive control, 6-propyl-2-thiouracil (PTU; 10 µM) resulted in a 99.9% inhibition of thyroid peroxidase activity. Pydiflumetofen had no effect on rat thyroid peroxidase activity at any concentration tested.

The *ex vivo* hepatic microsomal UDP-GT activity study was designed to evaluate the effect of treatment with pydiflumetofen on hepatic microsomal UDP-glucuronosyl transferase activity. Male rat liver samples were taken at termination of the 90-day dietary toxicity study where the rats were given diets containing 0, 18.6, 111, and 587 mg/kg/day active substance. Liver samples were not taken from the top dose group (16000 ppm or 1187 mg/kg/day).

Hepatic microsomal UDP-glucuronosyl transferase activity (using thyroxine as substrate), expressed as (i) specific activity, (ii) per gram of liver, (iii) per total liver and (iv) per relative liver weight, was significantly ($p < 0.01$) increased to 171, 194, 224 and 239% of control, respectively, by treatment with 111 mg/kg/day pydiflumetofen and to 288, 347, 421 and 486% of control, respectively, by treatment with 587 mg/kg/day pydiflumetofen ($p < 0.01$). Female livers were not tested.

3.2 Conclusion

The RMS concluded that pydiflumetofen was a positive inducer of rat hepatic microsomal UDP-glucuronosyl transferase activity as determined from assays that used thyroxine as substrate.

4. Consideration of the dose level selection in the toxicity studies and the carcinogenic potential and impact of 2,4,6 TCP

4.1 Background

The DS described uncertainties expressed in the DAR regarding the dose level selection employed in some of the toxicity studies based on pharmacokinetic data (see section 2.6.1 and B.6.1 in volume 3). They were critical of the additional pharmacokinetic studies that investigated the TK profile of the active substance with non-radiolabelled pydiflumetofen following single or repeated doses. Pydiflumetofen was extensively and rapidly metabolised and without an appropriate radiolabel it was not possible to follow the fate of several important metabolites including 2,4,6-trichlorophenol (2,4,6 TCP). The dose selection arguments put forward by the applicant did not consider one of the major circulating metabolites following administration of pydiflumetofen, i.e. 2,4,6 TCP. Its plasma concentration largely exceeded that of the parent. It would have been appropriate to also investigate the pharmacokinetics of 2,4,6 TCP following repeated or single oral administration of pydiflumetofen, especially since this metabolite is of toxicological concern (classified as Carc 2. H351, by the European Union, and similarly by other international regulatory bodies).

The applicant suggested that as the dose increased, the fraction of pydiflumetofen absorbed decreased and therefore systemic levels of metabolites were presumed to also not increase. The kinetic studies showed that systemic exposure to pydiflumetofen increased in an approximately proportional manner after both single and repeated doses up to 30 mg/kg (with oral absorption remaining constant at around 85-90% of the dose). Between 30 and 100 mg/kg systemic exposure also increased but in a non dose-proportional manner. As the dose increased beyond 100 mg/kg (oral gavage), 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 pydiflumetofen was the major component in faeces which contained up to 63% of the dose, but less than 0.2% was detected in bile. According to the applicant, 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. At very high doses, absorption was assumed not to be influenced by repeated administration and therefore systemic levels of metabolites would not be expected to increase.

The DS and RMS reserved most concern for the dose levels selected by the applicant for the long-term and reproductive toxicity studies. The applicant justified their dose selection based on TK data and dose limited absorption, i.e. with higher oral doses, less compound was absorbed into the blood. However, this argument was regarded as insufficient. This does not mean that systemic exposure does not continue to increase with doses higher than the highest dose levels selected by the applicant for the long-term and reproductive toxicity studies. On the contrary, comparison between plasma AUCs determined after administration in rats of the phenyl radiolabelled active substance at dose levels up to 1000 mg/kg/day, showed that systemic exposure still increased beyond 100 or 300 mg/kg/day:

1. by 4-fold between 100 - 1000 mg/kg bw/d
2. and by 1.7-fold between 300 - 1000 mg/kg bw/d

Also, increased systemic exposure at very high doses was confirmed by the short-term repeated dose studies where an increase in toxicity (liver and body weight effects) was observed with increasing doses beyond the maximal doses selected by the applicant for the long-term or reproductive toxicity studies.

The DS and RMS believed the doses of pydiflumetofen chosen for the long-term studies might not have been sufficiently high to cover the carcinogenic potential of 2,4,6 TCP in the rat. High systemic exposures of pydiflumetofen (and consequently 2,4,6 TCP) resulting from administration of doses higher than 300 mg/kg (males) have not been tested in the rat long-term study. Furthermore, female rats were only dosed up to 102 mg/kg, at which concerns for thyroid adenomas were already expressed in the 2-year rat dietary study.

Leukaemias were observed from an estimated dose of 250 mg/kg/day of 2,4,6 TCP in male rats in a long-term toxicity study from the NTP (NCI, 1979; see Additional key elements for a summary of this study). *Note*: it is not clear how this dose was calculated since no food consumption data was recorded for the original NCI study.

In the CLH report (2018) for pydiflumetofen, no tumours were observed following 2-year administration in male rats at a dose of up to 300 mg/kg/day. The DS believed that the maximum tolerated dose (MTD) was reached in the 2-year study in rats as the top dose in males (300 mg/kg/d) resulted in an 18% reduction of body weight. They considered that the carcinogenic potential of pydiflumetofen was thus appropriately assessed. To verify whether higher doses of pydiflumetofen (>300 and up to 1000 mg/kg bw/day) would have

covered the carcinogenic potential of 2,4,6 TCP, a risk analysis was performed by the RMS to estimate systemic exposure to 2,4,6 TCP. This considered the oral absorption of pydiflumetofen and the proportion of 2,4,6 TCP measured in plasma (% of AUC) after an oral administration of radiolabeled pydiflumetofen. The DS concluded that the higher tested dose of 300 mg/kg bw/d of pydiflumetofen in the long term rat study was actually not sufficiently high to elicit the carcinogenic potential of its major circulating metabolite 2,4,6 TCP (DAR, B.6.8.1.6, p614). They go on to state that a dose of about 1000 mg/kg/day would be required. They then further stated that the mammalian toxicity data package on pydiflumetofen had sufficiently assessed the toxicity of 2,4,6 TCP and its conjugates, but they did not explicitly state that the carcinogenicity of the metabolite has been adequately assessed.

4.2. Conclusions

It is clear that the RMS (as expressed in the DAR) had doubts over whether pydiflumetofen had been adequately tested in a high enough dose in the long-term or reproductive toxicity studies. They concluded on one hand that the highest dose (for males) in the long term rat study was actually not sufficiently high to elicit the carcinogenic potential of the metabolite 2,4,6 TCP; while on the other hand stating that the MTD was achieved and that the mammalian toxicity data package on pydiflumetofen had sufficiently assessed the toxicity of 2,4,6 TCP and its conjugates. It was not a very clear conclusion by the DS/RMS.

Comments received during public consultation

Two MSCA submitted comments.

Comment 1

This MSCA supported classification of pydiflumetofen with Carc 2, H351 based on a significant increase in liver cell adenoma and carcinoma in high dose male mice. They accepted a MoA involving CAR in the mouse but the lack of tumourigenicity in rats if the same MoA was operating was problematic. This could be due to (i) further modes of action of unknown human relevance are involved in tumour formation in male mice or (ii) the absence of a similar neoplastic effect in the rat might be due to the rather low dose levels (up to 100 or 300 mg/kg bw/day in female/male rats) employed in the long-term study. The MSCA were not convinced that the MTD was reached.

The DS responded that it considered that the MTD was reached. Significant treatment-related decreases were observed in body weight gains in high dose males (↓ 18%) and high dose females (↓ 13%). The DS agreed that the major metabolite 2,4,6-TCP, must be taken into consideration. The DS calculated that a pydiflumetofen dose of 1000 mg/kg bw/day could potentially give rise to a systemic exposure of TCP which might account for 25% of leukaemias observed in the rat (T25) based on the NTP study data performed with 2,4,6-TCP (NCI 1979). The relevance of this risk based assessment was questioned by RAC. The DS agreed with the proposed MoA leading to liver tumours in mice (through CAR activation) and that sufficient data was provided by mechanistic studies. The DS also mentioned concern regarding the use of SDHI (succinate dehydrogenase inhibitor) fungicides in agriculture.

Comment 2

The second MSCA commented with respect to the rat thyroid adenomas and the increase

in eosinophilic foci of cellular alteration in the liver of male mice from the long-term studies and their relevance in setting hazard-based endpoints such as NOAELs. They considered the thyroid adenomas to lie outside the HCD and be treatment related in the rat. They considered the preneoplastic foci to be treatment related at the 75 ppm (9.2 mg/kg/day) dose in male mice.

The DS responded that following peer review the most relevant HCD within a 5 year timeframe gave a background incidence of 0-5.8% (3/52) (as presented in Table 6.5-16 in the revised DAR) for rat thyroid adenomas. The increase in the incidence of thyroid follicular cell adenomas (3/51 (5.9%) vs 1/51 (2%) in control), observed at the highest tested dose for females, 1500 ppm (102 mg/kg/day), can be considered to be within the historical control data and not treatment related. In support of this they noted there were no preneoplastic lesions (hyperplasia) observed at 12 or 24 months in the long term study and no histopathological findings were observed in thyroids from females in other rat toxicity studies (28/90 days and 2 generation reproductive toxicity). Mechanistic data had shown that pydiflumetofen does not have a direct effect on thyroid peroxidase in the rat (*in vitro*) and therefore, pydiflumetofen was not acting via a direct effect on the thyroid.

The DS explained that during the peer review process under Regulation 1107/2009, new HCD for eosinophilic foci of cellular alteration in the liver of male mice from long-term studies was received (2007 – 2013, a total of nine 80-week mouse studies). This new data showed that the incidence of eosinophilic foci at 75 ppm (9.2 mg/kg/day equivalent dosing and incidence of 8%) was still outside the revised HCD range (0-6%). The DS noted that the increase in preneoplastic foci at the 9.2 mg/kg/day dose was not statistically significant; they occurred in terminal animals only, indicating there was no reduction in latency; and eosinophilic foci were not observed in the 90 day mouse study where only an increase in hepatic centrilobular hypertrophy was observed in the absence of hepatic necrosis. Taking several other factors into account, the DS did not consider this effect at 9.2 mg/kg/day to be treatment related and that a NOAEL could be set at 9.2 mg/kg/day.

During the second RAC member consultation of the opinion for pydyflumetofen which was opened some time before RAC-48, industry submitted three position papers regarding the adequacy of dose selection, the liver carcinogenic response in male mice and the delay in sexual maturation in rats in the 2-generation study.

The position paper on carcinogenicity was entitled - Company "Comments: Pydiflumetofen - Mechanism of Mouse Liver Carcinogenicity and Non-Relevance to Man". They concluded "*The mechanism by which Pydiflumetofen causes liver tumours in the male mouse has been clearly demonstrated as mediated by the constitutive androstane receptor (CAR). The key event in this MoA has been demonstrated to not occur in human. All relevant alternative MoAs have been excluded*". No new data was presented in this report. It outlined the work that was done and how the available data fitted with the CAR MoA to produce tumours in mice.

The position paper on dose selection was entitled – Company "Comments: Pydiflumetofen – Adequacy of Dosing in Toxicology Studies. The company concluded that "*Systemic exposure to pydiflumetofen becomes limited by absorption as the external dose increases, until there is no appreciable increase in internal exposure. At doses above the linear range of toxicokinetic exposure, the inherent hazard of the chemical cannot be assessed, as large quantities of unabsorbed test item residing in the GI tract may impact on food transit time and nutrient absorption, disrupting normal biological homoeostasis. Therefore, adverse effects observed at doses exceeding the inflexion point of linear kinetics are not related to the test-item, but may be related to biological stresses and not relevant to*

hazard characterisation. Therefore, based on the non-linear kinetics through absorption limited exposure, which has clearly been demonstrated for individual animals, studies have been more than adequately dosed (as the high doses were actually set above the point where linearity is lost and the inherent hazard of pydiflumetofen has been fully assessed".

Additional key elements

5.1 National Cancer Institute. (1979). Bioassay of 2,4,6-Trichlorophenol for Possible Carcinogenicity

5.1.1 Background & Summary

Dietary Carcinogenicity Studies in B6C3F1 Mice and Fischer 344 Rats. The studies were not conducted to a regulatory guideline and there was no claim to GLP compliance. Quality Assurance statements were not provided (DAR B.6.8.1.6, study K-CA 5.8.1/41).

The test substance was 2,4,6-TCP (96-97%). Bioassays of 2,4,6-trichlorophenol (2,4,6-TCP) for possible carcinogenicity were conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice for 2 years (50 animals/sex/test group; 20/sex in control groups). Low (5000 ppm) and high dose (10 000 ppm) test groups were employed for both rats and mice. Female mice began with 10 000 and 20 000 ppm but after 38 weeks were lowered due to reduced body weights to 2500 and 5000 ppm for 67 weeks; exposures averaged 5200 and 10 400 ppm. Food consumption data was not recorded so it was not possible to calculate the mean dose administered to the animal test groups.

Over the course of the studies, group mean body weights of all 2,4,6-TCP treated groups (both sexes of rats and mice) were decreased compared to controls.

Survival of 2,4,6-TCP treated groups (both sexes of rats and mice) was not reduced compared to concurrent controls. No increase in clinical signs were observed in 2,4,6-TCP treated groups relative to those in controls.

2,4,6-TCP was carcinogenic in male F344 rats, inducing lymphomas or leukaemias and it was also carcinogenic in both sexes of B6C3F1 mice, inducing liver hepatocellular carcinomas and/or adenomas.

5.1.2 Neoplastic findings

In male and female rats (table below), leukaemias were increased and dose related (the data for lymphomas was not so clear and is perhaps best described as equivocal). Differences between the low and high dose groups were not linear for combined incidences (50% vs 58% for males and 22% vs 26% for females). Incidences were statistically significantly greater than concurrent controls. The incidences were greater than the mean HCD incidences.

In mice, liver toxicity, including individual liver cell abnormalities, focal areas of cellular alteration, and focal and nodular areas of hyperplasia were commonly present in 2,4,6-TCP groups of mice. Hepatocellular carcinomas/adenomas were increased in both males and females, which were dose related, statistically significantly higher in all dose groups, and exceeded contemporary mean HCD rates. In addition, female but not male mice showed distinct but non-dose related increases in lymphoma relative to their control group.

5.1.3 Conclusions

2,4,6-TCP was carcinogenic in male Fischer 344 rats, inducing leukaemias. The test chemical was also carcinogenic in both sexes of B6C3F1 mice, inducing hepatocellular carcinomas and/or adenomas. Lymphomas also appeared in treated females.

***Table**: Lesions/Tumours induced by 2,4,6-TCP in two-year dietary exposure bioassays in Fischer 344 rats and B6C3F1 mice*

Dose group (ppm)	**Male rats**			**Female rats**		
	Control	**5000**	**10000**	**Control**	**5000**	**10000**
No. necropsied	20	50	50	20	50	50
Location Lesion: number of animals with lesion (%)						
Hematopoietic system						
Lymphoma	1 (5)	2 (4)	0	0	0	2 (4)
Leukemia	3 (15)[b]	23 (46)[b]	28 (56)[b]	3 (15)	11 (22)	10 (20)
Combined	4 (20)[b]	25 (50)[a]	29 (58)[b]	3 (15)	11 (22)	13 (26)
Historical controls: Male rats 11/255, 4% (Monocytic leukemia); female rats 42/420, 10% (Combined leukemia or lymphomas)						
	Male mice			Female mice[c]		
Dose group (ppm)	**0**	5000	10000	0	5214	10428
No. necropsied	20	49	47	20	50	48
Liver						
Hyperplasia	2 (10)	12 (24)	6 (13)	1 (5)	1 (2)	6 (13)
Adenoma	3 (15)[b]	22 (45)	32 (68)	1 (5)[b]	12 (24)[a]	17 (35)[b]
Carcinoma	1 (5)	10 (20)	7 (15)	0[b]	0	7 (15)
Combined	4 (20)[b]	32 (65)[b]	39 (83)[b]	1 (5)[b]	12 (24)[a]	24 (50)[b]
Historical controls (adenoma or carcinoma): Male mice 99/323 30.7%; female mice 14/324, 4.3%						
Hematopoietic system						
Lymphoma	2 (10)	3 (6)	1 (2)	2 (10)	12 (24)	7 (15)

[a] P values: P < 0.05
[b] P values: P < 0.01 d = decrease; P at control column = dose response trend; at dose groups = compared to controls.
[c] Average dietary levels for female mice: 38 weeks at 10000 and 20000 ppm, then 67 weeks at 2500 and 5000 ppm resulting in time-weighted averages 5214 and 10428 ppm.
[d] Decrease

The HCD from the performing laboratory were provided on selected primary tumours for studies conducted on or before 1979. The number of studies used to compile the HCD was not provided nor was there any information on those studies other than the combined tumour incidence.

5.2 Notes on absorption and metabolic profile of pydiflumetofen in rats

The proportion of pydiflumetophen administered oral decreases with increasing dose levels. High concentrations in male rats are assumed to be 22.4% absorbed. High concentrations in female rats are assumed to be 48% absorbed.

The study of *MacDonald and Jewkes* (2015) showed that the metabolite profile in plasma of rats following a single administration of [Phenyl U-^{14}C] or [pyrazole U-^{14}C] pydiflumetofen was similar, irrespective of the dose or gender. That is, approximately 55% of a systemic dose of parent represented the proportion of 2,4,6-TCP and related compounds in blood. It is noted that the major component of blood was not 2,4,6-TCP but its sulphate conjugate (≈ 40%). Any estimation of the systemic dose to 2,4,6-TCP assuming 55% of the pydiflumetofen dose is a gross overestimation for this metabolite yet

this estimate was used in the DAR for calculating potential exposure to 2,4,6-TCP and comparing the resulting values with estimates of the dose used in the NTP studies when they investigated 2,4,6-TCP in rats and mice.

Assessment and comparison with the classification criteria

6. Carcinogenicity

6.1 Introduction

Pydiflumetofen induced liver tumours in mice and thyroid tumours in rats, thus there is a need to consider whether classification for carcinogenicity is appropriate. There is no information from studies in humans to inform on carcinogenic potential and so classification in category 1A may be excluded from further consideration.

6.2 Rat thyroid tumours

In females there was a slight numerical increase (3/51 (5.9%) vs 1/51 (2%) in control) in the incidence of thyroid follicular cell adenomas at 102 mg/kg/day (1500 ppm), which did not reach statistical significance on pairwise comparison, or show a dose-related trend, and was at the upper bound limit of the most relevant historical control range of 0-5.8% (table below). There were no recorded thyroid follicular cell carcinomas at any dose. There was no substance-related effect on the incidence of C-cell tumours. There were no preneoplastic lesions observed. Some non-neoplastic microscopic findings were present. The incidence of 3/51 (5.9%) observed for follicular cell hyperplasia in females at the highest dose of 102 mg/kg/day was within the historical control data (range: 0- 6%). These findings are considered possibly related to administration of pydiflumetofen but are not sufficient for classification.

Table: *Summary of thyroid neoplastic findings in female rats*

Finding	**Dose Concentration (mg/kg/day) Females**				
	0	**10.2**	**31.0**	**102**	**Historical control data[#]**
Thyroids (no. examined)	51	52	51	51	439
Follicular cell adenoma	1 (2%)	0	0	3 (5.9%)	9 (2%) range 0-5.8%
Hyperplasia; Focal, Follicular cell	1 (2%)	3 (5.8%)	0	3 (5.9%)	12/323 (3.7%) range 0-6%
Thyroid masses at necropsy	0	0	0	2	--

Note: thyroid follicular cell adenomas were also present in males but their incidences at all dose levels was below the concurrent controls.

Regarding the metabolite 2,4,6-TCP, there is no data to indicate that a sufficiently high dose of parent active substance was tested in the long-term study to elicit the carcinogenic potential of one of the major circulating metabolites. The carcinogenicity of this metabolite has not been adequately assessed in either the long-term rat or mouse studies. The RMS estimated that a limit dose of 1000 mg/kg/day pydiflumetofen would

have been required to at least reach levels of 2,4,6-TCP sufficient to elicit a tumourigenic response comparable to 25% that observed in male rats in the NTP study with 2,4,6-TCP[4]. Whether such information is relevant to a classification proposal for pydiflumetofen needs to be considered. With the available data and the maximum tested dose of approximately 300 mg/kg/day in males there was no indication of a concern for leukaemia in the tested animals (the major carcinogenic effect of 2,4,6-TCP in male F344 rats). The raw data from the long-term feeding study for pydiflumetofen showed no evidence of leukaemia or perturbations of white blood cell and lymphocyte counts.

Two mechanistic studies were performed investigating effects on the thyroid (table below). A mechanistic study on increased phase II metabolic activity in the livers of male rats indicated an increase of approximately 2-5 fold over concurrent controls in the activity of microsomal UDP-glucuronosyl transferase (at doses of 111 and 587 mg/kg/day over 90-days). This may account for increased thyroxine clearance by the liver and place the thyroid under secondary feedback pressure to increase T4 output and alter the expected incidence of thyroid follicular tumours. However, the incidence of follicular cell adenomas in males in the top dose group did not exceed those in the concurrent controls. A major weakness of the study is that it did not investigate UDP-glucuronosyl transferase activity from female rat livers. Females were the more sensitive sex and ideally the mechanistic investigation should have been performed with female livers rather than those from males. A second mechanistic study (males only), found that pydiflumetofen had no direct effect on rat thyroid peroxidase activity at any concentration tested.

RAC can conclude on a classification only based on an assessment of all the data that has been presented in the available studies. In this context, the rat thyroid adenomas occur at the upper boundary limit of the HCD range. There was no supporting evidence from males that pydiflumetofen elicits a tumourigenic response. Thyroid tumours were not observed in mice. RAC notes that it is regrettable that a higher concentration was not investigated in the female rat and also notes that female livers were not analysed for increased phase II metabolic activity. RAC concludes that there is no firm evidence from the available data for a neoplastic response in the rat thyroid as a consequence of exposure to pydiflumetofen.

Table: *RAC Summary of mode of action studies investigating effects on the rat thyroid*

Endpoints investigated	Summary observations	Reference
1. In-vitro thyroid peroxidase activity. - microsomal preparation from five male rats - Table 40 CLH report	1. Pydiflumetofen had no effect on rat thyroid peroxidase activity.	Lake, (2014)
2. Ex-vivo 90-day rat oral (dietary) study - (Crl:WI(Han) strain - Male liver samples - Pydiflumetofen tested (0, 18.6, 111 and 587 mg/kg/day) - Total cytochrome P450 levels - Enzymes: hepatic microsomal UDP-GT	**a. Enzyme activity:** 1. Pydiflumetofen induced substantial hepatic microsomal UDP-GT activity (2-5 fold relative to control). **b. Hepatic microsomal protein:** 1. ↑ hepatic microsomal protein content; 114 and 122% of control at the two top doses.	Lake, (2015)

[4]National Cancer Institute. (1979). Bioassay of 2,4,6-Trichlorophenol for Possible Carcinogenicity. National Cancer Institute Technical Report Series No. 155, 1979. National Institutes of Health, Bethesda, Maryland 20014, USA.

activity - Table 40 CLH report		

6.3 Mouse liver tumours

In animals sacrificed at 18 months, an increased incidence of hepatocellular carcinomas and adenomas was observed in <u>males</u> administered pydiflumetofen at 45 and 288 mg/kg/day compared with the control group (table below). In animals that received ≥ 45 mg/kg/day a clear increase in multiplicity of tumours was observed. <u>No neoplastic findings were observed in female mice</u>. No increases in liver tumours were noted in the rat lifetime study.

Table: *Summary of hepatocellular neoplastic findings in the liver in males*

Finding	**Dose Concentration (mg/kg/day) Males**				
	0	**9.2**	**45.4**	**288**	**Historical control data#**
Liver (no. examined)	50	50	49	50	250
hepatocellular carcinoma [multiple]	2 (4%) [0]	3 (6%) [0]	4 (8.2%) [0]	10* (20.0%) [2]	19 (7.6%) range 6-10% [3-5/250]
hepatocellular adenoma [multiple]	4 (8.0%) [0]	6 (12.0%) [0]	9 (18.4%) [7]	22** (44.0%) [14]**	45 (18.0%) range 10-28% [5-14/250]
Liver masses at necropsy	5	9	14	20	

* 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 Charles River Edinburgh between 2007 and 2009.

In support of the findings in liver, hepatocellular carcinomas and adenomas correlated with liver masses observed at necropsy at ≥ 45 mg/kg/day. Pre-neoplastic lesions were apparent. There was an increasing numerical trend for a higher incidence of eosinophilic foci of cellular alteration in the livers of males with increasing dose (table below). The incidence was only significantly higher (Fisher's exact test) in males dosed at 288 mg/kg/day compared to the control group.

Table: *Comparison of histopathology findings in males (including presumptive preneoplastic lesions)*

Finding		**Dose Concentration (mg/kg/day) Males**				
		0	**9.2**	**45.4**	**288**	**HCD**
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	

focus of cellular alteration, eosinophilic	total	1	4	6	10**	7/250 (2.8%)
	minimal	0	0	1	1	Range: 0 – 6%
	mild	1	2	1	3	
	moderate	0	2	4	6	

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

In summary, pydiflumetofen was considered to cause carcinogenic effects in the livers of male mice that received ≥ 45.4 mg/kg/day where higher incidences of liver tumours (hepatocellular carcinomas and adenomas (multiple)) were observed. A clear dose response relationship was evident.

6.3.1 Mouse liver tumours: assessment of the Mode of Action

The tumour profile observed in pydiflumetofen carcinogenicity bioassays was typical of a non-genotoxic mechanism (single species, single sex and single organ involvement without decreased latency). MoAs involving induction of other CYP P450 isoforms or peroxisome proliferation may 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 showed that pydiflumetofen treatment did not result in any significant biochemical evidence for peroxisome proliferation within the liver hepatocytes (e.g. no ↑ CN^--insensitive palmitoyl CoA oxidase, and limited ↑ LAH). Hepatocellular cytotoxicity and subsequent regenerative proliferation, such as that caused by chloroform, is another mechanism by which carcinogenesis can occur. This mechanism is typically characterised by sustained diffuse necrosis and cellular proliferation. In this case it can be excluded for pydiflumetofen because the data from the i*n vivo* studies demonstrated a lack of hepatic damage and regenerative proliferation at all time points investigated.

There are various possible mechanistic explanations that can be considered for the carcinogenic response in mice and a limited investigation into these other modes of action was undertaken, which may be summarised as follows:

- genotoxicity → negative data in this case → conclusion: unlikely
- cytotoxicity → the liver was the target organ but there were no indications from histopathology to support this as a primary MoA. The incidences of focal/ multifocal necrosis and focal/ multifocal vacuolation were zero to low and similar across all treatment groups, or similar to controls. However, *in vitro* tests with human hepatocytes did show increased sensitivity relative to mouse hepatocytes, so the question regarding cytotoxicity as a factor still remains unresolved.
- PPARα receptor activation → limited to no effect in this case → conclusion: unlikely
- CAR/PXR receptor activation → positive data in this case → conclusion: plausible
- AhR receptor activation → very limited effect → conclusion: unlikely as a primary mechanism, possible crosstalk between receptors or limited induction

of Cyp1A/AhR

- Porphyria → no data
- Endocrine mediated proliferation → no data, no evidence from other studies.
- Immunosuppression → no data

Recognising that pydiflumetofen may be associated with a hepatocarcinogenic effect in mice, the applicant sponsored a series of mechanistic studies to investigate a possible non-genotoxic mode of action involving liver stimulation via constitutive androstane receptor (CAR) induction. The DS presented these studies and others from the plant protection DAR. Some of the key and associative events in this process are:

- CAR activation
- Altered gene expression specific to CAR activation
- Increased cell proliferation
- Inhibition of apoptosis
- Clonal expansion leading to altered foci
- Liver adenomas/carcinomas

Such a non-genotoxic mode of action leading to liver tumour formation in rodents has been considered of limited to no relevance to humans. The mechanistic data presented in the CLH report and the DAR suggests that the CAR activation model (table below) is the most plausible. The data from pydiflumetofen support a proposed MoA in male CD-1 mice involving the following key events:

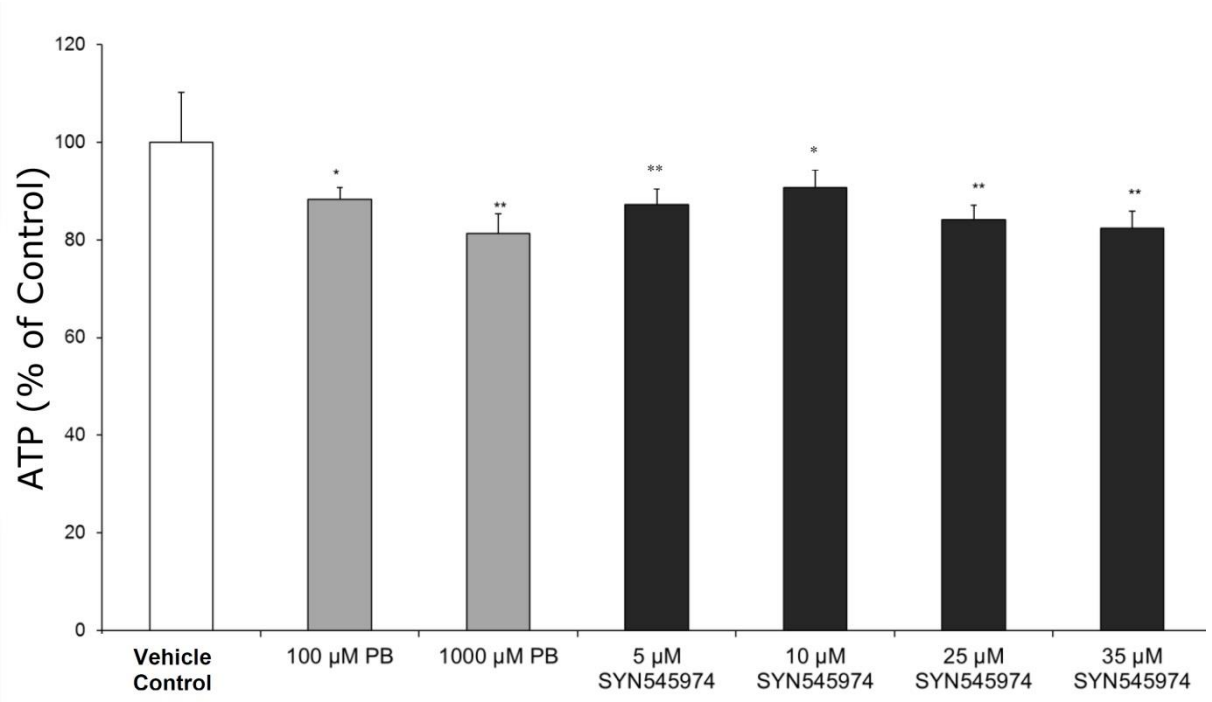
- Activation of the constitutive androstane receptor (CAR).
- An early, transient, increase in hepatocellular proliferation.
- Increased hepatocellular foci as a result of clonal expansion of spontaneously mutated (initiated) cells.
- Eventual progression to form liver tumours.

And the following associative events:

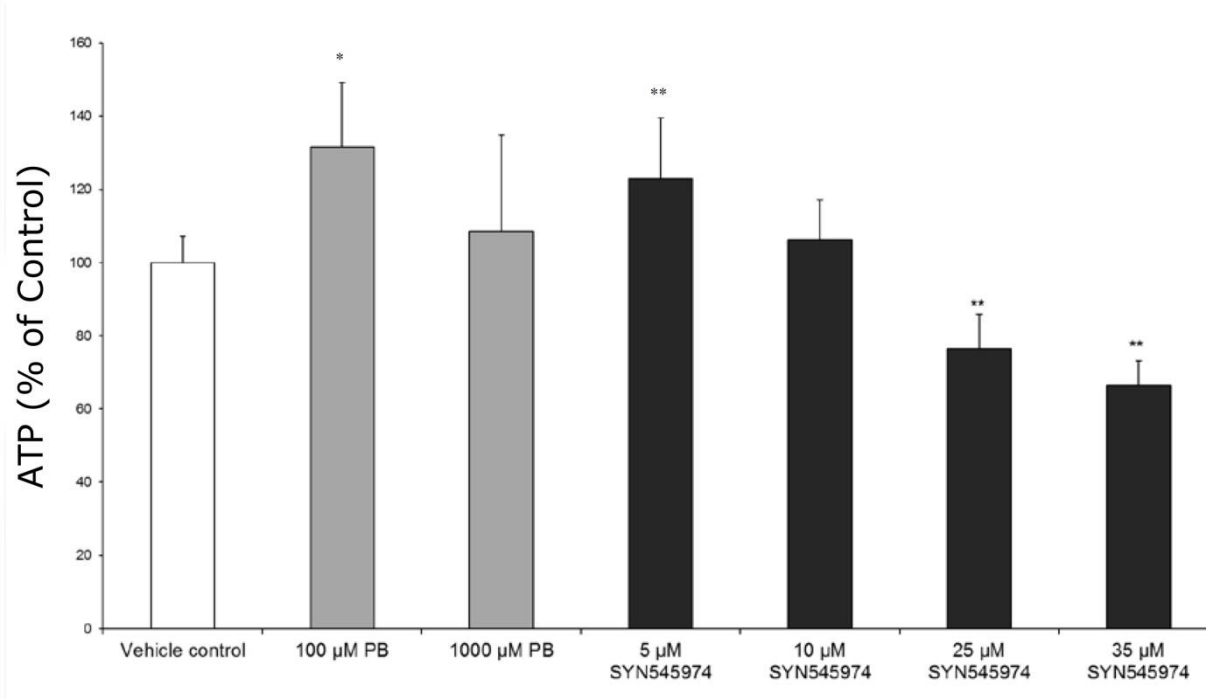
- Increased expression of genes encoding cytochrome P450s (CYPs), particularly Cyp2b/Cyp3a isoforms.
- Increased incidence of hepatocellular hypertrophy.
- Increased liver weight.

While no transgenic knockout animal or cell models nor mRNA induction studies were used in the investigation into the MoA, and this is a valid criticism of the pydiflumetofen data package, experimental data demonstrated that pydiflumetofen did not produce the key event of cell proliferation in human liver cells *in vitro*. However, it must be noted that the tests in *in-vitro* human hepatocytes were conducted with only one donor which limits the degree of certainty as to whether this effect matches the known species differences that have been demonstrated for other CAR activators. Also noted is the greater sensitivity of the human hepatocytes to cytotoxicity with pydiflumetofen treatment (figure below). Hepatocyte toxicity was assessed following 96 hours of culture and was indicated by ATP depletion. Significant cytotoxicity (as measured by decreases in ATP) was observed at

levels > 10 uM such that meaningful results for replicative DNA synthesis with higher concentrations of pydiflumetofen are lacking. In mouse hepatocytes there was also cytotoxicity but only at higher levels of pydiflumetofen as illustrated by a significant depletion of ATP (≥ 56.8% of control) and therefore cytotoxicity with concentrations ≥ 50 µM (preliminary study CXR1490). Human hepatocytes are more sensitive to pydiflumetofen toxicity. Based on such limited test samples it is difficult to conclude on a qualitative difference in the established CAR activation MoA for hepatocarcinogenesis between rodents and humans.



(A) Mouse hepatocytes. ATP content as mean % of control ± standard deviation, (n=6). A one-way ANOVA followed by the Dunnett test was performed on the results; * statistically different from control $p<0.05$; ** $p<0.01$



(B) Human hepatocytes. ATP content as mean % of control ± standard deviation, (n=6 replicates, 1 donor liver). A one-way ANOVA followed by the Dunnett test was performed on the results; * statistically different from control $p<0.05$; ** $p<0.01$.

Figure: *In Vitro* Hepatocyte cytotoxicity – ATP levels. (A) Pooled mouse hepatocytes. (B) Liver hepatocytes from a single human donor.

It must be further noted that data to investigate alternative modes of action is limited. It has not been adequately demonstrated with pydiflumetofen that other mechanisms are not also at work. There is some increase in EROD and LAH enzyme expression but no mRNA induction studies were performed to investigate alternative or CAR-supporting pathways. No positive controls for aryl-hydrocarbon receptor (AhR) activation/CYP1A induction (e.g. 3-methylcholanthrene, omeprazole) or LAH expression/CYP4A induction (e.g. fibrate drugs, other peroxisome proliferators) were investigated so it is not possible to put into context the small increases in EROD and LAH that were observed. Other effects of CAR activation such as the suppression of apoptosis were also not investigated, though it is recognised that some crosstalk with AhR (and/or CYP1A induction) may be indicative of apoptotic suppression. Consequently the data package supplied in support of the primary MoA is not as robust as some of those supplied in the past for other substances presented for consideration by RAC.

An explanation for the differential sensitivity between male and female mice with respect to the development of liver tumours is also lacking. The tumours were only observed in male mice. In the *ex-vivo* enzyme analysis of liver samples study (table below, #5) PROD activity in females is 50-80% reduced relative to males at day-7 and day-28, respectively, implying an attenuated CAR response in female mice; this however remains speculative.

6.3.2 The proposed Mode of Action for pydiflumetofen induced liver tumours

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, figure below). This promotes an environment which enables increased cell replication to occur, 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 a 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.

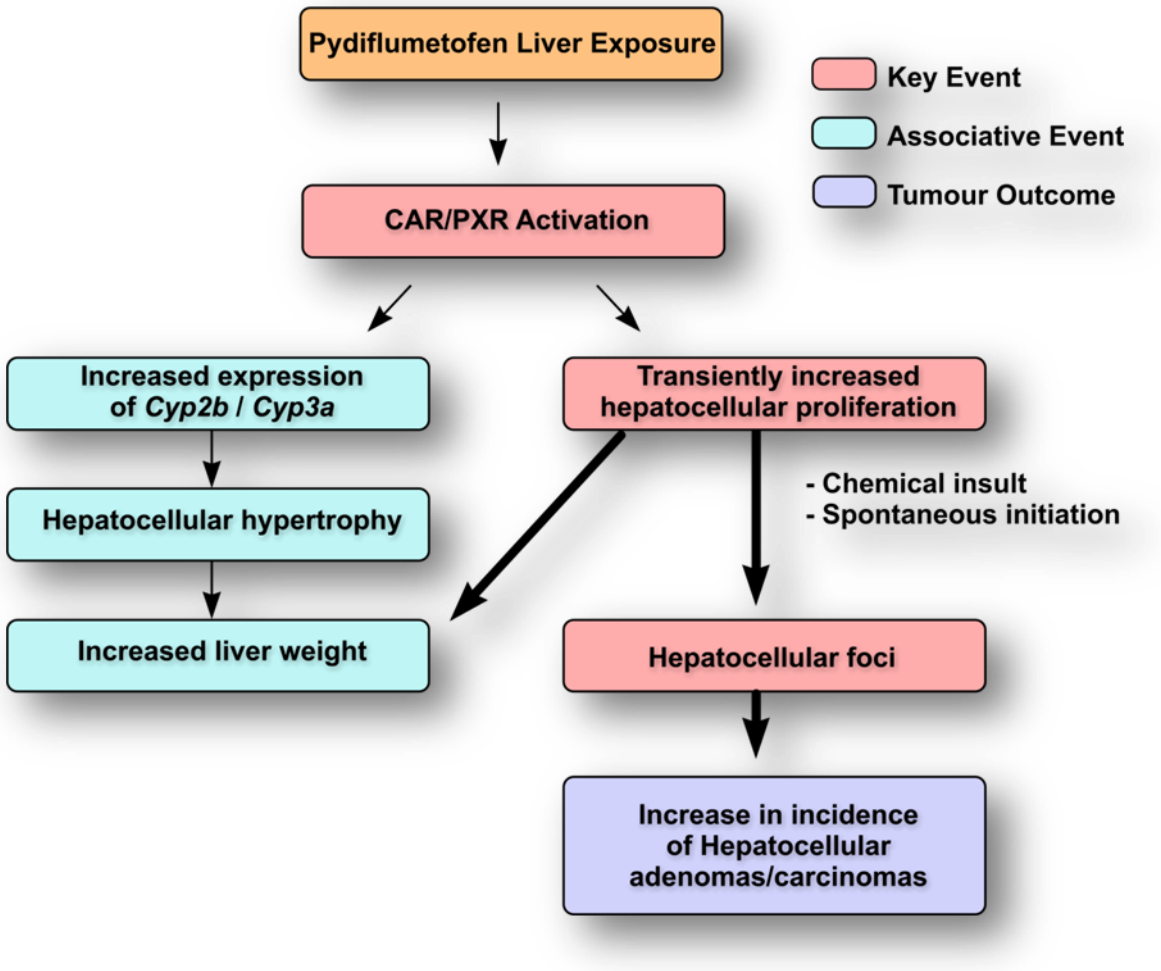


Figure: Mode of Action hypothesis for pydiflumetofen-induced liver tumour formation in male mice.

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.

The mechanistic studies showed the following results (see summaries in the table below):

1. Pydiflumetofen increased mouse and human hepatocyte activity of Phase I

xenobiotic metabolising enzymes consistent with activation of CAR/PXR nuclear receptors.

2. Pydiflumetofen was a direct activator of CAR from the mouse, rat and human, and was efficacious in all three species.
3. Pydiflumetofen did not increase hepatic peroxisomal β-oxidation (PCO) but did have a limited increase in LAH (surrogate for CYP4A).
4. Liver weight increased with concomitant hepatocellular hypertrophy.
5. Pydiflumetofen increased replicative DNA synthesis in a PB-like manner in mouse hepatocytes. However the increase was not that convincing and a clear dose response relationship is not evident (part A of the figure below)
6. Pydiflumetofen increased PROD activity in preference to BQ by about 5-fold *in vivo* in the mouse liver.
7. Pydiflumetofen did not increase replicative DNA synthesis in human hepatocytes although the study has been conducted with only one donor.
8. Pydiflumetofen had a small effect on EROD activity.

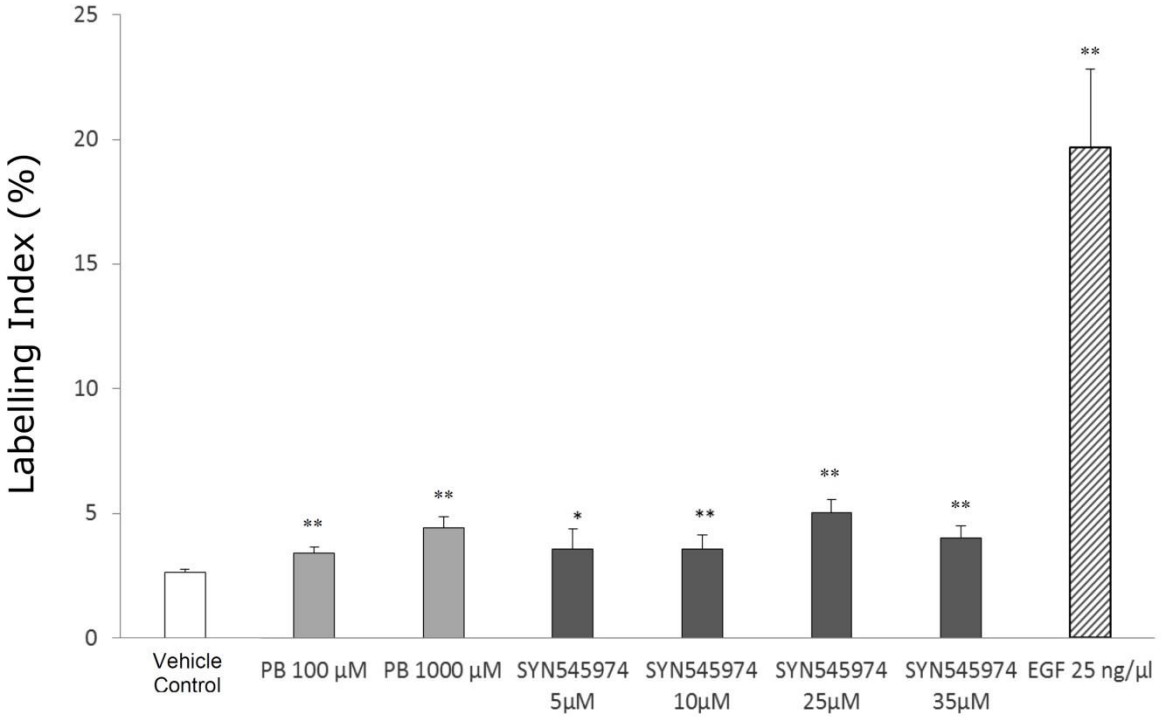
Table: *RAC Summary of mode of action studies investigating liver tumours*

Endpoints investigated	Summary observations	Reference
1. *In-vitro* CAR3 Transactivation Assay with Mouse, Rat and Human CAR - CAR 3 activation explored via cDNA expression vectors transfected into monkey COS-1 cells, along with necessary cofactors and a CYP2B6 response element-luciferase reporter construct. - Table 40 CLH report - Pydiflumetofen clearly interacts with and activates the CAR receptor construct from 3 species: the rat, mouse and human in a positive dose response manner. The data indicate that it is a generic CAR activator similar to Artemisinin (typically used as a reliable positive control for CAR activation in *in vitro* studies).	1. Pydiflumetofen is a direct human and rodent CAR activator. 2. Selective species positive controls robustly activated the appropriate CAR. **30 µM** rat CAR3 ↑ 42 fold mouse CAR3 ↑ 20 fold human CAR3 ↑ 15 fold **10 µM** rat CAR3 ↑ 37 fold mouse CAR3 ↑ 32 fold human CAR3 ↑ 13 fold **3 µM** rat CAR3 ↑ 14 fold mouse CAR3 ↑ 34 fold human CAR3 ↑ 5 fold **1 µM** rat CAR3 ↑ 2.8 fold mouse CAR3 ↑ 24 fold human CAR3 ↑ 1.5 fold	Omiecinski, (2014)
2. *In-vivo* 28-day mouse oral (dietary) study - CD-1 strain - Males only. - Evaluate liver effects - Treatment: 2, 7, and 28 days - Pydiflumetofen tested	**a. Liver:** 1. ↑ Abs and Rel wt at high dose 2. ↑ Centrilobular hepatic hypertrophy 3. ↑ Mitotic cells in liver after 2 days **b. Enzyme activity:** 1. Pydiflumetofen induced substantial PROD activity at all timepoints indicative of CAR activation.	Anonymous (2015)

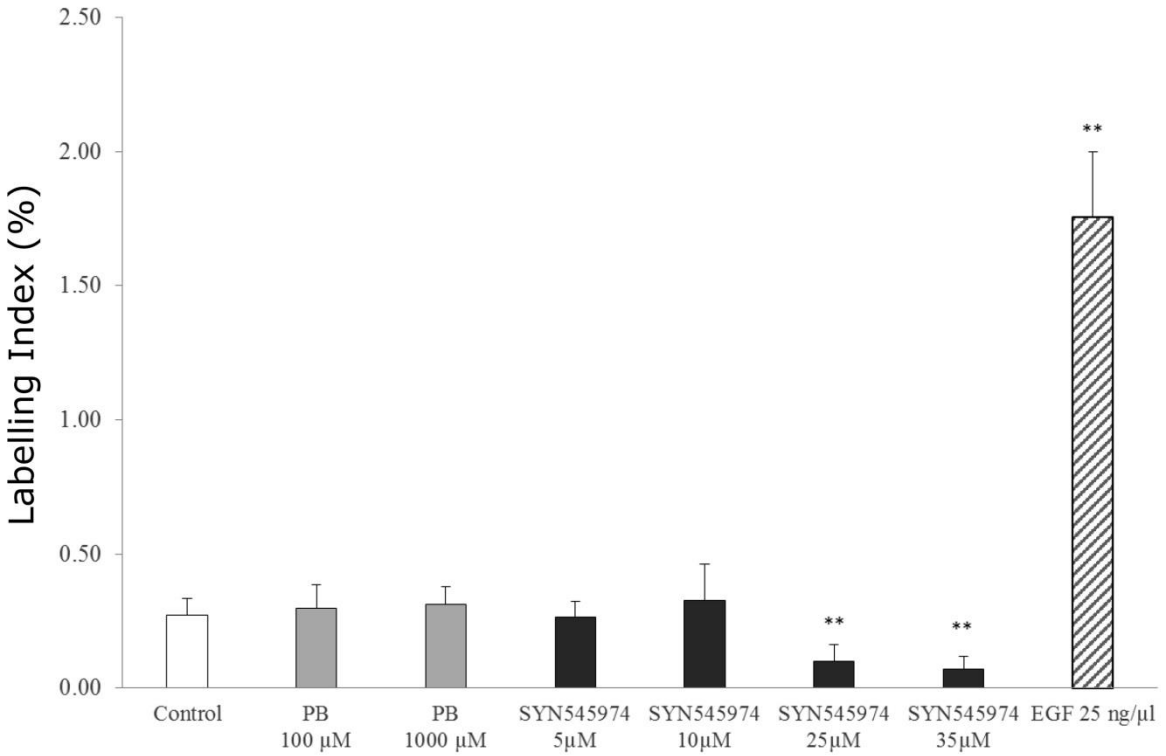
(0, 10, 324 mg/kg/day) - Total cytochrome P450 levels - Enzymes: PROD only. - Strong positive dose response for PROD activity - Brd immunostaining - Table 40 CLH report	**c. Replicative DNA Synthesis:** 1. ↑ BrdU incorporation with initial high burst at day 2, lower thereafter. **2250 ppm (324 mg/kg/day)** ↑ absolute liver weight 22% and 24% after 28 and 7 days respectively ↑ liver:body weight ratio 28% and 21% after 28 and 7 days respectively ↓ 40% AST activity 28 days ↑ cytochrome P450 approx. 2-fold after 2, 7 and 28 days ↑ PROD activity 28, 36 and 37-fold after 2, 7 and 28 days respectively (marker of Cyp2b activity) ↑ BrdU incorporation 14-, 5 and 6-fold after 2, 7 and 28 days respectively ↑ Centrilobular hepatic hypertrophy in 9/10, 9/10 and 10/10 animals after 2, 7 and 28 days respectively ↑ Mitotic cells in liver 7/10 after 2 days **75 ppm (10 mg/kg/day)** ↑ PROD activity 1.9. 1.6 and 2.4-fold after 2, 7 and 28 days respectively not statistically significant ↑ BrdU incorporation 3.1, 2.3- and 5.6-fold after 2, 7 and 28 days respectively	
3. *In-vitro* mouse hepatocytes - CD-1 strain - Cells from male animals - Pydiflumetofen, PB, EGF tested - low [pydiflumetofen] tested (5, 10, 25 and 35 µM) - Table 40 CLH report	**a. Cytotoxicity:** 1. ↓ ATP by 9-20% No dose effect up to 35 µM pydiflumetofen. **b. Enzyme induction:** 1. Pydiflumetofen induces PROD and BROD activity indicative of CAR and PXR activation. In absolute terms, mouse expression levels are higher than in human hepatocytes, typically > 200-fold. **c. Replicative DNA Synthesis:** 1. Pydiflumetofen increases labelling index in a PB-like manner, dependent on CAR activation, positive EGF control. The proliferative signal is much stronger in mouse hepatocytes relative to human hepatocytes, typically by about 10-fold. **All groups** ↓ 9.3-17.6% ATP as an indicator of cytotoxicity **35 µM** ↑ 50.6% hepatocyte proliferation as measured by replicative DNA synthesis ↓ PROD and BROD enzyme activities (not statistically significant), possibly indicating substrate competition or signs of cytotoxicity **25 µM** ↑ 89.8% hepatocyte proliferation as measured by replicative DNA synthesis ↓ PROD and BROD enzyme activities (not statistically significant) **10 µM** ↑ 71.3 and 97.0% respectively PROD and BROD enzyme activities **5 µM** ↑ 84.6 and 98.5% respectively PROD and BROD enzyme activities	Lowes (2015a)

4. *In-vitro* human hepatocytes - 1 male donor. - Pydiflumetofen, PB, EGF tested - low [pydiflumetofen] tested (5, 10, 25 and 35 µM) - Table 40 CLH report	**a. Cytotoxicity:** 1. ↓ ATP by 34% (i.e. 67% relative to control values) at 35 µM pydiflumetofen and ↓ ATP by 24% at 25 µM → significant cytotoxicity. **b. Enzyme induction:** 1. Pydiflumetofen induces PROD and BROD activity indicative of CAR and PXR activation. **c. Replicative DNA Synthesis:** 1. No effect on cell proliferation (S-phase of the cell cycle). Positive EGF control. **35 µM** ↓ 34 % ATP as an indicator of cytotoxicity ↑ 164 and 218% respectively PROD and BROD enzyme activities **25 µM** ↓ 24 % ATP as an indicator of cytotoxicity ↑ 155 and 326% respectively PROD and BROD enzyme activities **10 µM** ↑ 236 and 491% respectively PROD and BROD enzyme activities **5 µM** ↑ 191 and 237% respectively PROD and BROD enzyme activities	Lowes (2015b)
5. *Ex-vivo* Enzyme Analysis of Liver Samples - CD-1 mouse livers. - Treatment: 3, 7, and 28 days - Dietary study - [pydiflumetofen] 0, 500, 1500, 4000 and 7000 ppm - Table 40 CLH report	Alternate modes of action were only investigated via enzyme activity; there were no mRNA expression studies performed. No increase in peroxisome palmitoyl CoA oxidation and only minimal increase (<4fold) in microsomal LAH, indicating pydiflumetofen (SYN545974) is not a peroxisome proliferator. EROD (marker for Cyp1a activity), no biologically significant effects. Dose related increase in hepatic total P450 content to maximum of 1.8 and 1.6 fold (males and females) at 7000 ppm on day 28**.** **a. 28-day treatment:** 1. PCO: ↓ 0.7-0.5x (M-F) peroxisomal B-oxidation 2. total cytochrome P450: ↑ up to 2x 3. EROD: ↑ 1.7-1.4x (M-F) 4. PROD: ↑ 15-3x (M-F) 5. BQ: ↑ 3.5-2.5x (M-F) 6. LAH: ↑ 3.5-1x (M-F) **b. 7-day treatment:** 1. PCO: ↓ 0.7-0.4x (M-F) peroxisomal B-oxidation 2. total cytochrome P450: ↑ up to 2x 3. EROD: ↑ 2-1.3x (M-F) 4. PROD: ↑ 21-11x (M-F) 5. BQ: ↑ 5.4-2.5x (M-F) 6. LAH: ↑ 2-0.7x (M-F) **c. 3-day treatment:** 1. PCO: ↓ 0.7-0.8x (M-F) peroxisomal B-oxidation 2. total cytochrome P450: ↑ up to 2x 3. EROD: ↑ 3.6-2x (M-F) 4. PROD: ↑ 10-13x (M-F) 5. BQ: ↑ 4.4-3.5x (M-F) 6. LAH: ↑1.8-1.3x (M-F)	Anonymous (2012)

The available experimental data for pydiflumetofen indicate that the CAR-mediated MoA is the most plausible mechanism for induction of the mouse liver tumours. Mechanistic studies indicate a direct activation of CAR from different species (the rat, mouse and human) by pydiflumetofen. Results also demonstrated that the associative events of the proposed CAR mediated mechanism, i.e. induction of enzymes specific to CAR/PXR occur in human hepatocytes (but at a very low level relative to mouse enzyme induction). However, the key event, proliferation, (essential for subsequent tumour formation, figure below) is not observed in primary human hepatocytes at levels of pydiflumetofen of 5 and 10 µM. It is plausible from the available data that the liver carcinogenicity in mice proceeds via CAR activation, which is a tumour mechanism with little relevance to humans, however, significant uncertainties in the key study are a cause of concern and the level of increase in DNA replicative synthesis in mouse cells does not seem to follow a convincing dose response relationship. Indeed at the highest dose tested (35 µM) there is a drop in DNA replicative synthesis (part A of the figure below).



(A) Mouse hepatocyte replicative DNA synthesis - labelling index as mean ± standard deviation (n=5). A one-way ANOVA followed by the Dunnett test was performed on the results; * statistically different from control $p<0.05$; ** $p<0.01$.



(B) Human hepatocyte replicative DNA synthesis - labeling index as mean ± standard deviation (n=5). A one-way ANOVA followed by the Dunnett test was performed on the results; ** statistically different from control $p<0.01$. Concentrations of pydiflumetofen > 10 µM were cytotoxic and not informative on DNA replicative potential.

Figure: Mechanistic data - *In Vitro* Hepatocyte S-Phase labelling. (A) Pooled mouse hepatocytes. (B) Liver hepatocytes from a single human donor.

The *in vitro* hepatocyte studies (*Lowes 2015a, b*) with mice and human hepatocytes are key studies in the assessment of the MoA. The lack of proliferation in human hepatocytes seems to be considered as the ultimate proof for the proposed MoA. However, at the concentrations where proliferation of mice hepatocytes was shown (25 and 35 µM), there seems to be cytotoxicity in the human hepatocytes making the interpretation of the data rather difficult. Thus, following exposure of the human cells to 25 and 35 µM, the ATP-concentration decreased substantially (24-34% relative to controls) meaning that at least one quarter to one third of the human cells had died, thus it is doubtful if it would have been possible to detect potential proliferation. The data might indicate a rather large difference in sensitivity between rat and human hepatocytes to pydiflumetofen toxicity or that the human hepatocytes were in a worse condition than the mice hepatocytes, making them more vulnerable to toxic insult.

Details about the human donor (a human hepatocyte quality certificate was supplied in the original study report), indicated that the liver was from a middle aged (51yr) and healthy caucasian male with no history of tobacco, alcohol or drug abuse and with negative serology for viruses such as HIV-1 & 2; HTLV 1 & 2; CMV, HBV, and HCV.

Cytotoxicity was apparent with the human hepatocytes, since concentrations of pydiflumetofen >10 µM showed significant decreases in ATP relative to concurrent

controls. In mouse hepatocytes there was significant depletion of ATP content (≥56.8% of control) following the administration of pydiflumetofen at concentrations of 50 µM and higher (CXR1490; preliminary study). It appears that human hepatocytes are more sensitive to pydiflumetofen toxicity. Note, pydiflumetofen belongs to the class of succinate dehydrogenase inhibitors or SDHI fungicides, a mitochondrial complex II inhibitor and therefore is designed to inhibit oxidative phosphorylation and ATP production in target fungi.

There is no evidence to suggest that the human hepatocytes were in a worse condition than the mouse hepatocytes thereby rendering them more vulnerable to toxic insult. From the graphs in the figure above, it can be seen that they tolerated high doses of phenobarbital and 5 and 10 µM pydiflumetofen as well if not better than the mouse hepatocytes. There was some historical control data supplied with respect to the performance of human hepatocytes but it is not well documented. It is thought that the historical control data referred to the same hepatocyte donor source, i.e. donor 8210 as used in the human hepatocyte *in vitro* study. The S-phase basal labelling index was similar but cells from donor 8210 had lower responses to PB and EGF relative to the HCD and induced levels of PROD and BROD enzyme activity were also lower than HCD (table below).

Table: *In Vitro Hepatocyte Proliferation Index and Enzyme Activity Measurements in Male Human Hepatocyte Cultures used to investigate pydiflumetofen.*

Test Item	**Lowes (2015)**	**HCD (alternate study, same donor?)**
S-phase labelling index: Vehicle control PB (1000/500) EGF, 25 ng/ml	 0.27 ± 0.06 0.31 ± 0.06 / 1.76 ± 0.24	 0.33 ± 0.09 / 0.41 ± 0.09 4.50 ± 0.89
PROD activity (pmol resorufin/min/mg): Vehicle control PB (1000/500)	 0.11 ± 0.017 0.37 ± 0.045	 0.191 ± 0.022 0.463 ± 0.019
BROD activity: (pmol resorufin/min/mg) Vehicle control PB (1000/500)	 1.15 ± 0.06 8.53 ± 0.65	 1.49 ± 0.20 8.158 ± 0.441

Basal levels or responses may be a way to assess hepatocyte health. The table above illustrates some variability in response by human hepatocytes from the same donor, but without access to a more substantial database from several human donors it is difficult to assess the functional quality of the hepatocytes from the *Lowes* (2015b) study. For instance, the reduced response to 25 ng/mL EGF (1.76 vs 4.5); perhaps this could indicate a reduction in the health of the donor hepatocytes or just reflect the natural variation expected amongst hepatocytes to xenobiotics in their environment.

The data from the *in vitro* S-phase labelling study illustrated that the hepatocytes were capable of responding to a positive control proliferative stimulus (figure above). Typically the EGF proliferative response is weaker in human hepatocytes relative to rodent hepatocytes by about 10-fold. This has been seen for other *in vitro* human hepatocyte

proiferative assays investigating CAR agonists (e.g. silthiofam). The human hepatocytes appear to be sufficiently viable and able to tolerate high levels of phenobarbital and pydiflumetofen up to 10 µM. Higher levels of pydiflumatofen are clearly cytotoxic to the human hepatocytes so that the only reliable controls to indicate proliferative capacity and non-proliferative capacity lie with the positive control EGF and the negative control (in humans) with phenobarbital.

The main limitation with the *in vitro* human hepatocyte study is the reliance on cells from a single donor.

6.4 Dose selection

Dose selection is considered to be an issue. While the MTD was technically achieved, female rats could have been dosed higher than 102 mg/kg/day, certainly at a level at least equivalent to that of the males (≈ 300 mg/kg/day). Extensive explanations have been provided by the applicant and RMS in the DAR.

Dose levels were selected after evaluation of the rat subchronic (90 day) and pharmacokinetic studies with pydiflumetofen. 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 female rats.

The top dose level therefore was established where kinetic proportionality was lost due to dose-limited absorption of pydiflumetofen. Higher oral doses resulted in less and less compound being absorbed (per increment in dose) into the blood. However, this argument for the dosing regimen employed in studies may be regarded as insufficient because there is no plateau achieved in the increase in absorption. Systemic exposure continues to increase with doses higher than the highest dose levels selected for the long-term and reproductive toxicity studies. Comparison between plasma AUCs determined after administration of the phenyl radiolabelled active substance in rat at dose levels up to 1000 mg/kg/day, showed that systemic exposure still increased beyond 100 or 300 mg/kg/day:

i. by 4-fold between 100 - 1000 mg/kg bw/d

ii. by 1.7-fold between 300 - 1000 mg/kg bw/d

Further support for increased systemic exposure at very high doses was confirmed in the short-term repeated dose studies where an increase in toxicity (liver and body weight effects) was observed with increasing dose. A higher dose administered to female rats in the long-term study for instance, would have confirmed whether the increase in the thyroid follicular adenomas was a real substance related effect or not.

RAC agrees with the DS that technically the long-term rodent studies satisfy the MTD requirement. The DS did not explain the rationale behind the dose selection. They only noted the DAR RMS concern on whether the doses were sufficient to account for the presence of the primary metabolite 2,4,6 trichlorophenol (2,4,6-TCP). RAC considers all scientific approaches to dose selection as long as all the inherent hazards of the substance can be evaluated with confidence. The pharmacokinetic approach in this case did not provide the necessary confidence for concluding fully on toxicity and therefore also on classification. Top dose selection in the long-term and reproductive toxicity studies is not considered sufficient. RAC notes the concerns of the RMS on the dosing regimens

employed and considers that a dose beyond the linear range of absorption should have been tested because it was apparent that systemic exposure continued to increase with increasing dose.

6.5 Conclusions

6.5.1. Human Relevance

1. There is uncertainty regarding a possible treatment-related increase in thyroid adenoma /hyperplasia observed in female rats. The mechanistic investigation in males was inappropriate (since this was the less sensitive sex for thyroid effects) and the top dose for females was too low to adequately assess the tumourigenic response in this organ.
2. The available data shows that pydiflumetofen activates human CAR and induces Cyp2b/3a-related enzyme activity, indicating that CAR binding and activation is also relevant for humans. However, the central tenet that CAR-mediated hepatocarcinogenesis in rats and mice is of no relevance to liver tumour promotion in humans is based on the supposition that CAR-mediated gene activation in humans produces only a subset of responses observed in mice and rats. While CAR activation can occur in humans as it does in mice and rats, there is so far no evidence that human CAR regulates genes involved in cell growth leading to replicative DNA synthesis and liver hyperplasia.
3. It has not been adequately demonstrated in the case of pydiflumetofen that there are qualitative differences between humans and rodents, particularly in the ultimate key event of cell proliferation. The limited *in vitro* study with human hepatocytes showed significant cytotoxicity at concentrations > 10 µM so that no firm conclusions regarding cell proliferation can be made. Cells from only one donor were used and the results cannot be interpreted as being representative from a cross section of the human population.
4. It has not been adequately demonstrated with pydiflumetofen that other mechanisms are not also at work. There is some increase in EROD and LAH enzyme expression but no mRNA induction studies were performed to investigate alternate or CAR-supporting pathways. No positive controls for aryl-hydrocarbon receptor (AhR) activation/CYP1A induction (e.g. 3-methylcholanthrene, omeprazole) or LAH expression/CYP4A induction (e.g. fibrate drugs, other peroxisome proliferators) were investigated so it is not possible to put into context the small increases in EROD and LAH that were observed. Other effects of CAR activation such as the suppression of apoptosis were also not investigated though it is recognised that some crosstalk with AhR (and/or CYP1A induction) may be indicative of apoptotic suppression.
5. It has not been adequately demonstrated with pydiflumetofen that the CAR activation model was primarily responsible for the neoplastic response in mouse liver. No transgenic knockout animals or humanised receptor models were employed to provide further support for CAR-mediated liver tumours of little to no relevance to humans.

6.5.2 Conclusion on Carcinogenicity

1. The mechanistic data that are available indicate a mechanism-of-action (MoA) via CAR activation as the most plausible mechanism responsible for the liver tumours in male mice. The tumour profile observed in the pydiflumetofen carcinogenicity bioassay was typical of a non-genotoxic mechanism (single species, single sex and single organ involvement without decreased latency).
2. The data from the rat studies inadequately informs on thyroid carcinogenicity potential due to insufficient dosing and limited mechanistic investigation. The available thyroid data in the rat are not considered to be sufficient for classification. In female rats the incidence of benign follicular cell adenoma (3/51) and follicular cell hyperplasia (3/51) at the top dose of 102 mg/kg bw/day was at the upper boundary of the HCD range for that tumour. Some data suggests that it could be related to an increase in hepatic-mediated thyroxine clearance. Mechanistic studies were not performed using the more sensitive female animals. The maximum test dose to female rats in the rat carcinogenicity study was too low.
3. The data from the mouse study shows a strong liver carcinogenicity potential for pydiflumetofen in males. Several mechanistic studies were performed to show support for a CAR-mediated MoA for the mouse liver tumours. The concern for tumours arising from this MoA is only reduced if it is adequately demonstrated that (1) alternative MoAs are eliminated and (2) there are qualitative differences between humans and rodents, particularly regarding the ultimate key event, cell proliferation. It is the view of RAC that these two points have not been adequately addressed with the pydiflumetofen data. Therefore, even though there is reason to believe that a CAR-mediated mechanism of liver tumour promotion seems very plausible, the weight of evidence is weak and RAC cannot conclude on there being no hazard to humans. Classification with Carc 2 is proposed.

6.5.3. Classification into category 1A

There is no information from studies in humans to inform on carcinogenic potential and so classification in category 1A is not supported.

6.5.4 Classification into category 1B

The substance was not found to be genotoxic. Tumours were restricted to one organ (liver), to one species (mouse) and one sex (males), there was no evidence for a reduction in liver tumour latency. There was a progression to malignancy and there was an apparent dose response relationship. The incidence of thyroid adenomas (female rats) was within the historical control range. Overall the data was considered to show limited evidence of a carcinogenic effect and not sufficient to warrant classification in category 1B.

6.5.5. Classification into category 2

The data supports a category 2 classification for pydiflumetofen. The main weakness in the evidence base is that hepatocytes from only one donor were used to demonstrate that pydiflumetofen lacks proliferative ability in this tissue in humans. On the weight of the presented evidence, a CAR mode of action was considered to be the most plausible explanation for the increase in liver adenomas and carcinomas in the male mouse. However, the shortcomings in the hepatocyte studies, in combination with the other

uncertainties (such as selection of doses in both rats and mice, possible treatment-relationship of thyroid adenoma/hyperplasia observed in female rats, absence of MoA data for female rats, possible involvement of carcinogenic metabolite 2,4,6-TCP) need to be considered. A weight of evidence assessment by RAC considered that it was not conclusively shown that the tumours were of no relevance to humans. RAC concludes there is sufficient uncertainty to **warrant classification as Carc. 2** for pydiflumetofen.

6.5.4 No Classification

Overall RAC considers insufficient evidence has been presented to indicate no concern for human health; there is insufficient data to conclude on other alternative modes of action; and that whether the sole MoA for liver tumours in mice were secondary to hepatocellular proliferation induced by activation of the CAR/PXR nuclear receptors has not been adequately addressed.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies [equivalent to section 10.10.1 of the CLH report template]

Table 42: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two generation reproduction OECD 416 GLP acceptable Oral (continuous in diet) Rat, Crl:WI (Han) 24/sex/group (see also sections 2.6.3 and 2.6.6.3)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) Males: 0, 150, 750 & 4500 ppm Females: 0, 150, 450 & 1500 ppm Continuous in the diet	***Parental toxicity - Males*** 150 ppm (9.1 mg/kg/day, F0; 11.9 mg/kg/day, F1) No effects 750 ppm (46 mg/kg/day, F0; 59 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑ 9% ; F1: ↑ 12%) 4500 ppm (277 mg/kg/day, F0; 364 mg/kg/day, F1) F0: ↓ body weight gain (10% weeks 0-17); ↑ liver weight adjusted for bw (↑38%); ↑ incidence of hepatocyte hypertrophy (slight): 19/24 (control = 0/24 incidence); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24 (control = 1/24) F1: ↓ body weight gain (10% weeks 0-17); ↓ food consumption (8% weeks 0-17); ↑ liver weight adjusted for bw (↑42% males); ↑ incidence of hepatocyte hypertrophy (slight) 18/24 (controls = 0/24); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24(controls = 2/24). ***Parental toxicity - Females*** 150 ppm: (11.9 mg/kg/day, F0; 14.1 mg/kg/day, F1) No effects 450 ppm (36 mg/kg/day, F0; 42 mg/kg/day, F1) F0: ↑ liver weight adjusted for bw (↑ 6%) 1500 ppm (116 mg/kg/day, F0; 141 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑15% and F1: 19%) F0: ↑ incidence of hepatocyte hypertrophy (minimal) 8/24 (controls = 0/24)	***Anonymous*** (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
		NOAEL (parental) 750/450 ppm (46/36 mg/kg/day F0 generation pre-pairing) in males and females respectively ***Reproductive toxicity*** No effects at any dose level **NOAEL (reproductive) 4500/1500 ppm (277/116 mg/kg/day F0 generation pre-pairing) in males and females respectively.** ***Offspring toxicity - Males*** 750 ppm (59 mg/kg/day) No effects 4500 ppm (364 mg/kg/day) F1: delayed sexual maturation (45.9 days versus 43.0 days in controls) considered secondary to ↓ body weight ***Offspring toxicity - Females*** 450 ppm (42.4 mg/kg/day) No effects 1500 ppm (116 mg/kg/day) F1: delayed sexual maturation (33.0 days versus 30.3 days in controls). No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance **NOAEL (offspring) 4500/450 ppm (277/36 mg/kg /day F0 generation pre-pairing) in males and females respectively.**	

Table 43: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 44: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

PYDIFLUMETOFEN (SYN545974) has been evaluated for effects on reproduction and fertility in a 2-generation reproduction study (***Anonymous***, 2015) in rats. Wistar rats were administered PYDIFLUMETOFEN (SYN545974) continuously in the diet at inclusion levels of 0, 150, 750 and 4500 ppm for males and 0, 150, 450 and 1500 ppm for females.

Paternal toxicity (reduced body weight gain) was observed in males at the highest dose level tested of 4500 ppm (equivalent to 277 mg/kg/day) but there was no effect on sexual function of fertility. There was no effect of treatment on sperm parameters for males from either generation or on the quantification of the F1 generation ovarian follicles in females.

Sexual maturation was slightly delayed for F1 generation males given 4500 ppm and females given 1500 ppm. The delay in sexual maturation in males was secondary to reduced body weight gain and not a direct effect of treatment with PYDIFLUMETOFEN (SYN545974). In female, the delay was not secondary to bodyweight effects. However, this can be considered questionable as there was no effect on related parameters such as oestrus

cycling, mating performance or fertility and no effect on ano-genital distance of F1 generation pups. In a conservative approach, this finding was taken into account to derive the NOAEL of the study. Overall, the NOAEL for systemic toxicity was 46/36 mg/kg/day for males and females respectively, the NOAEL for reproductive toxicity was 277/116 mg/kg/day for males and females respectively (both based on F0 pre-pairing period dose intake) and the NOAEL for offspring toxicity was 277/36 mg/kg/day for males and females respectively.

There were no short-term toxicity studies relevant for toxicity on sexual function and fertility i.e. there was no indication of any adverse effects on the reproductive organs including spermatogenesis from routine histopathology. The short-term toxicity of PYDIFLUMETOFEN (SYN545974) was evaluated by the oral route in rats, dogs and mice and by the dermal route in rats. In general, the effects included a reduction in body weight gain and minor changes in food consumption and utilization. The target organs were the liver and thyroid (increased liver weight and hypertrophy in both organs).

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

In the classification system, adverse effects on sexual function and fertility include, but are not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

There were no effects to warrant classification of PYDIFLUMETOFEN (SYN545974) as a reproductive toxicant.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 45: Summary table of animal studies on adverse effects on development

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL (for parent, offspring and for developmental effects) - target tissue/organ - critical effects at the LOAEL**	**Reference**
Developmental toxicity OECD 414 GLP Acceptable Oral (gavage) Rat, Crl:CD (SD) 24 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 30 or 100 mg/kg/day on days 6-19 of gestation Vehicle: 1% CMC (w/v)	*Maternal toxicity* 100 mg/kg/day: No effects at highest dose tested Maternal NOAEL 100 mg/kg/day *Developmental toxicity* 100 mg/kg/day: No effects at highest dose tested Developmental NOAEL 100 mg/kg/day	***Anonymous*** (2015)
Preliminary developmental toxicity Non-guideline Non-GLP Supplementary Oral (gavage) Rat, Crl:CD (SD) 6 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.6%) 0, 100, 200, 500 or 1000 mg/kg/day on days 6-19 of gestation Vehicle: 1% CMC (w/v)	*Maternal toxicity* 1000 mg/kg/day: No effects at highest (limit) dose tested *Developmental toxicity (no skeletal examination)* 1000 mg/kg/day: No effects at highest (limit) dose tested	***Anonymous*** (2011)
Developmental toxicity OECD 414 GLP Acceptable Oral (gavage)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 100 or 500 mg/kg/day on days 6-27 of gestation	*Maternal toxicity* 500 mg/kg/day: No effects at highest dose tested Maternal NOAEL 500 mg/kg/day *Developmental toxicity* ≥ 100 mg/kg/day:	***Anonymous*** (2015b)

<table>
<tr><th>Method, guideline, deviations[1] if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results
- NOAEL/LOAEL (for parent, offspring and for developmental effects)
- target tissue/organ
- critical effects at the LOAEL</th><th>Reference</th></tr>
<tr><td>Rabbit, New Zealand White
24 mated females/group</td><td>Vehicle: 1% CMC (w/v)</td><td>Increased incidence of one skeletal variant (rib costal cartilage interrupted) without clear dose response. No historical control data available
<table>
<tr><td></td><td colspan="4">Dose level (mg/kg/day)</td></tr>
<tr><td></td><td>0</td><td>10</td><td>100</td><td>500</td></tr>
<tr><td>Observations</td><td colspan="4">Rib: one or more: costal cartilage interrupted (variant)</td></tr>
<tr><td>Fetuses</td><td>8/163 (4.4%)</td><td>8/142 (5%)</td><td>14/132 (14%)</td><td>12/154 (8%)</td></tr>
<tr><td>Litters</td><td>6/22 (27.3%)</td><td>6/18 (33.3%)</td><td>12/19 (63%)*</td><td>10/21 (47.6%)*</td></tr>
</table>
Developmental NOAEL 10 mg/kg/day</td><td></td></tr>
<tr><td>Preliminary developmental toxicity
Non-guideline
Non-GLP
Supplementary
Oral (gavage)
Rabbit, New Zealand White
10 mated females/group</td><td>PYDIFLUMETOFEN (SYN545974) (purity 99.3%/ 98.5%)
0, 250, 500 or 1000 mg/kg/day on days 6-27 of gestation
Vehicle: 1% CMC (w/v)</td><td>Maternal toxicity
<u>1000 mg/kg/day</u>:
↓ body weight 35% days 6-28
Maternal NOAEL 500 mg/kg/day

Developmental toxicity (no skeletal examination)
<u>1000 mg/kg/day</u>:
No effects at highest (limit) dose tested
Developmental NOAEL 1000 mg/kg/day</td><td>Anonymous (2015c)</td></tr>
<tr><td>Two generation reproduction
OECD 416
GLP
acceptable
Oral (continuous in diet)
Rat, Crl:WI (Han)
24/sex/group
(see also sections 2.6.3 and 2.6.6.3)</td><td>PYDIFLUMETOFEN (SYN545974) (purity 98.5%)
Males: 0, 150, 750 & 4500 ppm
Females: 0, 150, 450 & 1500 ppm
Continuous in the diet</td><td>Only data for offspring developmental toxicity are presented
<u>Offspring toxicity - Males</u>
<u>750 ppm (59 mg/kg/day)</u>
No effects
<u>4500 ppm (364 mg/kg/day)</u>
F1: delayed sexual maturation (45.9 days versus 43.0 days in controls) considered secondary to ↓ body weight
<u>Offspring toxicity - Females</u>
<u>450 ppm (42.4 mg/kg/day)</u>
No effects
<u>1500 ppm (116 mg/kg/day)</u>
F1: delayed sexual maturation (33.0 days versus 30.3 days in controls). No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance
NOAEL (offspring) 4500/450 ppm (277/36 mg/kg /day F0 generation pre-pairing) in males and females respectively.</td><td>Anonymous (2015)</td></tr>
</table>

Table 46: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No evidence of adverse health effects in humans				

Table 47: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No relevant studies				

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

For the prenatal developmental toxicity study in the rat, dose levels of 0, 10, 30 and 100 mg/kg/day were evaluated in a GLP study, conducted to current OECD Test Guideline No. 414 (***Anonymous***, 2015). An initial reduction in body weight and food intake in response to the onset of dosing with 100 mg/kg/day on day 6, was resolved by day 9 with no overall adverse effects on either parameter. There was no evidence of developmental toxicity at any dose level. The incidences and intergroup distribution of major, minor and variant foetal abnormalities were considered not to be related to administration of PYDIFLUMETOFEN (SYN545974). Based on the results of this study the NOAEL for maternal and embryo-fetal development is considered to be 100 mg/kg/day. In addition, a preliminary developmental toxicity study was conducted in the rat at doses of 0, 100, 200, 500 or 1000 mg/kg/day. No maternal or developmental toxicity was seen at the highest dose tested.

For the prenatal developmental toxicity study in the rabbit, dose levels of 0, 10, 100 and 500 mg/kg/day were evaluated in a GLP study, conducted to current OECD Test Guideline No. 414 (***Anonymous***, 2015c). No maternal effects were observed in the study. A marginally increased incidence of rib cartilage variant (one or more costal cartilage interrupted) was observed at 100 and 500 mg/kg/day without a clear dose response. The absence of available historical control data leads difficult the interpretation of this findings. However in a conservative approach, the NOAEL for embryo-fetal development was considered to be 10 mg/kg/day. In addition, a preliminary developmental toxicity study was conducted in the rabbit at doses of 0, 250, 500 and 1000 mg/kg/day. The maternal NOAEL was 500 mg/kg/day based on decrease in maternal body weight gain at 1000 mg/kg/day. No developmental toxicity was seen at the highest dose level.

In the 2-generation study in rat (***Anonymous***, 2015), sexual maturation was delayed for F1 generation males given 4500 ppm and females given 1500 ppm. In males, the delay in sexual maturation was secondary to reduced body weight gain and not a direct effect of treatment with PYDIFLUMETOFEN (SYN545974). In female, the delay was not secondary to reduced body weight gain. However, this effect was considered questionable as there was no effect on related parameters such as oestrus cycling, mating performance or fertility and there was no effect on ano-genital distance of F1 generation pups. In a conservative approach, this finding was considered to derive the NOAEL (offspring) of the study at 36 mg/kg/day.

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

In the classification system, adverse effects on development of the offspring include any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation.

In the rat prenatal developmental toxicity studies, compliant with current test guidelines, no maternal or developmental toxicity was seen. In the rabbit, a marginally increased incidence of a skeletal variant was observed at 100 and 500 mg/kg/day in the absence of maternal toxicity. However, no clear dose response was observed and no historical control data regarding this finding were submitted by the Applicant. In a conservative approach for the risk assessment, this effect was considered to derive the NOAEL of the study. However, this is only a minor defect with no consequence on post-natal survival or development and according to the CLP regulation, variants may not lead to classification if considered to be of low toxicological significance.

In the 2-generation study in rat (***Anonymous***, 2015), sexual maturation was slightly delayed for F1 generation females given 1500 ppm and this delay was not secondary to reduced body weight gain. Although this effect has been considered to derive the NOAEL of the study, they remain questionnable for the following reasons:

(i) no subsequent effect on related parameters such as oestrus cycling, mating performance or fertility were observed in the F1 generation pups

(ii) There was no effect on ano-genital distance of F1 generation pups

(iii) No effect on endocrine or reproductive organs were observed in all the available repeated toxicity studies database (rat, mice, dog)

In conclusion, the RMS is of opinion that these findings (skeletal variant and sexual maturation) are not sufficiently convincing to be a basis for classification of PYDIFLUMETOFEN (SYN545974) as a developmental toxicant.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 48: Summary table of animal studies on effects on or via lactation

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
Two generation reproduction OECD 416 GLP acceptable Oral (continuous in diet) Rat, Crl:WI (Han) 24/sex/group (see also section 2.6.3 and 2.6.6.1)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) Males: 0, 150, 750 & 4500 ppm Females: 0, 150, 450 & 1500 ppm Continuous in the diet	***Parental toxicity - Males*** 150 ppm (9.1 mg/kg/day, F0; 11.9 mg/kg/day, F1) No effects 750 ppm (46 mg/kg/day, F0; 59 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑ 9% (males) ; F1: ↑ 12%) 4500 ppm (277 mg/kg/day, F0; 364 mg/kg/day, F1) F0: ↓ body weight gain (10% weeks 0-17); ↑ liver weight adjusted for bw (↑38% males) and ↑15% females); ↑ incidence of hepatocyte hypertrophy (slight): males 19/24 (control = 0/24 incidence); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24 (control = 1/24) in males. F1: ↓ body weight gain (10% weeks 0-17); ↓ food consumption (8% weeks 0-17); ↑ liver weight adjusted for bw (↑42% males and ↑ 17% females); ↑ incidence of hepatocyte hypertrophy (slight) 18/24 (controls = 0/24); ↑ incidence of thyroid follicular hypertrophy (minimal) 7/24(controls = 2/24). ***Parental toxicity - Females*** 150 ppm: (11.9 mg/kg/day, F0; 14.1 mg/kg/day, F1) No effects 450 ppm (36 mg/kg/day, F0; 42 mg/kg/day, F1) F0: ↑ liver weight adjusted for bw (↑ 6%) 1500 ppm (116 mg/kg/day, F0; 141 mg/kg/day, F1) F0 & F1: ↑ liver weight adjusted for bw (F0: ↑15% and F1: 19%) F0: ↑ incidence of hepatocyte hypertrophy (minimal) 8/24 (controls = 0/24) **NOAEL (parental) 750/450 ppm (46/36 mg/kg/day F0 generation pre-pairing) in males and females respectively** ***Reproductive toxicity*** No effects at any dose level **NOAEL (reproductive) 4500/1500 ppm (277/116 mg/kg/day F0 generation pre-pairing) in males and females respectively.** ***Offspring toxicity - Males*** 750 ppm (59 mg/kg/day) No effects 4500 ppm (364 mg/kg/day) F1: delayed sexual maturation (45.9 days versus 43.0 days in	***Anonymous*** (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL**	**Reference**
		controls) considered secondary to ↓ body weight ***Offspring toxicity - Females*** 450 ppm (42.4 mg/kg/day) No effects 1500 ppm (1416 mg/kg/day) F1: delayed sexual maturation (33.0 days versus 30.3 days in controls). No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance **NOAEL (offspring) 4500/450 ppm (277/36 mg/kg /day F0 generation pre-pairing) in males and females respectively.**	

Table 49: Summary table of human data on effects on or via lactation

Type o data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No evidence of adverse health effects in humans				

Table 50: Summary table of other studies relevant for effects on or via lactation

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies				

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

The two generation reproduction study (***Anonymous***, 2015) has been described previously. The results showed that administration of 1500 ppm for females (equivalent to 116 mg/kg/day, F0; 141 mg/kg/day, F1) increased liver weight adjusted for body weight in both generations. In addition, the F0 females also showed an increased incidence of hepatocyte hypertrophy. Increased liver weight adjusted for body weight in both generations was also observed in females at the lower dose of 450 ppm (equivalent to 36 mg/kg/day, F0; 42 mg/kg/day, F1).

There was no indication of impaired nursing behaviour or decreased pup viability during lactation and no effect on pup growth to weaning. The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the chemical via the milk or to the quality of the milk.

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

The classification is intended to indicate when a substance may cause harm due to its effects on or via lactation and is independent of consideration of the reproductive or developmental toxicity of the substance. There were no effects to warrant classification of PYDIFLUMETOFEN (SYN545974) for effects on or via lactation.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

According to CLP criteria, no classification is required for reproductive toxicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Pydiflumetofen was evaluated for reproductive and developmental toxicity in a multi-generation reproductive toxicity study in the rat (*Anon., 2015*) and in pre-natal developmental toxicity studies in the rat (Preliminary study; *Anon, 2011* and Main study; *Anon., 2015*) and rabbit (Preliminary study; *Anon., 2015a* and Main study; *Anon., 2015b*). All studies were guideline (OECD 416 and OECD 414) and GLP compliant.

1. Sexual function and fertility

In a two-generation reproduction toxicity study, four groups of 24 male and 24 female rats of the Crl:WI(Han) strain were administered pydiflumetofen orally *via* the diet at 0, 150, 750 or 4500 ppm (males) or 0, 150, 450 or 1500 ppm (females) from 10 weeks before pairing and until necropsy.

No treatment-related deaths or clinical signs occurred in either the P or F_1 generation parents or litters. Body weight gain was slightly (but stat. sig.) reduced in P and F_1 generation males at 4500 ppm during the initial weeks of the pre-pairing periods, with slight effects on food intake only seen during the F_1 generation. There was no effect of treatment on female body weight gain at any concentration, in either generation.

Relative liver weights were increased in males at 750 and 4500 ppm in both generations, in the P generation females at 450 ppm and in the P and F_1 generation females at 1500 ppm. Microscopic findings in the liver (diffuse hepatocyte hypertrophy) were only seen at 4500 ppm in P and F_1 generation males and 1500 ppm in P generation females only. Microscopic findings in the thyroid (minimal follicular epithelial hypertrophy) were seen in males of both generations given 4500 ppm.

A slight increase in oestrous cycling at 1500 ppm in the P generation females was within the HCD range and was not observed in the F_1 females; fertility and mating performance or gestation length were not affected for either generation at any dietary concentration. All pregnant females gave birth to live litters with a similar number of pups born, and there was no effect of treatment on the postnatal survival of P or F_1 generation litters to Day 21 of age.

Sexual maturation was slightly delayed for F_1 generation males given 4500 ppm and females given 1500 ppm which was considered by the DS to be possibly related to treatment. The delay was associated with reduced body weight gain in males but not in females. However, this was considered to be of questionable relevance as there was no effect on related parameters such as oestrus cycling, mating performance or fertility and no effect on ano-genital distance of F1 generation pups. Sperm parameters for males from either generation or on the number of the F1 generation ovarian follicles were unaffected.

In parental males the LOAEL for systemic toxicity was 4500 ppm (P/F_1 - 276.6/363.8 mg/kg/day, respectively), based on a reduction in body weight gain during the early pre-pairing period and increased liver and thyroid weights associated with microscopic changes (diffuse hepatocyte hypertrophy and thyroid follicular epithelial hypertrophy). In females, the LOAEL was 1500 ppm (P/F_1 - 116.2/140.6 mg/kg bw), based on increased liver weight

associated with hepatocyte hypertrophy.

The reproductive NOAEL was considered to be in excess of 4500 ppm for P and F_1 generation males (276.6 and 363.8 mg/kg/day, respectively) and 1500 ppm for females in the P (116.2 mg/kg/day (pre-pairing)) and F_1 generations (140.6 mg/kg/day (pre-pairing)).

The NOAEL for offspring toxicity was considered to be in excess of 4500 ppm for P and F_1 generation males (276.6 and 363.8 mg/kg/day, respectively) and 450 ppm for female in the P (36.1 mg/kg/day (pre-pairing)) and F_1 generations (42.4 mg/kg/day (pre-pairing)) based on the delayed sexual maturation observed at 1500ppm.

It was the conclusion of the DS/RMS that there were no effects to warrant classification of pydiflumetofen for fertility. In addition, the DS/RMS was of the opinion that the findings (skeletal variants and sexual maturation) were not sufficiently convincing to be a basis for classification of pydiflumetofen as a developmental toxicant.

2. Development

Developmental toxicity was investigated in the rat and the rabbit in GLP and guideline compliant studies with preliminary range-finding studies for both.

2.1 Rat studies

A preliminary developmental toxicity study was conducted in the rat at doses of 0, 100, 200, 500 and 1000 mg/kg/day. The only treatment related effect was a transient reduction in bodyweight gain at 500 mg/kg/day and a slight body weight loss for females given 1000 mg/kg/day on day 6 to 7 of gestation. No maternal or developmental toxicity was seen at the highest dose tested.

For the main developmental toxicity study in the rat, dose levels of 0, 10, 30 and 100 mg/kg/day were evaluated (*Anon., 2015*). An initial reduction in body weight and food intake in response to the onset of dosing with 100 mg/kg/day on day 6 was resolved by day 9 with no overall adverse effects on either parameter. There was no evidence of developmental toxicity at any dose level. The incidences and intergroup distribution of major, minor and variant foetal abnormalities were considered not to be related to administration of pydiflumetofen. Based on the results of this study, the NOAEL for maternal toxicity is considered to be 30 mg/kg/day and the NOAEL for embryo-foetal development is considered to be 100 mg/kg/day.

In the 2-generation study in rats (*Anon., 2015*), sexual maturation was delayed in F_1 generation males given 4500 ppm and in females given 1500 ppm. In males, the delay in sexual maturation was considered by the DS to be secondary to reduced body weight gain and not a direct effect of treatment with pydiflumetofen. In females, the delay was not secondary to reduced body weight gain. In a conservative approach, this finding was considered sufficient to establish the NOAEL (offspring) of the study at 36 mg/kg/day but was not considered to be of sufficient toxicological significance to be relevant for classification because:

i. There was no subsequent effect on related parameters such as oestrus cycling, mating performance or fertility observed in the F1 generation pups.

ii. There was no effect on ano-genital distance of F1 generation pups

No effect on endocrine or reproductive organs were observed in all the available repeated

toxicity studies database (rat, mice, dog)

2.2 Rabbit studies

A preliminary developmental toxicity study was conducted in the rabbit at doses of 0, 250, 500 and 1000 mg/kg/day. The maternal NOAEL was 500 mg/kg/day based on decrease in maternal body weight gain at 1000 mg/kg/day. No developmental toxicity was seen at the highest dose level.

For the prenatal developmental toxicity study in the rabbit, dose levels of 0, 10, 100 and 500 mg/kg/day were evaluated (*Anon., 2015c*). No maternal effects were observed in the study. A marginally increased incidence of rib cartilage variant (one or more costal cartilage interrupted) was observed at 100 and 500 mg/kg/day without a clear dose response relationship but above the HCD from the conducting laboratory. In a conservative approach, the NOAEL for embryo-foetal development was considered to be 10 mg/kg/day.

In conclusion, the RMS was of the opinion that these findings in rats (skeletal variants and sexual maturation) are not sufficiently convincing to be a basis for classification of pydiflumetofen as a developmental toxicant.

Comments received during public consultation

Industry (comment 7)

This comment referred to the observation of a statistically significantly increased foetal (but not litter) incidence of a rib variant 'one or more costal cartilage interrupted' at 100 and 500 mg/kg bw/day in the main rabbit developmental toxicity study (*Anon., 2015c*).

Historical control data were submitted by the industry (*Manton, J., 2018*) which refers specifically to the background occurrence of the rib variant 'one or more costal cartilage interrupted'. In the table below the incidence in 54 studies from the relevant supplier is described from 2007 to 2017.

Table. *Variant skeletal findings with statistical significance*

Observations	**Dose levels (mg/kg bw/day)**			
	0 (control)	**10**	**100**	**500**
Skeletal examination				
No. of foetuses (F)	*163*	*142*	*132*	*154*
No. litters (L)	*22*	*18*	*19*	*21*
Rib: one or more: costal cartilage interrupted (variant)	F: 8 (4.4%) L: 6 (27.3%)	F: 8 (5%) L: 6 (33.3%)	F: 14 (14%) L: 12 (63%)*	F: 12 (8%) L: 10 (47.6%)*
HC data (Manton, J., 2018)	**54 studies (2007-2017):** **Foetal range 4-13 (2.6% - 9.4%)** **Litter range 4-9 (25% -42.8%)**			

The individual study data indicated that this variation occurred in 8 of 54 studies; between 2.6 and 9.4% of foetuses were affected in 4 – 9 litters. It was not clear whether the remaining studies had either zero incidence of the variant or it was not recorded or not looked for.

After the public consultation, industry submitted three position papers addressing the adequacy of dose selection, the liver carcinogenic response in male mice and the delay in sexual maturation in rats in the 2-generation study.

The position paper on the delay in sexual maturation was entitled - Company "Comments: Pydiflumetofen - Explanation of Developmental Delays in Rat Multigeneration Study". They concluded:

1. "*Reductions in body weight during post-natal development are known to cause delays in the onset of puberty. It is established in the scientific literature that growth rate is of greater importance than arrival at a particular fixed weight in determining the onset of puberty. For both the high dose F1 female and male pups there is clear evidence of a delay in growth during the post-natal period, and this can explain any apparent difference in the time of sexual maturation in these groups.*
2. *Further evidence that administration of pydiflumetofen does not directly perturb sexual maturation comes from the F2 generation which were examined on PND 0 for differences in anogenital distance (AGD). There was no difference in AGD in the F2 generation at any dose level. Furthermore, no other endpoint on the study, which could indicate abnormal progression through puberty, was different from the control group (e.g. there was no effect on oestrus cyclicity in the females, and no effect on time to mating or overall mating performance).*
3. *The individual animal data do not show the treated groups, including the highest doses tested, to fall outside of a normal physiological range.*
4. *Taking the above together there are compelling reasons to consider that any apparent specific changes in sexual maturation are unrelated to pydiflumetofen*".

MSCA (Comment 8)

A Member State considered that the following malformations/variations in the rat and rabbit developmental toxicity studies may be treatment-related and relevant for classification.

1. Cleft palate/palatine and/or malformed palate at 100 mg/kg bw/day in the rat main study and at 1000 mg/kg bw/day in the rabbit range-finding study.
2. rib variants (costal cartilage) in rat at 100 mg/kg bw/day and rabbit at 100 and 500 mg/kg bw/day.

They also argued that the test substance was insufficiently tested as minimal toxicity was seen at the highest dose tested in the rat which was within the linear kinetic phase of the absorption rate. Likewise it was noted that in the rabbit the AUC was only slightly increased with increased oral dosing (AUC increase by only 1.2 and 4.2 fold following a dose increase of 10 (dose of 100 mg/kg/day) and 50 fold (dose of 500 mg/kg/day), respectively) while a single incidence of malformed palate was seen in litters of dams tested to 250 and 1000 mg/kg bw in the preliminary study.

Assessment and comparison with the classification criteria

3. Assessment of reproductive and developmental studies

3.1 Fertility

3.1.1 Introduction

The potential reproductive toxicity of pydiflumetofen was investigated in a GLP and Guideline compliant study up to dietary levels of 4500 ppm (P/F_1; 276.6/363.8 mg/kg bw) in males and 1500 ppm (P/F_1 ; 116.2 and 140.6 mg/kg/bw) in females.

3.1.2 Dose selection

In this study, minimal systemic toxicity was evident at any dose level, with no clinical signs or mortalities. This, it could be argued, may be a deviation from OECD guidelines where (according to OECD 416) dose selection criteria specify "*..the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering*". Minimal transient effects on weight gain (males), increased relative liver weight with diffuse hepatocyte hypertrophy at doses ≥ 750 ppm were seen in males and at 450 ppm in females. Minimal thyroid follicular cell hypertrophy was seen (males). The toxicokinetic profile of pydiflumetofen following repeated gavage or dietary dosing in rats was determined and used to support dose level selection for studies including the 2-gen study based on linear *versus* non-linear kinetics in rat.

It was argued in the DAR (Vol 3CA B6.1 (iii) p.17) that the dose selection based on TK data is only applicable to the parent as the additional studies were performed using a non-radiolabelled method which does not permit following the fate of the metabolites. The active substance is extensively metabolised, hence circulating levels of parent are extremely low compared to the metabolites, especially 2,4,6 TCP and its sulphated conjugate. The applicant considered that the non-proportionality of pydiflumetofen kinetics with increasing dose (due to dose limited absorption) would be reflected by non-proportionality in the formation of all metabolites. However, the non-proportionality of pydiflumetofen 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 higher doses than the maximal dose employed for the reproductive toxicity studies (100-300 mg/kg/day (female-male) in rat studies.

This is confirmed both by the available toxicokinetic and toxicity studies performed. Table 6.1-3 in the DAR (reproduced below) presents a comparison between the plasma AUCs measured after oral administration in rat of phenyl radiolabeled pydiflumetofen (permitting a follow-up of the active substance 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.

Table: *Comparison of AUC0-t for Total Radioactivity in Plasma Following a single Oral Administration of [phenyl-U-14C]- pydiflumetofen to Male and Female Rats*

Dose (mg/kg)	**Fold increase**		**PLASMA**	
			AUC(0-t)(*ng*	**Fold increase**
5		Male	8830	
		Female	10200	
5		Male	8705	
		Female	7890	
100	20	Female	113000	14
300	60	Male	316000	36
1000	200	Male	546000	62
		Female	450000	44

Therefore, the maximal doses used in the multi-generation study were not optimal and this raises the issue of whether the endpoints were fully investigated or indeed if the study was truly OECD 416 guideline compliant with regard to the selection criteria for determining the highest dose.

3.1.3 Parental effects

Effects of treatment at the higher doses consisted of ;

1. reduced body weight gain in P and F_1 generation males given 4500 ppm during the initial weeks of pre-pairing, with slight effects on food intake only in the F_1 generation. There was no effect of treatment on female body weight gain at any dietary concentration, in either generation.
2. no effects on fertility and mating performance or gestation length for either generation at any dietary concentration. Sperm parameters were unaffected. All pregnant females gave birth to live litters with a similar number of pups born, and there was no effect of treatment on the postnatal survival of P or F_1 generation litters to Day 21 of age.
3. oestrous cycling; high dose females had slightly longer and fewer oestrous cycles during the 21 days before the pairing period ($p<0.05$). However, cycling parameters for all females were within the normal range (DAR Table 6.6.1-6). This effect may have been driven by 2 females with longer mean cycle length (5 and 4.5 days) and was not reproduced in the F_1 generation. Therefore, any differences are biologically insignificant and unrelated to treatment.
4. An increase in liver and thyroid weights was associated with microscopic changes (diffuse hepatocyte hypertrophy and thyroid follicular epithelial hypertrophy) for both the P and F_1 generations (see the highlighted in the table below).

***Table**. P Generation Oestrous cycle length and periodicity*

Observation		**Dose Group (ppm)**			
		0	**150**	**450**	**1500**
No. of oestrous cycles (over 21 days)	Mean	4.8	4.7	4.6	4.5
	SD	0.4	0.5	0.5	0.5
	N	24	24	24	24
Oestrous cycle length (days)	Mean	3.93	3.95	3.95	4.05*
	SD	0.10	0.13	0.11	0.24
	N	24	24	24	24

***Table**. Intergroup comparison of selected organ weights in P animals*

		Dietary Concentration (ppm)							
		Males				**Females**			
		0	**250**	**750**	**4500**	**0**	**150**	**450**	**1500**
Liver	Absolute	13.10	13.62	14.23 *(+9%)*	**17.19**** *(+31%)*	13.35	13.44	13.96 *(+5%)*	**15.28**** *(+14%)*
	Adjusted	12.96	13.12	**14.18**** *(+9%)*	**17.88**** *(+38%)*	13.24	13.38	**14.05*** *(+6%)*	**15.36**** *(+16%)*
	Relative‡	3.09	3.14	3.37 *(+9%)*	4.27 *(+38%)*	4.89	4.94	5.20 *(+6%)*	5.67 *(+16%)*
Kidney	Absolute	2.63	2.62	2.62	2.72	2.07	2.08	2.10	2.14
	Ajusted	2.61	2.55	2.61	**2.82**** *(+8%)*	2.05	2.07	2.12	2.15
	Relative‡	0.62	0.61	0.62	0.68	0.76	0.76	0.78	0.80
Thyroid	Absolute	0.022	0.024	0.024	**0.025*** *(+14%)*	0.017	0.016	0.017	**0.015*** *(-12%)*
	Adjusted	0.022	0.023	0.024	**0.026**** *(+18%)*	0.017	0.016	0.017	**0.015*** *(-12%)*
	Relative‡	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006

*statistically significant p<0.05
**statistically significant p<0.0

Table 20. *Intergroup comparison of selected organ weights in F[1] animals*

		Dietary Concentration (ppm)							
		Males				Females			
		0	250	750	4500	0	150	450	1500
Liver	Absolute	13.06	13.39	13.93	**16.47**** *(+26%)*	11.30	11.09	12.01	**13.24**** *(+17%)*
	Adjusted	12.43	12.82	**13.93**** *(+12%)*	**17.63**** *(+42%)*	11.26	11.09	12.05	**13.23**** *(+17%)*
	Relative‡	3.02	3.10	3.35 *(+11%)*	4.27 *(+41%)*	4.47	4.43	4.83	5.30 *(+18%)*
Kidney	Absolute	2.59	2.54	2.56	2.60	2.12	2.06	2.09	2.15
	Ajusted	2.52	2.47	2.56	**2.74**** *(+9%)*	2.11	2.06	2.10	2.15
	Relative‡	0.60	0.59	0.62	0.68	0.84	0.82	0.84	0.86
Thyroid	Absolute	0.020	0.022	0.022	**0.023*** *(+15%)*	0.019	0.020	0.019	0.019
	Adjusted	0.019	**0.022*** *(+16%)*	**0.022*** *(+16%)*	**0.024**** *(+26%)*	0.018	0.020	0.019	0.019
	Relative‡	0.005	0.005	0.005	0.006	0.007	0.008	0.008	0.008

*statistically significant p<0.05
**statistically significant p<0.01

3.1.4 Offspring effects

There were no deaths or clinical signs considered related to treatment in either F_1 or F_2 pups. Anogenital distance was not affected in either generation.

All litter and viability parameters were unaffected by treatment. Mean body weight from day 7 and mean weight gains at 1500 ppm were slightly (but stat. sig.) lower over Days 1 – 21 in the F_1 generation litters only (table below).

Table 21. *F_1 and F_2 intergroup comparison of bodyweights/body weight gains*

Lactation Day	Dose Group (ppm)							
	0	150	450	1500	0	150	450	1500
	F_1 Pups - male & female				F_2 Pups - male & female			
1	6.69	6.64	6.72	6.45	6.23	6.45	6.60	6.35
4a	10.17	9.98	10.30	9.27	8.16	7.81	8.76	8.05
7	16.83	15.99	16.50	**14.89****	12.60	12.32	13.47	12.95
14	33.97	32.75	33.11	**29.91****	30.32	29.58	30.93	29.33
21	51.63	50.63	50.28	**46.29****	48.94	47.27	49.60	47.32
D1-21	44.95	43.96	43.57	**39.84****	42.44	40.70	42.93	40.69

a - Before standardisation (culling)
** - Statistically different from control, p<0.01

3.1.4.1 Sexual maturation F_1 Pups

Exposure to pydiflumetofen delayed the age of onset of preputial separation in males and vaginal opening in females (table below). Both effects were statistically significant but only the preputial separation was outside the historical control range for males at the highest dose. There is no evidence to determine if the delays have been caused by direct effects on the genital tract or by effects on systemic endocrine function.

Rat 2-gen study – Points to note:

- *In Males:* Sexual maturation was delayed (stat. sig.) at 4500 ppm (45.9 days versus 43.0 days in controls). This was considered by the DS to be related to lower body weight in males given 4500 ppm indicating that the delay in sexual maturation of males was secondary to reduced body weight gain during the lactation period. However, the decrease in body weight was not dose-related amongst the treated groups and varied between 9-12% lower than controls in the high dose group. The association of the reduced preputial separation with reduced weight may be considered equivocal in this case.
- *In Females:* Sexual maturation was also delayed (stat. sig. $p<0.01$) at 1500 ppm (30.3 and 33.0 days for control and 1500 ppm females, respectively). There was no association with reduced body weight gain in female pups during the lactation period.
- There is no evidence to conclude that the delay in sexual maturation is secondary to a reduction in the rate of body weight development and not a direct effect of pydiflumetofen. Prior experience with other substances considered by RAC (e.g. fluxapyroxad) where there were very clear and more pronounced reductions in the rate of post-natal body weight development across two-generations showed little to no effect on pubertal milestones, i.e. no delay in time to BPS in males or vaginal opening in females. On this basis time-to-puberty endpoints in the context of post-natal body weight changes need to be assessed carefully.
- The rat 2-gen study does not fully inform on these endpoints, given that the high dose selected for females was too low. Pubertal data was only available for one generation, the P-generation offspring (F1 juveniles). There were no data available for the F1-generation offspring (F2 juveniles) for puberty endpoints because the pups were sacrificed on PND-21.

Table. *Sexual maturation and body weights (g); F_1 pups only.*

Males						
Observation			**Dose Group (ppm)**			
			0	**150**	**750**	**4500**
Preputial Separation	Day of age	Mean	43.0	43.2	44.1	45.9**
		SD	2.4	2.7	2.3	3.5
		N	24	24	24	24
	Body weight	Mean	191.9	189.8	193.3	183.9
		SD	18.3	16.3	15.5	12.7
		N	24	24	24	24
Females						
Observation			**Dose Group (ppm)**			
			0	**150**	**450**	**1500**
Vaginal Opening	Day of age	Mean	30.3	31.3	31.8	33.0**
		SD	2.1	2.6	2.1	2.5
		N	24	24	24	24
	Body weight	Mean	97.2	100.3	103.7	105.7
		SD	11.1	16.5	12.4	11.8
		N	24	24	24	24

** - Statistically different from control, $p<0.01$

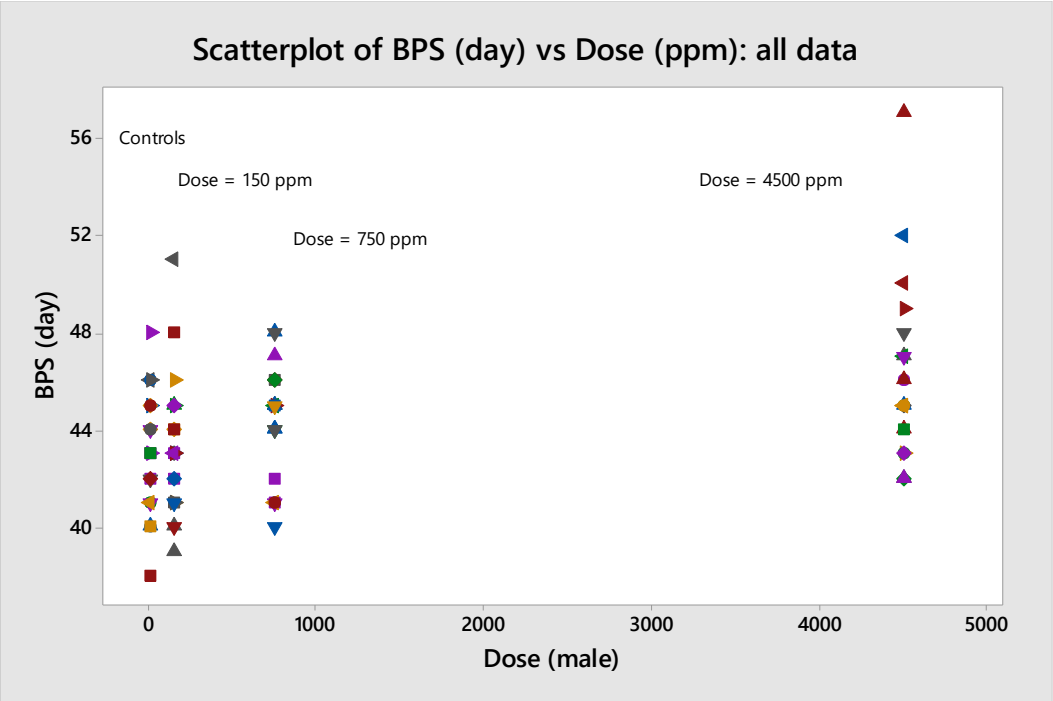
Offspring toxicity

Offspring toxicity		
Males	750 ppm (59 mg/kg/day)	No effects
	4500 ppm (364 mg/kg/day)	F1: delayed sexual maturation (45.9 days versus 43.0 days in controls)
Females	450 ppm (42.4 mg/kg/day)	No effects
	1500 ppm (116 mg/kg/day)	F1: delayed sexual maturation (33.0 days versus 30.3 days in controls). No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance

The vaginal opening was delayed by around 3 days and the delay was statistically significant at 1500 ppm ($p<0.01$), although within the range of the historical control data submitted. The age of vaginal opening is dependent on both body weight at weaning and body weight on the day of vaginal opening (*Edwards and Kay, 1985*). There was no effect on related parameters such as oestrous cycling (in the F_1 generation), mating performance or fertility, and there was no effect on ano-genital distance of F_1 generation pups. However, as weight gain was not affected, the delay in vaginal opening may be due to the delay in development during the lactation period and a relationship to treatment also cannot be excluded (see DAR Table 6.6.1-21). Moreover, the delay is more than 2 days which should be considered as treatment related and generally adverse, unless it is seen as a delay in general growth. RAC noted that effects were seen in both sexes, outside the HCD range (for males) and cannot be explained on the basis of bodyweight change alone, therefore the effects were considered to be clearly treatment related. Reproductive organ weights were unaffected by treatment and there were no abnormalities found at necropsy which were related to treatment.

3.1.4.2 Detailed examination of Pubertal Data

Simple scatterplots (figure below) of the pubertal raw data are not very informative with respect to comparing dose groups with the controls.



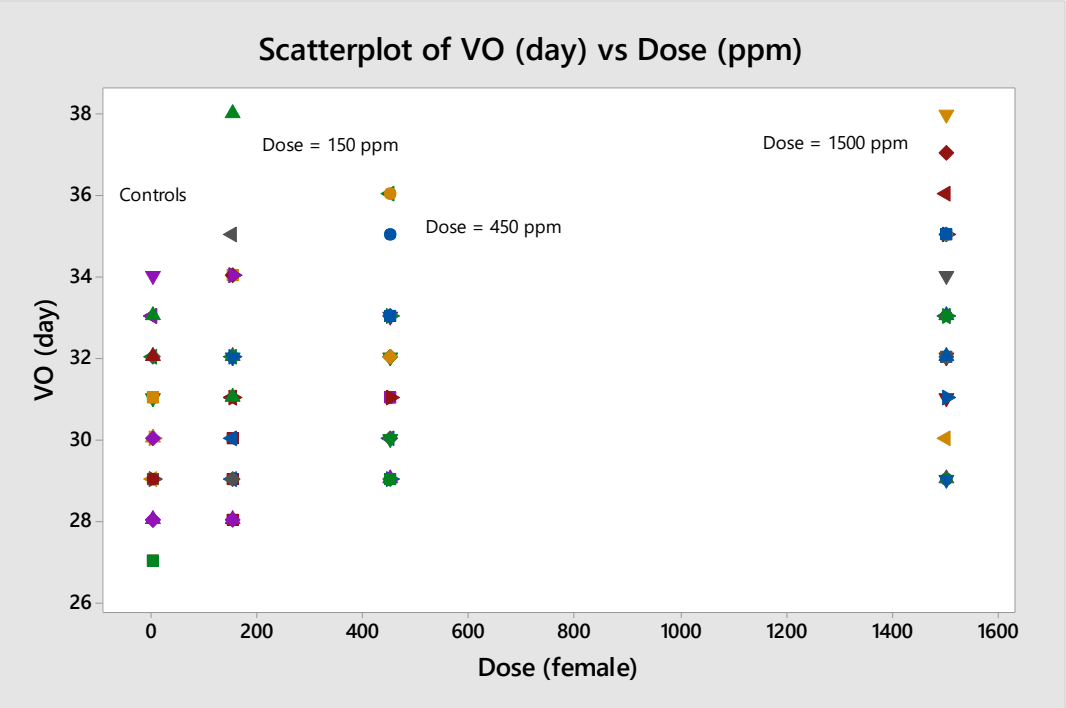
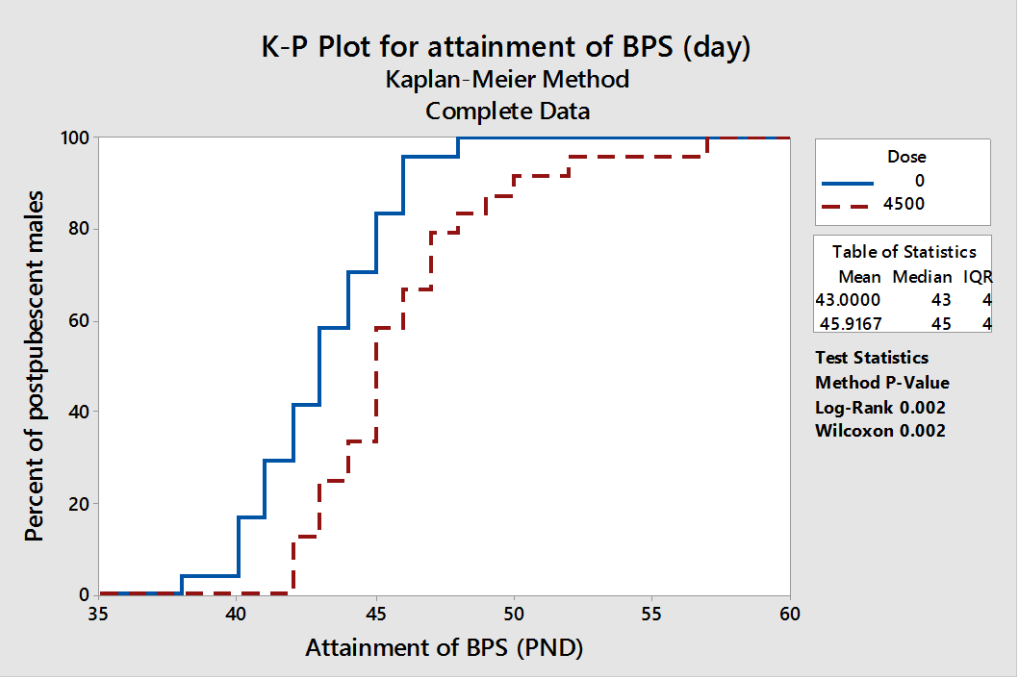


Figure: simple scatterplots of all individual pubertal day of attainment data.

A more convenient way to compare all the data within a dose group to that of the controls is to generate Kaplan-Meier Curves for data with differing times-to-event endpoints. Pubertal data is ideal to compare in this way because the measured endpoint is a fixed, timed event, i.e. post natal day of BPS in males, post natal day of vaginal opening in females. In this case the comparative analysis depends upon the whole "curve" and is not unduly disrupted by isolated data points (figure below).



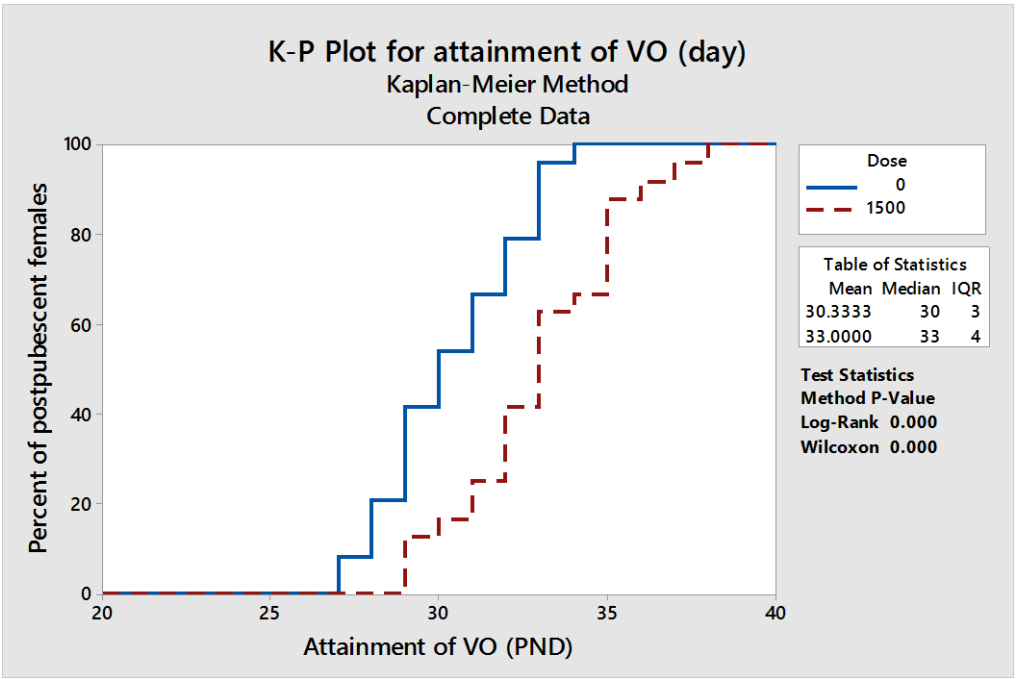


Figure: Kaplan-Meier Curves for Pubertal data endpoints.

Seqani historical control data for balanopreputial reparation and vaginal opening

The mean value at 4500 ppm for the postnatal day of balanopreputial separation sits outside of the Seqani mean HCD range (2008-2014: 43.0 – 45.3 days). This is driven largely by 1 of the 24 males in this group, which is not representative of the remaining 23 treated males and background control animals. Exclusion of this male as an outlier from the 4500 ppm dose group gives a mean value of 45.4 days, which is at the upper boundary of the HCD range. The use of Kaplan-Meier plots, however, allows for the comparison of all data points and still shows a significant delay in BPS associated with the

high dose group animals. If the HCD are confined to within +/- 5 years of the 2-gen rat study, then the Seqani (see the table below) HCD (3 studies) for mean attainment of BPS becomes 43.0 – 43.5 days, illustrating that the PND of BPS in the high dose group may be even more significant because it now falls well outside the upper boundary of the HCD.

Summary of Sequani historical control data (six studies: 2008 – 2014)

The raw data was not available, only summary data from the original study report, for the rat 2-gen study (2015).

HCD from performing lab

Year	BPS	BW	VO	BW
Jan. 2008	45.2	184.8	32.7	92.3
Mar. 2009	45.0	177.8	34.1	98.1
Jan. 2009	45.3	193.4	31.8	89.3
Jan. 2014	43.0	172.3	31.3	88.1
Feb. 2014	43.5	183.4	29.3	89.4
Feb. 2014	43.3	179.6	29.9	85.7
mean	44.2	181.9	31.5	90.5
sd	1.0	6.5	1.6	3.9

range (all years):	38-52	25-39
range (2014):	38-47	25-35

3 studies within the relevant date range (+/- 5 years)

3 older studies had greater delays in BPS and VO

Other sources of historical control data

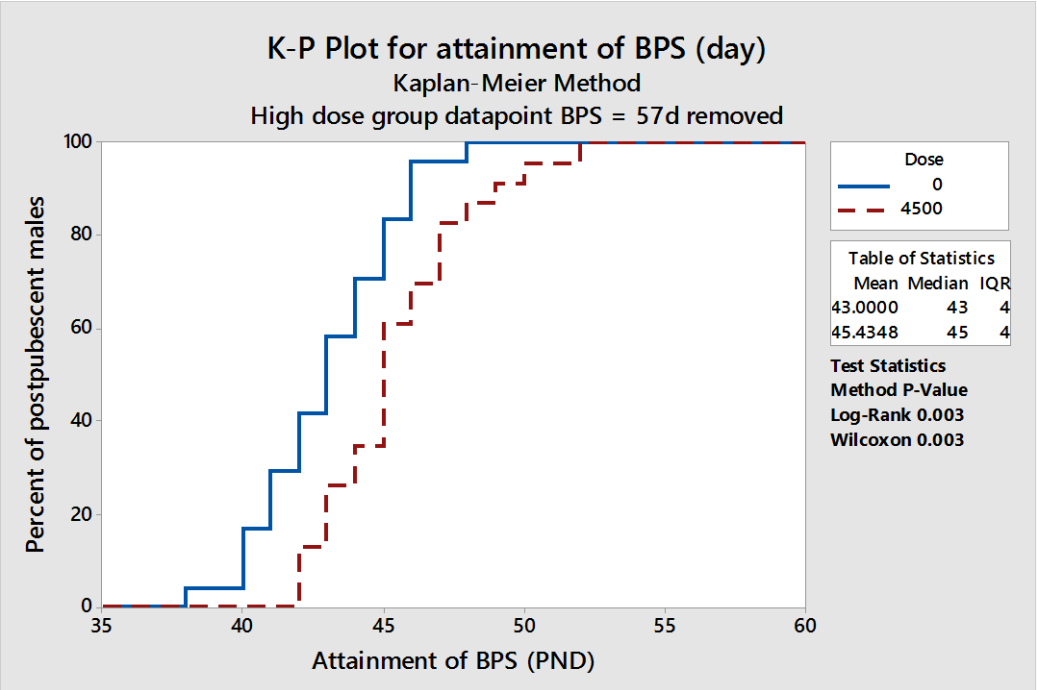
Sexual maturation data for Wistar rats was also available from the 2018 report "Reproductive Toxicology Historical Control Data in Rats" published by Charles River laboratories. Data from six studies was available but no date for the studies was provided.

Males: BPS: mean = 44.7 days; range 43.3 – 45.9 days, n = 87 animals

Females: VO: mean = 31.8 days; 30.7 – 32.5 days, n = 85 animals.

Revised analysis of BPS in the high dose group:

Exclusion of one male as an outlier from the 4500 ppm group gives a mean value of 45.4 days, which is at the upper boundary of the HCD range. The use of Kaplan-Meier plots (figure below) enables the comparison of all data points and still shows a significant delay in BPS associated with the high dose group animals.



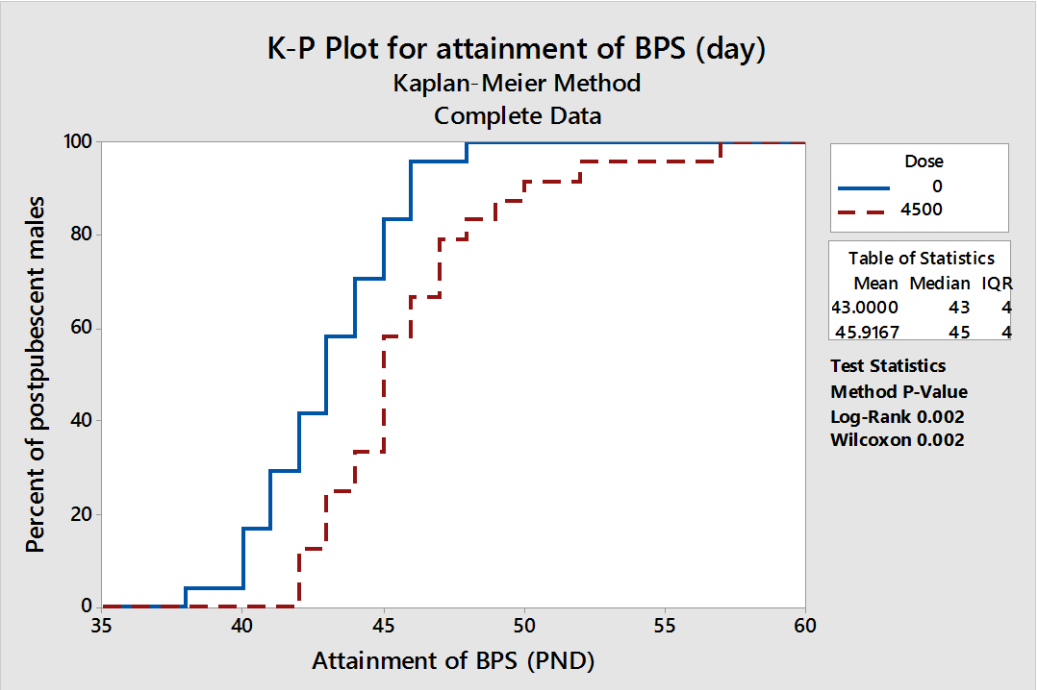


Figure: Kaplan-Meier Curves for adjusted BPS and original BPS pubertal data.

3.1.4.3 Comparison with the Criteria for Classification

Under CLP (annex I: 3.7.1.3) it is recognised that adverse effects on sexual function and fertility include effects on the onset of puberty. This criterion is satisfied for pydiflumetofen, since it delays the time of onset for balanopreputial separation (BPS) in males and vaginal opening in females. The classification criteria as specified under section 3.7.2 of CLP indicate classification as Repr. 2 is appropriate when there is some evidence from animal studies "..*of an adverse effect on sexual function and fertility,..*"

Published literature generally shows that delays in pubertal endpoints by substances due

to endocrine-mediated mechanisms occur together with numerous other effects. For example, known anti-androgens responsible for significant delays in BPS in males include flutamide, prochloraz, and vinclozolin but the effects are not solely confined to one specific event but occur together with other evidence that may include changes in nipple retention, anogenital distance/anogenital index and sex organ weights, as well as gross and histopathological findings. In female rats, atrazine, propazine and esfenvalerate prolong or delay vaginal opening by a number of days, often through centrally acting mechanisms that perturb the hypothalamic-pituitary control responsible for puberty attainment. These other effects are not apparent in rats treated with pydiflumetofen. The fact that both male and female pubertal endpoints are delayed may indicate a more general central acting mechanism or general toxicity. There is no mechanistic data however to explain the delayed attainment of the pubertal endpoints.

Reductions in body weight during post-natal development are known to cause delays in the onset of puberty. It is established in the scientific literature that growth rate is of greater importance than arrival at a particular fixed weight in determining the onset of puberty. For both the high dose F1 female and male pups there is evidence of a delay in growth amounting to a 9-12% reduction relative to concurrent controls during the initial (PND 1-21) post-natal period. However, much larger effects seen for another substance (fluxapyroxad) previously considered by RAC across two generations did not show these effects on pubertal age endpoints, therefore it is recognised that growth rate data must be interpreted with care.

The key question is whether the effects on pubertal attainment alone are sufficient to justify classification for such a significant adverse hazard to human reproduction. The weight of the available evidence suggests an absence of such a hazard (no alteration on the timing of the first dioestrus and of oestrus or mating performance or fertility have been reported; no effect on ano-genital distance of F1 generation pups; no effect on endocrine or reproductive organs were observed in any of the available repeated toxicity studies; no effect on follicle counts in the ovaries).

3.1.4.4 Increased pup death in the F1 generation litters (F2 pups)

Table 9.2 in the original study report summarises F1 generation litters (F2 pups). Curiously the [Missing (presumed cannibalised)] and [Found dead/killed prematurely] entries show very high numbers of lost pups when compared with the data for the F0 generation litters (F1 pups, Table 9.1 of the study report) (see the highlighted in the tables below). Also, in the summary of pregnancy and litter data (for the F1 generation parents) it was stated that 4, 4, 1 and 5 females in the groups given 0, 150, 450 or 1500 ppm, respectively, showed total litter loss between days 2 and 9 of age. Since these litter losses were spread across the groups, including controls, and showed no dose response relationship, they were considered not to be treatment related. However, no explanation has been provided for this effect and hence they do raise questions regarding the quality of the original study.

3.1.5 Conclusion

Dietary administration of pydiflumetofen up to 4500 ppm for males and 1500 ppm for females for two successive generations, was well tolerated. There was a slight reduction in early body weight gain of males at 4500 ppm of both the P and F_1 generations. Microscopic changes were seen in the liver (diffuse hepatocyte hypertrophy) and thyroid (follicular epithelial hypertrophy) of high dose P and F_1 generation males; the liver changes were also seen for high dose P generation females and generally correlated with organ weight

changes.

RAC agrees with the DS that the maximal dose level tested should have been higher than the linear kinetics dose range although effects on liver and thyroid weight and histology were apparent at the higher doses in males and less so in females.

There were no adverse effects on reproductive performance, mating behaviour or conception. A statistically significant delay in vaginal opening without reduced body weight was observed in females given 1500 ppm which may be associated with a delay in development during the lactation period or other undefined effects. A statistically significant delay in preputial separation in males at 4500 ppm may have been associated with a slight but not statistically significant reduction in body weight, but again other factors may be involved. RAC noted that effects are seen in both sexes, at the upper boundary of the historical control data (for males) and these cannot be explained by bodyweight changes alone, therefore an association with treatment cannot be excluded.

3.2 Development

3.2.1 Rat

3.2.1.1 Preliminary study

A preliminary study investigated the effects of the test item on the pregnant rat and on embryonic and foetal development (Days 6 to 19 of gestation), in order to select dose levels for a subsequent developmental toxicity study.

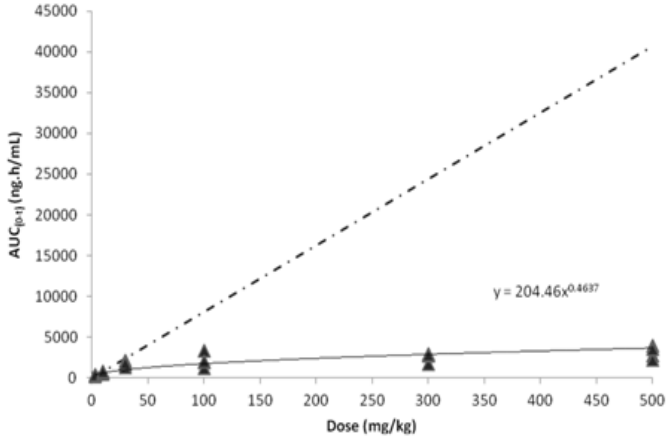
Oral administration to 6 females per group of 0, 100, 200, 500 and 1000 mg/kg bw/day was well tolerated. Reduction in body weight gain for females given 500 mg/kg/day and body weight loss for females given 1000 mg/kg/day, were observed between Days 6 to 7 of gestation as the only maternal findings.

There was no effect of treatment on the mean number of corpora lutea, mean number of implantations, the extent of pre- or post-implantation losses or on the mean number of live foetuses. All pregnant females had litters with live foetuses at scheduled necropsy. There was no effect of treatment on mean foetal weights, mean litter weights or the percentage of male foetuses. Mean placental and gravid uterus weights were similar across the groups.

Relevant findings

Significant effects on weight gain from 500 mg/kg bw/day. On the basis of this data, a higher dose should have been selected in the main study to properly assess the hazard according to the OECD guideline, even though above 100 mg/kg/d dosage, the total systemic increase becomes non-linear with an increase by 1.3 fold when the dose increases by 3 (from 100 to 300 mg/kg/d) and by 1.5 fold when the dose increases by 5 (from 100 to 500 mg/kg/d).

Relationship between AUC(0-t) and dose in female rats following daily oral administration of pydiflumetofen for 7 days (Figure 6.1.1-3 of the DAR):



3.2.1.2 Main study

Time-mated female Crl:CD (SD) rats were dosed by oral gavage from Day 6-19 of gestation within the vehicle, 1 % carboxymethylcellulose, at dose levels of 10, 30 or 100 mg/kg/day.

Relevant findings

There were no clinical signs of toxicity, no mortalities, no treatment-related findings at necropsy.

An initial reduction in weight gain (and food consumption) on day 6 was resolved by day 11. Weight gain was not affected otherwise.

The uterine/implantation data were unaffected by the administration of pydiflumetofen. There was no effect of treatment on mean foetal, litter or placenta weights.

Some major abnormalities were noted

Major foetal abnormalities (table below) were noted in:

- 1 foetus from the control group
- 1 foetus from the 10 mg/kg/day group
- 2 foetuses from two litters in the 30 mg/kg/day group
- 2 foetuses from one litter in the 100 mg/kg/day group.

Table. *Summary of major foetal abnormalities*

Dose (mg/kg/day)	Dam	Fetus	Findings
0	16	L5	Diaphragmatic hernia
10	48	L8	Multiply malformed fetus
30	66	L4	Anophthalmia; oribital cavity reduced in size; malformed cervical neural arch; absent cervical neural arches; bent scapula
30	69	L12	Scapulae severly bent; humerii malformed; femurs bowed
100	82	L1	Exencephaly; open eye; cleft palate; malformed inter-parietals, parietals, frontals and nasals; cleft palatine
		R3	Exencephaly; open eye; malformed palate; malformed parietals and frontals; absent inter-parietals; cleft palatine

Controls: Diaphragmatic hernia was seen in one control foetus.

10 mg/kg bw/day: The one affected foetus had multiple severe malformations both external and internal including skeletal and sort tissues. This is considered to be a spontaneous malformation.

30 mg/kg be/day: 2 foetuses/2 litters with multiple malformations. Specific HCD were not presented for anophthalmia; oribital cavity reduced in size; malformed cervical neural arch or absent cervical neural arches. However, there were no additional occurrences in the 100 mg/kg bw/day dose level or in the preliminary study up to 1000 mg/kg bw/d.

Following a request from EFSA (February 2018), additional HCD have been submitted by the applicant (Crl: CD (SD) rat data (19 studies) from pre-natal developmental toxicity studies after consolidation of other contract research organisations (CRO's) under Charles River Laboratories since 2016). Bent scapula was identified as a major abnormality which can occur as a background finding (3/337 litters).

100 mg/kg bw/day: Multiple malformations of the skull and oral cavity including cleft palate and exencephaly were seen in two foetuses of a single litter. Some of the malformations observed were compared to the HC data (which were considered relevant by the RMS (*Davies 2015*) for litter incidence in cleft palate/ malformed palate/exencephaly/open eye in studies performed between 2008 and 2012 (table below); cleft palate, exencephaly and open eye were within the range of the background data (*Davies, 2015*).

Table. *Summary of major foetal external abnormalities*

Observations	Type	Dose levels (mg/kg bw/day)				HCD range: incidence (group mean %)		
		0 (control)	10	30	100	Sequani [a]	CR Horsham [b]	CR Montreal [c]
External examination								
No. of foetuses (F)		*311*	*271*	*317*	*296*			
No. litters (L)		*24*	*21*	*24*	*23*			
Head: eye- uni- or bilateral: open	Major	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 2 (0.7%) # L: 1 (4.3%)	F: 0-1 (0-0.4%) L: 0-1 (0-5.3%)	F: 0-1 (0-0.4%) L: 0-1 (0-5.6%)	F: 0-2 (0-0.32%) L: 0-2 (0-4.2%)
Brain: exencephaly	Major	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 2 (0.7%) # L: 1 (4.3%)	-	F: 0-1 (0-0.3%) L: 0-1 (0-5%)	F: 0-2 (0-0.36%) L: 0-2 (0-5%)
Oral cavity: palate: cleft or malformed	Major	F: 0 (0%) L: 0 (0%)	F: 1 (0.3%) L: 1 (4.8%)	F: 0 (0%) L: 0 (0%)	F: 2 (0.67%) # L: 1 (4.3%)	F: 0-1 (0-0.4%) L: 0-1 (0-5.3%)	F: 0-1 (0-0.3%) L: 0-1 (0-4.2%)	F: 0-4 (0-0.34%) L: 0-4 (0-4.76%)
Runted foetus	Minor	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 1 (0.3%) L: 1 (4.2%)	F: 0 (0%) L: 0 (0%)	-	-	-

Two foetuses from the same litter present malformations of the oral cavity (cleft palate/ malformated palate, cleft palatine) associated with malformations of the head (inter-parietals, parietals, frontals and nasals), exencephaly and open eye

However, further HCD on exencephaly in the Crl:CD (SD) rat was submitted during Peer Review on request from EFSA. This HCD demonstrated that the spectrum of malformations apparent in the pydiflumetofen prenatal developmental toxicity study in the rat at both 30 or 100 mg/kg bw/day are considered to be spontaneous in origin and have been observed for this strain in control females at similar incidences within the historical control data, and

therefore, are considered to incidental to pydiflumetofen administration (see the highlighted in the table above).

Table. *A Comparison of Foetal Gross External Malformations from the Rat Developmental Toxicity Study with pydiflumetofen (Davies, 2015) and HCD Data in Crl: CD [SD] Rats.*

		Reference	**Sequani Limited, Ledbury**	**Charles River Laboratories, Montreal**	**Charles River Laboratories, Lyon**	**Charles River Laboratories, Ashland**	**MARTA (MARTA, 1996)**	**Ema *et al.*, 2014**
		Year	2007 to 2015	2007 to 2017	2012-2017	2016-2017	1988-1991	2001-2010
		Strain	Crl: CD [SD]	Crl: CD [SD]	Crl: OFA [SD]	Crl: CD [SD]	Crl: CD [SD]	Crl: CD [SD]
Malformation	**Study incidence**	**Dose (mg/kg bw/day)**						
Anophthalmia	1/21 litter 1/271 foetuses	30	0/478 litters 0/6108 foetuses	2/1462 litters 2/19277 foetuses	1/563 litters 1/2158 foetuses	0/288 litters 0/3628 foetuses	16/6102 litters 16/88270 foetuses	10†/5702 litters 10/39192 foetuses
Exencephaly	1/24 litters 2/296 foetuses	100	0/478 litters 0/6108 foetuses	1/1462 litters 1/19277 foetuses	1/563 litters 1/7079 foetuses	0/288 litters 0/3628 foetuses	16/6102 litters 26/88270 foetuses	7†/5747 litters 7/79960 foetuses
Open eye	1/24 litters 2/296 foetuses	100	1/478 litters 1/6108 foetuses	1/1462 litters 1/19277 foetuses	0/563 litters 0/7079 foetuses	0/288 litters 0/3628 foetuses	6/6102 litters 9/88270 foetuses	4†/5747 litters 4/79960 foetuses
Cleft palate	1/24 litters 1/296 foetuses	100	2/478 litters 2/6108 foetuses	2/1462 litters 2/19277 foetuses	0/563 litters 0/7079 foetuses	1/288 litters 3/3628 foetuses	9/6102 litters 9/88270 foetuses	9†/5747 litters 9/79960 foetuses
Malformed palate (High arched palate)	1/24 litters 1/296 foetuses	100	1/478 litters 1/6108 foetuses	0/1462 litters 0/19277 foetuses	0/563 litters 0/7079 foetuses	0/288 litters 0/3628 foetuses	2/6102 litters 4/88270 foetuses	0/5747 litters 0/79960 foetuses
Malformed or absent nares	1/24 litters 1/296 foetuses	100	0/478 litters 0/6108 foetuses	1/1462 litters 1/19277 foetuses	0/563 litters 0/7079 foetuses	0/288 litters 0/3628 foetuses	5/6102 litters 5/88270 foetuses	4†/5747 litters 4/79960 foetuses

Exencephaly was reported to occur in this strain of rat and its occurrence in two foetuses from the same litter in the group given 100 mg/kg/day, but was not considered to indicate an adverse effect of treatment. **Note:** the background incidence of foetuses with multiple malformations as described is, however, not available.

A number of variations/abnormalities were significantly increased at 100 mg/kg bw/day.

- Absent costal cartilage (a skeletal abnormality/variation) was observed in rats (also seen in rabbits) ($p<0.05$) at 100 mg/kg/day, just within the available HCD.
- In the groups receiving 30 or 100 mg/kg/day, there was a significant ($p<0.05$) increase, compared with the control group, in the number of litters with foetuses showing 'small area of liver protruding into the thorax', which is considered to be a minor abnormality, as there is no hole in the diaphragm and there is no consequence on post-natal survival and development. This finding is a background lesion that occurs sporadically.
- Left sided umbilical artery was increased at this dose. The increase was within the HCD range provided.

Table. *Summary of minor foetal abnormalities and variants from external/ visceral/ skeletal examination (F: foetal incidence (group mean %); L: litter incidence (group mean %))*

Observation	**Dose levels (mg/kg bw/day)**				
	0	**10**	**30**	**100**	**HCD range from Sequani d/e (incidence (group mean %))**
Umbilical artery (left side)	F: 1 (0.6%) L: 1 (4.2%)	F: 2 (1.5%) L: 2 (9.5%)	F: 1 (0.6%) L: 1 (4.2%)	F: 6 (3.8%) L: **5 (22%*)**	F: 0-8 (0-5.4%) [e] L: 1-7 (12-32%)
Liver ≥ 1 lobe: small area protruding into the thorax	F: 0 (0%) L: 0 (0%)	F: 1 (1.0%) L: 1 (4.8%)	F: 4 (2.6%) **L: 4 (16.7%)***	F: 3 (2.1%) **L: 3 (13%)***	F: 0-3 (0-2.7%) [e] L: 0-3 (0-15.8%)
Rib ≥1 costal cartilage absent	F: 0 (0%) L: 0 (0%)	F: 0 (0%) L: 0 (0%)	F: 2 (1.2%) L: 2 (8.3%)	F: 2 (1.5%) L: 2 **(8.7%)***	F: 1-5 (0.7-1.6%) L: 1-2 (4.5 -9.5%)[ǂ]

The number of foetuses showing these minor or variant abnormalities was very small and within the background data ranges and their incidences do not indicate an adverse effect on foetal development.

The foetal malformations and variations seen in the rat main study lack a dose-response relationship and statistical significance when compared with the controls, and were not apparent in the previous preliminary dose-range finding study (*Anon., 2011*) at significantly higher doses of pydiflumetofen (100, 500 and 1000 mg/kg bw/d). This demonstrates a distinct lack of dose concordance; no major foetal abnormalities were observed at up to 1000 mg/kg bw/d in the preliminary rat study. These findings were not considered treatment related by the RMS.

3.2.1.3 Conclusion

RAC can agree with the argument of the RMS.

3.2.2 Rabbit data

3.2.2.1 Preliminary study

Preliminary study: 0, 250, 500 or 1000 mg/kg/day on days 6-27 of gestation in NZW rabbits.

The highest dose was well tolerated with a slight initial reduction in body weight gain (GD 6-12) ($p<0.05$ to $p<0.01$). No statistically significant effect on body weights was observed over the duration of the study (Days 6 to 28) after correction for gravid uterine weight.

Foetal effects

All pregnancy related endpoints were unaffected.

A number of malformations were recorded, the majority occurring in the controls. Two foetuses in treated groups had abnormalities of the head; one foetus in Group 2 (250 mg/kg/day) with cheilognathopalatoschisis (cleft lip, jaw and palate) and one foetus in Group 4 (1000 mg/kg/day) with proboscis, cyclopia and oral cavity and jaw abnormalities.

***Table**. Summary of major abnormalities*

Dose (mg/kg/day)	Dam	Fetus	Findings
0	41	R1	Thoracogastroschisis; left forelimb amelia; facial cleft on left side; microencephaly, pinna right low set; pinna left anotia. Left eye anophthalmia; left kidney pelvic; ovary bilateral ectopic; heart – persistent truncus arteriosus; intraventricular septum absent; descending aorta enlarged severely.
	44	R5	Heart severely enlarged; transposition of the great vessels; intraventricular septum absent; both lungs sevelery reduced in size
	46	R1	Left pinna malformed; abdomen – fissure of body wall
	48	L6	Interrupted aortic arch
250	53	R2	Eye bilateral open; nares absent; cheilognathopalatoschisis; pinna bilateral low set.
	55	R1	Both lungs severely reduced in size.
1000	75	R4	Proboscis; cyclopia; nares absent; oral cavity reduced opening; agnathia; malformed palate; microglossia. Interrupted aortic arch.

Although very rare, these abnormalities were considered by the RMS/DS to be isolated incidences and unrelated to treatment.

3.2.2.2 Main study

Administration of pydiflumetofen, once daily, by oral gavage, to pregnant New Zealand White rabbits from GD 6 to Day 27, inclusive, at dose levels of 10, 100 or 500 mg/kg/day was well tolerated with no clinical signs, no effect on body weight or pregnancy.

Foetal effects

Major foetal abnormalities were noted in 5/3 foetuses/litters in the control group, 4/3 foetuses /litters in the group given 10 mg/kg/day, 3/3 foetuses/litters in the group given 100 mg/kg/day and in 2/2 foetuses/litters in the group given 500 mg/kg/day.

Malformations related to skeletal and neural arches were observed in 2 foetuses at 100 mg/kg/day and were also observed in one foetus at 500 mg/kg/d (table "Summary of major foetal abnormalities" below). However, all these skeletal malformations are within the HCD provided by the conducting laboratory (13 studies performed between 2009 to 2013, table " Summary HCD for malformations related to skeletal and neural arches", below)

Table: *Summary of major foetal abnormalities*

Dose (mg/kg/day)	Dam	Fetus	Findings
0	8	R3	Flattened right maxillary region of the head; malformed forelimbs; arthrogryposis; malrotated hindlimbs; enlarged right orbital cavity
	17	R1	Severely enlarged aortic arch; transposition of the great vessels, absent intraventricular septum
	22	L3	Severely enlarged aortic arch
		R1	Pulmonary valvular artesia; absent intraventricular septum
		R3	Duplicated sternebrae; sternal and xiphoid cartilage duplicated on 1st and 4th sternebrae
10	34	L1	Interrupted aortic arch; incomplete intraventricular septum
		R1	Interrupted aortic arch; absent intraventricular septum; severely enlarged pulmonary arch, entire heart & superior vena cavas; severely fused 1st to 6th sternebrae
	38	R6	Severely enlarged aortic arch
	46	R5	Pulmonary valvular artesia
100	61	L1	Malrotated fore-limbs; severely bent scapula; bowed radii, ulna, tibia and fibula
	65	L4	Malformed and discontinuous lumbar cord; malrotated hidlimbs; filamentous tail; centrally placed kidneys; undescended testes; 10 thoracic vertebrae; 10 pairs of ribs; 10th centra absent; 10th neural arches malformed & fused; absent lumbar sacral & caudal vertebrae
	72	L3	Spina bifida; bifid 7th lumbar to 4th sacral neural arches; malformed 1st to 4th sacral cartilaginous spinous processes; severely fused 6th to 8th caudal centra
500	76	R2	Severely fused 4th to 5th thoracic centra; 4th & 5th right ribs arising drom the same neural arch
	80	L1	Diaphragmatic hernia; small lungs; right sided descending aorta.

Table: *Summary HCD for malformations related to skeletal and neural arches*

External and fresh examination: other major abnormalities

Observations	Dose levels (mg/kg bw/day)				HCD Sequani from the conducting laboratory[#] Mean % (range)	
	0	10	100	500	N = 13 studies (2009-2013)	
Spina bifida	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.7%) L: 1 (5.3%)**	F: 0 (0%)	F: 0.03% (0-0.7%) L: 0.41 (0-5.6%)	
Severely fused 4th to 5th thoracic centra	F: 0 (0%)	F: 0 (0%)	F: 0 (0%)	F: 1 (0.6%) L: 1 (4.8%)	Thoracic centra: one or more: major fusion	F: 0.11% (0-0.7%) L: 0.97% (0-5.3%)
Bifid 7th lumbar to 4th sacral neural archs	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.7%) L: 1 (5.3%)**	F: 0 (0%)	lumbar neural arch: one or more :bifid	F: 0.03% (0-0.7%) L: 0.48% (0-5.6%)
4th & 5th right ribs arising from the same neural arch	F: 0 (0%)	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.6%) L: 1 (4.8%)**	Ribs: one or more: arising from same neural arch	F: 0.05% (0-0.7%) L: 0.48% (0-5.3%)
Absent lumbar sacral & caudal vertebrae	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.7%) L: 1 (5.3%)**	F: 0 (0%)	Sacral centra: absent Caudal vertebrae: absent	F: 0.06% (0-0.7%) L: 0.48 (0-5.3%) F: 0.06% (0-0.6%) L: 0.48 (0-4.8%)

<u>*Diaphragmatic hernia:*</u> The increased incidence of diaphragmatic hernia observed in one foetus at 500 mg/kg/d (1/154 (0.6%)) is well within the range of the HCD (0-1.6% (liver diaphragmatic hernia); 0-1.3% (stomach diaphragmatic hernia), see table below. The

DS is of opinion that this finding may be considered incidental and not treatment related.

Table: *Diaphragmatic hernia*

Observations	Dose levels (mg/kg bw/day)				HCD Sequani from the conducting laboratory[#] Mean % (range)
	0	10	100	500	N = 36 studies (2010-2017)
No. of foetuses (F)	*163*	*142*	*132*	*154*	*3277*
No. litters (L)	*22*	*18*	*19*	*21*	*385*
Liver: diaphragmatic hernia	F: 0 (0%)	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.6%)** **L: 1 (4.8%)**	F: 0.18% **(0-1.6%)** L: 1.6% **(0-14.3%)**
Stomach: diaphragmatic hernia	F: 0 (0%)	F: 0 (0%)	F: 0 (0%)	**F: 1 (0.6%)** **L: 1 (4.8%)**	F: 0.06% **(0-1.3%)** L: 0.5% **(0-11%)**

Skeletal variations: Costal cartilage variant: A marginally increased incidence of one cartilage variant (one or more costal cartilage interrupted (rib)) was observed in the groups given 100 mg/kg/day or 500 mg/kg/day compared with control. HCD for the costal cartilage variant from 54 rabbit prenatal developmental studies performed by the conducting laboratory were submitted and have been assessed by the DS. Taking into account limitation to a time-frame of +/- five years with respect to the completion date of the rabbit developmental study with pydiflumetofen (42 studies performed between 2007 to 2017), the increase at the highest dose of 500 mg/kg/day is within the spontaneous background data range for foetal incidence (8% vs 9.4%) and slightly above the range for litter incidences (47.6% *vs* 44.5%). At 100 mg/kg bw/day, the increase in both litter and foetal incidences is above the HCD ranges (table with variant skeletal findings with statistical significance). The litter is the unit of interest in reproductive and developmental toxicity studies and the highest litter incidence was 9 (44.5%) in 2/8 studies from the HCD. The litter incidence in both the 100 and 500 mg/kg bw/day was greater than this (12 (63%) and 10 (47.6%), respectively).

The possibility of a relationship to treatment cannot be excluded. However, costal cartilage variant can be considered spontaneous in origin in NZW rabbits. In addition, this finding is not defined as a malformation, but rather, as variations in cartilage development which do not impact normal growth or function. Attention is drawn to the absence of other associated changes in any rib parameters and the absence of a dose response relationship. There is no retardation of foetal growth and development associated with pydiflumetofen at ≥ 100 mg/kg bw.

3.3 Adverse effects on or via lactation

In the two generation reproduction study as described, the administration of 1500 ppm in the top dose group for females (equivalent to 116 mg/kg/day, F0; 141 mg/kg/day, F1) increased the relative liver weight in both generations. There were no clinical signs of toxicity, no mortalities and no treatment-related findings at necropsy, amounting to no evidence to suggest biologically significant maternal toxicity. There was no indication of impaired nursing behaviour or decreased pup viability during lactation and no effect on pup growth to weaning. The results of the study do not indicate any direct, adverse effect on the offspring due to transfer of the active substance via the milk or to reductions in the quality of the milk.

3.4 Comparison with the criteria

3.4.1 Consideration of Category 1A classification

According to the CLP criteria, classification in Category 1A is largely based on evidence

from human data, which were not present in the CLH report. Therefore, classification as Repr. 1A is not warranted.

3.4.2 Consideration of Category 1B or 2 classification

Categories 1B and 2 are reserved for presumed and suspected human reproductive toxicants, respectively, and must be based on the presence of **clear** (Category **1B**) or **some** (Category **2**) evidence of alterations in sexual function, fertility, or development. In addition, the evidence for both hazard categories must be present in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other concurrent toxic effects.

3.4.2.1 Reproduction

Argument against classification

While the specific findings for sexual maturation in both sexes appear related to treatment, there was no effect on key downstream parameters of sexual function such as oestrus cycling, mating performance or general fertility and no effect on ano-genital distance. In addition, there was no effect observed on endocrine or reproductive organs in any of the available repeated toxicity studies (in rat, mice or dogs).

Argument for classification

Minimal parental toxicity was demonstrated when Wistar rats were administered pydiflumetofen up to 4500 ppm (277 mg/kg bw) in males and 1500 ppm (116 mg/kg bw/day) in females in a 2 generation study.

In the 2 generation rat study a statistically significant delay in vaginal opening without reduced body weight at the time was observed in females given 1500 ppm. A statistically significant delay in time to preputial separation in males at 4500 ppm was also observed along with a slight but not statistically significant reduction in body weight. Minor changes in postnatal body weight are not believed to account for this effect. There was no evidence to suggest the delays were caused by direct effects on the genital tract or by effects on systemic endocrine function. However, an association with treatment cannot be excluded; the effects, i.e. delays in the onset of both male and female puberty (data available in one generation only), may be regarded as a potential fertility retardation but it must be noted that these effects were observed in the absence of adverse effects on downstream reproductive performance, mating behaviour, conception, etc. Nevertheless, regulation 1272/2008 defines effects to be considered for classification in point 3.7.1.3 where it states that such adverse effects on sexual function and fertility include (but are not limited to) "…adverse effects on onset of puberty…". Another source of uncertainty comes from the fact that the maximal dose level tested could have been higher (particularly in females) than that proposed from the findings of the toxicokinetic and metabolic studies. Higher doses could potentially have led to more reproductive effects and so the available studies cannot fully inform on other potential reproductive effects by pydiflumetofen. Considering all of the available data and the limitations in the available studies, classification in Category 1B is not proposed on the basis of adverse effects on the onset of puberty alone.

All studies were technically conducted to guidelines but the data do not fully inform on all reproductive endpoints simply because of dosing shortcomings. The early culling of the F2 generation offspring means that data for pubertal onset is confined to a single generation

of animals. An argument can be made that higher doses should have been tested in both the 2 generation study and in the rat main developmental toxicity study for greater confidence and robustness of the data. Consideration was given to the fact that there were no downstream consequences on fertililty parameters in the 2-gen study and that no classification in a higher category could be supported. However, regulation 1272/2008 defines effects to be considered for classification in point 3.7.1.3 and mentions that adverse effects on sexual function and fertility include (but are not limited to) "…adverse effects on onset of puberty…". As a consequence, delays in the onset of puberty in two sexes may be considered as a case for Category 2 for fertility effects .

From a purely technical point of view pydiflumetofen satisfies the criteria for an adverse effect that is considered in CLP for classification purposes. However, central to all the decisions of RAC is the consideration of the weight of evidence of all the data available . In the case of pydiflumetofen, RAC considers that classification in category 2 for fertility is appropriate.

In summary, delayed pubertal effects were seen in both sexes, at the upper boundary of the HCD in the case of males which cannot be explained on the basis of body weight change alone, therefore these effects are considered treatment related, clearly impact on the development or time to attainment of puberty (which may potentially impact on human fertility or reproductive function) and therefore are considered sufficient for Category 2 classification.

3.4.2.2 Developmental toxicity

Table: *Summary of relevant data*

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Developmental toxicity OECD 414 GLP Acceptable Oral (gavage) Rat, Crl:CD (SD) 24 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 30 or 100 mg/kg/day on days 6-19 of gestation Vehicle: 1% CMC (w/v)	*Maternal toxicity* 100 mg/kg/day: Initial reduction in BWG between first days of dosing (Day 6 to day 10). Maternal NOAEL 30 mg/kg/day *Developmental toxicity* Malformations and variations reported were not considered related to treatment 100 mg/kg/day: No effects at highest dose tested	*Anon.*, (2015)
Preliminary developmental toxicity Non-guideline Non-GLP Supplementary Oral (gavage) Rat, Crl:CD (SD) 6 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.6%) 0, 100, 200, 500 or 1000 mg/kg/day on days 6-19 of gestation Vehicle: 1% CMC (w/v)	*Maternal toxicity* 500 and 1000 mg/kg/day: Initial reduction of BWG during the first day of dosing (Day 6-7). *Developmental toxicity (no skeletal examination)* 1000 mg/kg/day: No effects at highest (limit) dose tested	*Anon.* (2011)
Developmental toxicity OECD 414	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 100 or 500	*Maternal toxicity* 500 mg/kg/day: No effects at highest dose tested	*Anon.*, (2015b)

<table>
<tr>
<td>GLP
Acceptable
Oral (gavage)
Rabbit, New Zealand White
24 mated females/group</td>
<td>mg/kg/day on days 6-27 of gestation
Vehicle: 1% CMC (w/v)</td>
<td>Maternal NOAEL 500 mg/kg/day
Developmental toxicity
<u>≥ 100 mg/kg/day</u>:
-malformations reported at all dose levels including controls were not considered related to treatment.
-Increased incidence of one skeletal variant (rib costal cartilage interrupted) without clear dose response but increased incidence above the HCD.
<table>
<tr><td></td><td colspan="4">Dose level (mg/kg/day)</td></tr>
<tr><td></td><td>0</td><td>10</td><td>100</td><td>500</td></tr>
<tr><td>Observations</td><td colspan="4">Rib: one or more: costal cartilage interrupted (variant)</td></tr>
<tr><td>Fetuses</td><td>8/163 (4.4%)</td><td>8/142 (5%)</td><td>14/132 (14%)</td><td>12/154 (8%)</td></tr>
<tr><td>Litters</td><td>6/22 (27.3%)</td><td>6/18 (33.3%)</td><td>12/19 (63%)*</td><td>10/21 (47.6%)*</td></tr>
</table>
HCD: foetus: 0-13 (0-9.4%) / Litters: 0-9 (0-44.5%)
Developmental NOAEL 10 mg/kg/day</td>
<td></td>
</tr>
<tr>
<td>Preliminary developmental toxicity
Non-guideline
Non-GLP
Supplementary
Oral (gavage)
Rabbit, New Zealand White
10 mated females/group</td>
<td>PYDIFLUMETOFEN (SYN545974) (purity 99.3%/ 98.5%)
0, 250, 500 or 1000 mg/kg/day on days 6-27 of gestation
Vehicle: 1% CMC (w/v)</td>
<td>Maternal toxicity
<u>1000 mg/kg/day</u>:
↓ body weight 35% days 6-28
Maternal NOAEL 500 mg/kg/day

Developmental toxicity (no skeletal examination)
Malformations reported at all dose levels including controls were not considered related to treatment
<u>1000 mg/kg/day</u>:
Developmental NOAEL 1000 mg/kg/day</td>
<td>Anon., (2015c)</td>
</tr>
<tr>
<td>Two generation reproduction
OECD 416
GLP
acceptable
Oral (continuous in diet)
Rat, Crl:WI (Han)
24/sex/group
(see also sections 2.6.3 and 2.6.6.3)</td>
<td>PYDIFLUMETOFEN (SYN545974) (purity 98.5%)
Males: 0, 150, 750 & 4500 ppm
Females: 0, 150, 450 & 1500 ppm
Continuous in the diet</td>
<td>Only data for offspring developmental toxicity are presented
<u>Offspring toxicity - Males</u>
<u>4500 ppm (364 mg/kg/day)</u>
F1: delayed sexual maturation (45.9 days versus 43.0 days in controls) concurrent with slight ↓ body weight
<u>Offspring toxicity - Females</u>
<u>1500 ppm (116 mg/kg/day)</u>
F1: delayed sexual maturation (33.0 days versus 30.3 days in controls). No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance.</td>
<td>Anon., (2015)</td>
</tr>
</table>

<u>Arguments for classification/non-classification</u>

In the preliminary rat study, there were no adverse findings on development up to 1000 mg/kg bw/day. A number of foetuses with multiple malformations were observed sporadically in treated groups of the main study where exposure was up to 100 mg/kg bw/day. The findings were compared to a variety of sources of historical control data during the EFSA Peer Review and were generally within the ranges assessed.

In the rabbit, a considerable number of malformed foetuses were recorded across the doses from control to 1000 mg/kg bw. No particular dose or treatment-related pattern was apparent with the exception of a rib variant (one or more costal cartilage interrupted) the incidence of which was elevated in foetuses/ litters from 100 mg/kg bw/day. The increase at the highest dose of 500 mg/kg/d was slightly above the range for litter incidences of

the appropriate HCD (47.6% vs 44.5%). At 100 mg/kg bw/d, the increase in both litter and foetal incidences was above the HCD ranges (see table with variant skeletal findings with statistical significance above). The highest litter incidence of the HCD was 9 (44.5%) in 2/8 studies from the HC data. The litter incidence in both the 100 and 500 mg/kg bw/day was greater than this (12 (63%) and 10 (47.6%), respectively). The possibility of a treatment relationship cannot be excluded. However, costal cartilage variant can be considered spontaneous in origin in NZW rabbits. In addition, this finding is not defined as a malformation, but rather as variations in cartilage development and do not impact on normal growth or function. Classification for development is not proposed on the basis of these findings.

Conclusion

RAC considers that classification in category 2 for fertility is appropriate.

RAC proposes no classification for Development.

2.6.7 Summary of neurotoxicity

The need for classification related to neurotoxic effects have been considered in the appropriate sections i.e. STOT SE (2.6.2.10), STOT RE (2.6.3)

Table 51: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
Acute oral neurotoxicity study OECD Guideline 424 GLP Acceptable Rat Han-Wistar (RccHan™ WIST) 10/ sex/group (see also section 2.6.2.10)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100 (females only), 300 (males only), 1000 or 2000 mg/kg Single oral (gavage) dose Vehicle: 1% CMC (w/v)	**100 mg/kg bw (females)** No differences from control. **1000 mg/kg bw (females)** 1/10 females at 1000 mg/kg showed marked clinical signs and was euthanized ~3.25 hours post dose *Clinical signs at 6 hours post dose:* recumbency 1/9, piloerection 4/9, reduced activity 2/9, abnormal gait 1/9, skin cold to touch 1/9; pupillary reflex impaired 1/9 and mydriasis 1/9; ↓ 2.6% body temperature; ↓ decrease in locomotor activity (48%, 66% in mean distance travelled and number of rearings) *Clinical signs after 1 day:* No differences from control. **2000 mg/kg bw (females)** *Clinical signs at 6 hours post dose only:* Hunched posture 2/10, piloerection 4/10, reduced activity 1/10, abnormal gait 1/10; ↓ 3.1% body temperature; ↓ decrease in locomotor activity (59%, 81% in mean distance travelled and number of rearings, respectively) *Clinical signs after 1 day:* No differences from control. No treatment –related histopathological findings **NOAEL General toxicity and neurotoxicity: 2000 mg/kg (males) / 100 mg/kg (females)**	***Anonymous*** (2015a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
Acute oral neurotoxicity study (modified females only) OECD Guideline 424 Acceptable Rat Han Wistar (RccHan™: WIST) 10/ females/group (see also section 2.6.2.10)	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100, 300 or 1000 mg/kg Single oral gavage dose Vehicle: 1% CMC (w/v)	**<u>100 mg/kg bw</u>** *Clinical signs* ~ 2-5 hours post dose: in 2/10 animals the following were observed; ruffled fur, eyes half closed and ventral recumbency. *Clinical signs* 6 hours post dose*:* piloerection 1/10, skin cold to touch 2/10; impaired extensor thrust reflex 2/10; ↓ 0.8% body temperature; ↓ decrease in locomotor activity 4%, in mean distance travelled (no difference in number of rearings) **<u>300 mg/kg bw</u>** *Clinical signs* 6 hours post dose*:* ↓ 1.1% body temperature; ↓ decrease in locomotor activity (26%, 37% in mean distance travelled and number of rearings) **<u>1000 mg/kg bw</u>** *Clinical signs* ~ 3 hours post dose: in 1/10 animals the following were observed; ruffled fur, eyes half closed and ventral recumbency. *Clinical signs* 6 hours post dose: piloerection 1/10, skin cold to touch 1/10, tremor 1/10, impaired extensor thrust reflex 1/10; ↓ 1.1% body temperature; ↓ decrease in locomotor activity (28%, 41.0% in mean distance travelled and number of rearings) **NOAEL General toxicity and neurotoxicity (females):100 mg/kg**	***Anonymous*** (2015b)
13-week dietary study in rats OECD 408, GLP Acceptable Rat:Han wistar (Crl:WI(Han)) 10/sex/group (see also sections 2.6.3)	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) 0, 250, 1500, 8000 or 16000 ppm Actual doses 0, 18.6, 111, 587 and 1187 mg/kg/day (males) and 0, 21.6, 127, 727 and 1324 mg/kg/day (females) Continuous in the diet for 13 weeks.	Only results on functional observation battery parameters (FOB) are presented **<u>≥ 250 ppm (males 18.6 mg/kg/day, females 21.6 mg/kg/day)</u>**: No treatment-related effects on FOB parameters: detailed clinical observations, tests for reflexes and other stimuli, grip strength, landing foot splay, body temperature or on motor activity.	***Anonymous*** (2015)
2 year chronic toxicity/ carcinogenicity OECD 453 GLP Acceptable Rat: Han Wistar Crl: WI (Han) 64/sex (52/sex/group main group, 12/sex/group interim kill after 12 months) (see also	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) Males 0, 200, 1000 & 6000 ppm; Females 0, 150, 450 & 1500 ppm Actual doses 9.9, 51.0 and 319 mg/kg/day (males); 10.2, 31.0 and 102 mg/kg/day (females). Continuous in the diet for 104 weeks	Only functional observation battery parameters (FOB) are presented **<u>≥ 6000 ppm (males 319 mg/kg/day) and ≥ 1500 ppm (females 102 mg/kg/day):</u>** No treatment-related effects on FOB parameters: detailed clinical observations, tests for reflexes and other stimuli, grip strength, landing foot splay, body temperature or on motor activity.	***Anonymous*** (2015a)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results: - NOAEL/LOAEL - target tissue/organ -critical effect at LOAEL**	**Reference**
section 2.6.3)			

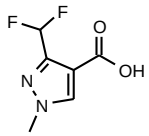
The acute neurotoxicity of PYDIFLUMETOFEN (SYN545974) has been evaluated in the rat. In the acute study, single gavage doses of 0, 300 (males) or 100 (females), 1000 and 2000 mg/kg produce clinical signs of toxicity and effect on body temperature and locomotor activity (LMA) at dose levels ≥ 1000 mg/kg only in females on the day of treatment. No effects were observed in males. A subsequent modified acute neurotoxicity study in female rats only, with single oral gavage doses of 0, 100, 300 and 1000 mg/kg, produce the same effects from 300 mg/kg. All signs of toxicity were resolved by day 2.

It should be noted that in the 90-day toxicity study in rat (***Anonymous*** 2015), no effect were observed on detailed clinical observations, functional observation battery (FOB) parameters or on locomotor activity (LMA) up to the highest dose tested (16000ppm equivalent to 1322-1174 mg/kg bw/d in males and females; respectively). In addition, there were no treatment effects in the FOB parameters or on motor activity following administration of PYDIFLUMETOFEN (SYN545974) at doses levels up to 6000ppm in males (319 mg/kg/day) and 1500 ppm in females (102 mg/kg/day) in the 104-week toxicity study in rat (***Anonymous*** 2015a).

2.6.8 Summary of other toxicological studies

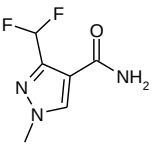
2.6.8.1 Toxicity studies of metabolites and impurities

- **Metabolite CSAA788670**



CSAA798670 is a common metabolite to a number of SDHI molecules and toxicity studies performed on this metabolite have been assessed during the peer-review of other pyrazole active substances (sedaxane, fluxapyroxade, benzovendiflupyr).
Metabolite CSAA798670 presented low oral acute and short-term toxicity, no adverse effects were observed up to 1000 mg/kg bw/day (limit dose) in a 90-day dietary study in rats; no adverse effect (regarding maternal and developmental toxicity) was observed in a developmental toxicity study in rabbits up to the highest dose tested of 250 mg/kg bw/day, but CSAA798670 produced high maternal toxicity at 500 mg/kg bw/day in a developmental range-finding study. No genotoxic potential is attributed to the metabolite. CSAA798670 was found to be less toxic than the parent compound. If reference values are needed for this metabolite, no acute reference dose (ARfD) is allocated and the Acceptable Daily Intake (ADI) is 0.25 mg/kg bw/day, based on the NOAEL of 250 mg/kg bw/day from the developmental toxicity study in rabbits with an assessment factor (AF) of 1000 applied, to account for the limited database available (no long-term, multigeneration or rat developmental toxicity study available).

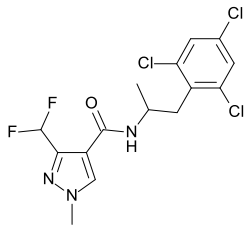
- **Metabolite SYN508272:**



Metabolite SYN508272 presented a higher acute oral toxicity than the parent PYDIFLUMETOFEN (SYN545974). In a 28-day toxicity study in rat, reductions in body weight and food consumption were observed at 143 mg/kg bw/day (males)/243.5 mg/kg bw/day (females). Metabolite SYN508272 was showed to be clastogenic *in vitro* in a chromosome aberration test in human lymphocytes; however, no genotoxic potential was attributed to the metabolite *in vivo* in a rat micronucleus assay in bone marrow cells.

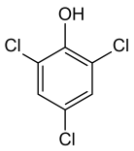
After an oral administration of PYDIFLUMETOFEN (SYN545974) in rat, SYN508272 was the major pyrazole specific metabolite detected in plasma accounting for up to 14.8% of total radioactivity AUC (TRA) with its precursor SYN548263 accounting for up to 8.1% TRA (***Anonymous*** (2015); see section B.6.1.1). Therefore, the mammalian toxicity database on PYDIFLUMETOFEN (SYN545974) also assesses the toxicity of the metabolite SYN508272 and risk assessment endpoints for PYDIFLUMETOFEN (SYN545974) are considered appropriate also for SYN508272. Therefore, if reference values are needed, the reference values of the parent PYDIFLUMETOFEN (SYN545974) are applicable for the metabolite SYN508272

- **Metabolite SYN545547:**



SYN545547 is a metabolite of PYDIFLUMETOFEN (SYN545974), which has been identified in animal commodities, primary and rotated crops. A comparative QSAR analysis has been conducted on SYN545547. Based on the structural similarity to parent and absence of any additional QSAR alerts (especially for genotoxicity), the Threshold of Toxicological Concern (TTC) for non-genotoxic compounds ('Cramer Class III') are considered appropriate for assessing dietary exposure to SYN545547.

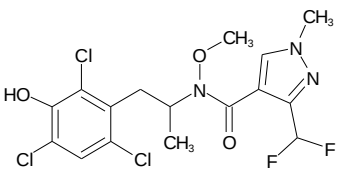
- **Metabolite 2,4,6-Trichlorophenol (2,4,6-TCP)**



2,4,6-trichlorophenol (2,4,6-TCP) and its related metabolites (hydroxyl TCP sulphate and 2,4,6-TCP sulphate) were identified in animal commodities and are included in the definition of residue only for animal commodities. Toxicological data regarding this substance are available in the published literature as well as in reviews from regulatory agencies and an overview of them are provided in the volume 3 section B.6.8.2. The 2,4,6-TCP has been classified as carcinogen by several international bodies: Carc. Cat. 2 H351 by the European Union (ATP0); carcinogen group 2B by IARC or carcinogen group B2 by US-EPA. This classification is based on the increase incidence of monocytic leukemia observed in male rats and liver tumours observed in mice in long term toxicity studies.

In the rat and mouse, 2,4,6-TCP was the major circulating metabolite of PYDIFLUMETOFEN (SYN545974) in plasma. Thus, it could be considered that the mammalian toxicity data package on PYDIFLUMETOFEN (SYN545974) has sufficiently assessed the toxicity of 2,4,6 TCP and its conjugates. However, as previously mentioned, the RMS questioned on the possibility that the doses of PYDIFLUMETOFEN (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 (see section 2.6.1 and 2.6.5). In this context, a rationale based on margins of safety was added by the RMS in section 2.6.10.1 and in volume 3 section B.6.8.1. This rationale confirms that the mammalian toxicity data package on PYDIFLUMETOFEN (SYN545974) has sufficiently assessed the toxicity of 2,4,6 TCP and its conjugates and risk assessment endpoints for PYDIFLUMETOFEN (SYN545974) can be considered appropriate also for 2,4,6 TCP.

- **Metabolite SYN547897**



SYN547897 is a metabolite of PYDIFLUMETOFEN (SYN545974), which has been identified in animal commodities in goat liver and goat kidney. After an oral administration of PYDIFLUMETOFEN (SYN545974) in rat, SYN547897 was detected in plasma accounting for up to 4.3% of total radioactivity AUC (TRA) and for up to 0.9% in urine. Based on the structural similarity to parent and it presence in rat metabolism, genotoxicity data generated on PYDIFLUMETOFEN (SYN545974) are considered appropriate to assess SYN547897. Therefore, the Threshold of Toxicological Concern (TTC) for non-genotoxic compounds ('Cramer Class III') are considered appropriate for assessing dietary exposure to SYN547897, if necessary.

2.6.8.2 Supplementary studies on the active substance

A small number of investigative studies have been conducted using PYDIFLUMETOFEN (SYN545974) as the test item. Samples of liver from the 90 day study in the male rat were analysed for UDP-GT activity and it was concluded that PYDIFLUMETOFEN (SYN545974) in the male rat was an inducer of hepatic microsomal UDP-GT. However, PYDIFLUMETOFEN (SYN545974) was not an inhibitor of rat thyroid peroxidase activity *in vitro.*

According to Commission Regulation (EU) No 283/2013 supplementary studies on the immunotoxicological potential are required for an active substance when they are necessary to further clarify observed effects on the immune system. PYDIFLUMETOFEN (SYN545974) does not fulfil these criteria and specific studies on immunotoxicity would not be required. However, the toxicity database has been evaluated for endpoints considered relevant for the identification of potential immunotoxicity. It was concluded that PYDIFLUMETOFEN (SYN545974) has no immunotoxic disruption potential.

2.6.8.3 Endocrine disrupting properties

All of the relevant data for PYDIFLUMETOFEN (SYN545974) for potential endocrine disruption in mammalian species have been evaluated using a weight of evidence approach proposed by the European Chemical Industry Council (CEFIC) Endocrine Modulators Steering Group (EMSG), structured according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters. PYDIFLUMETOFEN (SYN545974) has been extensively tested, with the relevant data from the regulatory studies covering a wide range of study types *in vitro* and *in vivo*. These data fall into the levels 2, 4 and 5 of the OECD Conceptual Framework. It was concluded that PYDIFLUMETOFEN (SYN545974) has no endocrine disruption potential.

2.6.9 Summary of medical data and information

PYDIFLUMETOFEN (SYN545974) has only been manufactured at pre-production scale at a Syngenta plant in Münchwilen, Switzerland since 2011. Formulation activities have taken place at Syngenta R&D sites at Jealott's Hill, UK, Greensboro, US and Münchwillen, Switzerland. Field trials have also taken place globally including EU, South-Africa, Australia, New Zealand, China, Japan, Korea, Taiwan, USA, Canada, Argentina, Chile, Brazil and Mexico.

The Occupational Health group of Syngenta has maintained a data base of incidents involving chemical exposure of workers since 1983. From 1994 data has been collected from all our manufacturing, formulation and packing sites around the world. A query of the Syngenta internal database in June 2015 for PYDIFLUMETOFEN (SYN545974) produced zero records of adverse health effects reported during AI manufacture, subsequent formulation and field trials.
As PYDIFLUMETOFEN (SYN545974) is still under development, no commercial sales have been made and therefore there are no observations following human exposure. There are no epidemiology studies or monitoring programs performed in humans.

PYDIFLUMETOFEN (SYN545974) is of low acute toxicity. Intoxication is only likely if large quantities are ingested. In animal studies, minor clinical signs of toxicity were evident at 5000 mg/kg bw. The same would be expected to occur in humans if similar dose levels were consumed. However, no cases of intoxication with PYDIFLUMETOFEN (SYN545974) have been observed.

2.6.10 Toxicological end points for risk assessment (reference values)

Table 52: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
Rat Han wistar (Crl:WI(Han))	13-week dietary toxicity study OECD 408, GLP 10/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) 0, 250, 1500, 8000 or 16000 ppm Actual doses 0, 18.6, 111, 587 and 1187 mg/kg/day (males) and 0, 21.6, 127, 727 and 1324 mg/kg/day (females) Continuous in the diet for 13 weeks.	≥1500 ppm: Male: Liver weight increase ≥ 20% of control associated with centrilobular hypertrophy and blood clinical chemistry parameter changes (ALP decrease). Follicular cell hypertrophy in the thyroid gland. Female: Liver weight increase ≥ 15% of control associated blood clinical chemistry parameter changes (ALP decrease). ≥ 8000 ppm: reduction in body weight gain (> 20%) and food utilisation in males and females. Liver weight increase ≥ 20% of control associated with centrilobular hypertrophy in females. Follicular cell hypertrophy in the thyroid gland in females.	250 ppm (18.6 mg/kg/day males, 21.6 mg/kg/day females)	1500 ppm (111 mg/kg/day males, 127 mg/kg/day females)	***Anonymous*** (2015)
Mice CD-1 (Crl:CD-1)	13 week dietary toxicity Study OECD 408 GLP 10/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 99.5%) 0, 100, 500, 4000 and 7000 ppm Actual dose 0, 17.5, 81.6, 630 and 1158 mg/kg/day (males) and 0, 20.4, 106, 846 and 1483 mg/kg/day (females) Continuous in the diet for 13 weeks	≥ 100 ppm: Liver weight increase ≥ 15% of control associated with hepatocyte hypertrophy and blood clinical chemistry parameter changes (dose-related increase cholesterol) in male. ≥4000 ppm: Liver weight increase ≥ 50% of control associated with hepatocyte hypertrophy and blood clinical chemistry parameter changes (increase cholesterol and triglycerides) in female There was a reduction in bodyweight gain with isolated statistical significance in males at all doses but without dose response in males (by 47%, 5%, 28%, 26%, respectively with increasing doses).	Male 100 ppm (17.5 mg/kg/day male) Female 500 ppm (105.9 mg/kg/day)	Male 500 ppm (81.6 mg/kg bw/d) Female 4000 ppm (846 mg/kg/day female)	***Anonymous*** (2015)
Dog Pure-breed Beagles	13-week oral (capsule) toxicity OECD 409, GLP 4/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 30, 300, 1000 mg/kg/day Capsule administration. No vehicle 13-week duration	≥300 mg/kg bw/day: blood chemical changes (ALP (>200%) and triglyceride increases), liver weight increase (30%) but without corresponding liver histopathological findings observed in males. 1000 mg/kg bw/day: reduced body weight gain, food consumption, blood chemical changes in females (elevated ALP in females and elevated ALP and TG in males, increase liver weight (>40%) associated with liver hepatocyte hypertrophy in both sexes.	Males: 30 mg/kg bw/day Females: 300 mg/kg bw/day	Males: 300 mg/kg bw/day Females: 1000 mg/kg bw/day	***Anonymous*** (2015a)
Dog Pure-breed Beagles	52-week oral (capsule) toxicity OECD 452	PYDIFLUMETOFEN (SYN545974) (purity 98.5%)	300 mg/kg/d (males): higher alkaline phosphatase levels evident at all time-points and higher liver weights (+35%) but not associated with histopathological changes.	Males 100 mg/kg bw/day	Males 300 mg/kg bw/day	***Anonymous*** (2015a)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
	GLP 4/sex/group	0, 30, 100, 300 mg/kg/day Capsule administration. No vehicle 52-week duration		Females 300 mg/kg bw/d	Females None	
Rat Crl:CD (SD)	Developmental toxicity OECD 414 GLP Oral (gavage) 24 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 30 or 100 mg/kg/day on days 6-19 of gestation Vehicle: 1% CMC (w/v)	Maternal: Marginal effects on bodyweight and food consumption were seen at 100 mg/kg/day during gestation days 6-9. Fetal: None.	Maternal: 100 mg/kg/day Fetal: 100 mg/kg bw/day	Maternal: >100 mg/kg/day Fetal: >100 mg/kg bw/day	***Anonymous*** (2015)
Rabbit New Zealand White	Developmental toxicity OECD 414 GLP Oral (gavage) 24 mated females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 10, 100 or 500 mg/kg/day on days 6-27 of gestation Vehicle: 1% CMC (w/v)	Maternal: None. Fetal: Increased incidence of one skeletal variant (rib costal cartilage interrupted) at 100 and 500 mg/kg/day without clear dose response. No historical control data available	Maternal: 500 mg/kg bw/day Fetal: 10 mg/kg bw/day	Maternal: >500 mg/kg bw/day Fetal: 100 mg/kg bw/day	***Anonymous*** (2015b)
Rat Crl:WI (Han)	Two generation reproduction OECD 416 GLP Oral (continuous in diet) 24/sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) Males: 0, 150, 750 & 4500 ppm Females: 0, 150, 450 & 1500 ppm Continuous in the diet	750ppm (males)-450 ppm (females): Adaptive responses observed in the liver (slight increased liver weight (<10%) without hepatocellular hypertrophy) in males at ≥ 750 ppm in both generations and in P generation female ≥450 ppm. 4500 ppm (males)-1500 ppm (females): - Reduction in overall cumulative body weight gains (0 to 17 weeks) in P and F1 male generations. - Increased liver weight (≈38% in male and ≈16% in female) in P and F1 generations associated with hepatocellular hypertrophy in P generation (male and female) and in F1 generation (male). - Increased thyroid weight in P generation (male and female) and in F1 generation (male) associated with follicular hypertrophy in the males of the P and F1 generations.	Parental: Males 750 ppm (46 mg/kg bw/day) Females 450 ppm (36.1 mg/kg bw/day)	Parental: Males 4500 ppm (276.6 mg/kg bw/day) Females 1500 ppm (116.2 mg/kg bw/day)	***Anonymous*** (2015)
			No effects on reproduction observed.	Reproduction: Males ≥ 4500 ppm (276.6 mg/kg bw/day) Females ≥ 1500 ppm (116.2 mg/kg bw/day)	Reproduction: None	

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
			In female F1 pups, a delayed sexual maturation (33 days vs 30.3 days in controls) was observed at 1500 ppm and was not secondary to bodyweught effects. No subsequent effect on oestrus cycling, mating performance or fertility and no effect on ano-genital distance	Offspring: Males ≥ 4500 ppm (276.6 mg/kg bw/day) Females 450 ppm (31.6 mg/kg bw/day)	Offspring: Males: None Females : 1500 ppm (116 mg/kg bw/day)	
Rat Han Wistar Crl: WI (Han)	2 year chronic toxicity/ carcinogenicity OECD 453 GLP 64/sex (52/sex/group main group, 12/sex/group interim kill after 12 months)	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) Males 0, 200, 1000 & 6000 ppm; Females 0, 150, 450 & 1500 ppm Actual doses 9.9, 51.0 and 319 mg/kg/day (males); 10.2, 31.0 and 102 mg/kg/day (females). Continuous in the diet for 104 weeks	1000 ppm (males): Lower body weight and body weight gain, food utilization, hepatocyte hypertrophy and increased liver weight. 1500 ppm (females): Lower body weight and body weight gain, slight liver increase associated with minimal hepatocellular hypertrophy 6000 ppm (males): Lower body weight and body weight gain, food consumption and food utilization, increased severity of liver findings (grossly prominent liver lobular architecture at 52 and 104 weeks and hepatocyte cytoplasmic eosinophilic inclusions at 104 week) No treatment related neoplastic findings.	Males 200 ppm (9.9 mg/kg bw/day); Females 450 ppm (31 mg/kg bw/day)	Males 1000 ppm (51 mg/kg bw/day) Females 1500 ppm (102 mg/kg bw/day)	***Anonymous*** (2015)
Mouse CD-1 (ICR)	Carcinogenicity OECD 451 GLP Acceptable 50/sex /group	PYDIFLUMETOFEN (SYN545974) (purity 98.5% w/w) 0, 75, 375 & 2250 ppm Actual doses 0, 9.2, 45.4 and 287.9 mg/kg/day for males and 0, 9.7, 48.4 and 306.2 mg/kg/day for females Continuous in the diet for 80 weeks	375 ppm (males): increase incidences of hepatocellular carcinomas and adenomas at ≥ 375 ppm, eosinophilic foci of cellular alteration and centrolobular hypertrophy in males only. 2250ppm (males): decrease in body weight and body weight gain and food utilization during the early stages of the study. Hepatocellular carcinomas and adenomas correlating to liver masses observed at necropsy, eosinophilic foci of cellular alteration, centrilobular hypertrophy and liver weight increase. 2250 ppm (females): Decrease in body weight and body weight gain and liver weight increase. Not oncogenic in female mice.	Males 75 ppm (9.2 mg/kg bw/day) Females 375 ppm (48.4 mg/kg bw/day)	Males 375 ppm (45.4 mg/kg bw/day) Females 2250 ppm (306.2 mg/kg bw/day)	***Anonymous*** (2015a)
Rat Han-Wistar (RccHan™ WIST)	Acute oral neurotoxicity study OECD Guideline 424 GLP 10/ sex/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100 (females only), 300 (males only), 1000 or 2000 mg/kg Single oral (gavage) dose Vehicle: 1% CMC (w/v)	**General toxicity** ≥1000 mg/kg: Males: slight body weight loss on day 1. No further effect on body weight/body weight gain during the course of the study. Females: clinical signs of toxicity on day 1 (recumbency, hunched posture, piloerection, reduced activity, abnormal gait, skin cold to touch and	**General toxicity and neurotoxicity:** 2000 mg/kg (males) 100 mg/kg (females)	**General toxicity and neurotoxicity:** > 2000 mg/kg (males) 1000 mg/kg (females)	***Anonymous*** (2015)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL	LOAEL	Cross reference
			mydriasis 3 to 6 hours post dose). Marked clinical signs ~3.5 hours post-dose in one female at 1000 mg/kg which was euthanized. No dose response in number of clinical signs or severity. **Neurotoxicity** ≥1000 mg/kg: Males: no effect Females: Lower mean body temperature and decrease in locomotor activity (mean distance travelled and number of rearings). No clinical or behavioural signs of toxicity evidents in any rats after day 1.			
Rat Han Wistar (RccHan™ WIST)	Acute oral neurotoxicity study (modified females only) OECD Guideline 424 10/ females/group	PYDIFLUMETOFEN (SYN545974) (purity 98.5%) 0, 100, 300 or 1000 mg/kg Single oral gavage dose Vehicle: 1% CMC (w/v)	**General toxicity** ≥100 mg/kg: clinical signs of toxicity on day 1 (recumbency, piloerection, reduced activity, decreased rearing, skin cold to touch and impaired extensor thrust reflex). However, no dose response evident for clinical signs presence or severity. **Neurotoxicity** ≥ 300 mg/kg: Lower mean body temperature and decreased locomotor activity (distance travelled and number of rears) was observed at ≥300 mg/kg. 2/10 animals were also affected at 100 mg/kg. No clinical or behavioural signs of toxicity were evident in any rats after day 1.	**General toxicity and neurotoxicity:** 100 mg/kg (females)	**General toxicity and neurotoxicity:** 300 mg/kg (females)	***Anonymous*** (2015a)

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

The acceptable daily intake (ADI) is typically derived from the NOAEL in the most susceptible species in long term toxicity and multi-generation reproduction toxicity studies with the application of an appropriate uncertainty factor. The dietary route of exposure is considered the most relevant for derivation of this end-point. Table 67 summarizes the relevant no effect levels to be considered for the purpose of deriving the ADI for PYDIFLUMETOFEN (SYN545974).

The lowest NOAEL in the long-term studies was 9.2 mg/kg bw/day from the 80 week mouse carcinogenicity study. An uncertainty factor of 100 is proposed for derivation of the ADI:

ADI = 9.2 mg/kg/day /100 = <u>0.092 mg/kg/day</u>

Liver tumours were observed in the male mouse only in long-term study performed with PYDIFLUMETOFEN (SYN545974). However, data conclude the CAR MOA has no relevance to humans and in the absence of a relevant genotoxic, carcinogenic, teratogenic, neurotoxic and immunotoxic potential of PYDIFLUMETOFEN (SYN545974) the use of an uncertainty factor of 100 (10x for intra- and interspecies variation each) is considered justified and sufficiently protective.

RMS consideration regarding the carcinogenic potential of 2,4,6 TCP and the dose level selection for PYDIFLUMETOFEN (SYN545974) toxicity studies:
As previously mentioned, the RMS had some reservations regarding the dose level selection which has been proposed by the applicant based on pharmacokinetic data (see section 2.6.1 and B.6.1 in volume 3). The RMS questioned on the possibility that the doses of PYDIFLUMETOFEN (SYN545974) selected by the applicant for the long-term studies might be not sufficiently high to cover the carcinogenic potential of 2,4,6 TCP (the major circulating metabolite), especially in rat. Indeed, no tumors were observed in rat following a 2-year administration of PYDIFLUMETOFEN (SYN545974) in male rats at dose up to 300 mg/kg/day 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). However, the MTD was reached in the 2-year study in rat with PYDIFLUMETOFEN (SYN545974), as the top dose in the male (300 mg/kg/d) and the top dose in the female (100 mg/kg/d) resulted in a 18% and 9% reduction of body weight, respectively. It can be thus reasonably considered that the carcinogenic potential of PYDIFLUMETOFEN (SYN545974) was appropriately assessed.

To consolidate this conclusion, a rationale was also proposed by the RMS based on margins of safety. Thus, systemic exposure of 2,4,6 TCP (and related compound) have been estimated for higher doses of PYDIFLUMETOFEN (SYN545974) (>300 and up to 1000mg/kg bw/day) to verify whether these non-experimentally tested doses would have actually covered the carcinogenic potential of 2,4,6 TCP. Taking into consideration the dose-limited oral absorption, the systemic exposure of 2,4,6 TCP estimated following an oral administration of PYDIFLUMETOFEN (SYN545974) in rats at 1000 mg/kg b.w./day, would give a value close to the dose of TCP which causes 25% leukemia **in** the rat (T25) in the NTP study (see detailed calculations in volume 3 B.6). If we consider this T25 value as the estimated LOAEL where a carcinogenic potential might be observed after 2-year exposure in rat, a margin of safety of 100 (1000/9.9) and of 10000 (1000/0.092) against, respectively, the NOAEL of the study and the ADI, can be calculated. These Margins of safety are considered sufficient as no indication of a genotoxic mode of action for PYDIFLUMETOFEN (SYN545974) or 2,4,6 TCP have been highlighted.

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Calculation of the ARfD, in the absence of a specific study designed to determine this endpoint, is based on a consideration of the NOAELs for “acute effects” observed in studies ranging from acute to sub-chronic exposure durations. Relevant NOAELs may be derived from studies involving administration of a single dose or from repeat dose studies in which effects are noted during the initial days of dosing. The studies of relevance to establishing an ARfD for PYDIFLUMETOFEN (SYN545974) are summarised in Table 67.

Looking across the studies summarised in Table 67, PYDIFLUMETOFEN (SYN545974) caused effects after a

single high dose in the acute neurotoxicity studies. Transient clinical signs and effect on body temperature and locomotor activity (LMA) were observed in female rats after single gavage dose of 1000 mg/kg bw and above in the main study. In the modified study (females only) the same effects were observed at ≥ 300 mg/kg bw. All signs of toxicity were resolved by day 2.

For the prenatal developmental toxicity study in the rabbit, a marginally increased incidence of rib cartilage variant (one or more costal cartilage interrupted) was observed at 100 and 500 mg/kg/day but without a clear dose response. The absence of available historical control data leads difficult the interpretation of this finding. However in a conservative approach, the NOAEL for embryo-fetal development was considered to be 10 mg/kg/day.

It is proposed thus that the ARfD should be based on the NOAEL of 10 mg/kg/day from the prenatal developmental toxicity study in rabbit with an uncertainty factor of 100.

ARfD= 10 mg/kg/day /100 = <u>0.1 mg/kg/day</u>

Remark: The applicant considered the functional effects (decrease motor activity) observed from 100 mg/kg as general toxicity, only transient (observed only after day 1) and consequently non-adverse. Moreover, he considered that no treatment related adverse effects were observed in the developmental toxicity studies. On this basis, he proposed an ARfD of 1 mg/kg/day based on the rat developmental toxicity study with an uncertainty factor of 100.

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

Considering all available sub-chronic toxicity studies available with PYDIFLUMETOFEN (SYN545974) (Table 67), the male mouse seems to be the most sensitive species with a NOAEL of 17.5 mg/kg bw/day in the 90-day mouse studies although the 90-day study in rat gave similar NOAELs of 18.6-21.6 mg/kg bw/day. However, as a marginally increased incidence of rib cartilage variant (one or more costal cartilage interrupted) was observed at 100 and 500 mg/kg/day (no clear dose-response, no historical control data available) in the prenatal developmental toxicity in rabbit, it is thus proposed that the AOEL should be based on the NOAEL of 10 mg/kg/day from this study in rabbit with an uncertainty factor of 100. As demonstrated by the comparative intravenous and oral absorption study, the oral bioavailability value was 85-90%. No correction is therefore required for the extent of oral absorption.

AOEL = 10 mg/kg bw/day/100 = <u>0.1 mg/kg bw/day</u>

Remark: The Applicant proposed a higher AOEL of 0.46 mg/kg bw/d based on the NOAEL derived in the 2-generation study, taking into account of the differences in dose spacing between the 2-generation and 90-day studies in rat since he considered that the NOAEL in the 90-day study in male mouse was 81 mg/kg bw/d instead of 17.5 mg/kg bw/d (as proposed by the RMS).

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

An acute AOEL of 0.1 mg/kg bw/day is proposed. This value is derived on the same basis as the ARfD and taking into account that no correction for the extent of oral absorption is required.

AAOEL = 10 mg/kg bw/day/100 = <u>0.1 mg/kg bw/day</u>

2.6.11 Summary of product exposure and risk assessment

Operators

Applications in grape and tomato have been presented as the worst case for high and low crops respectively to

estimate exposure to operators applying A19649B using a vehicle-mounted sprayer. According to the EFSA model the total systemic exposure to PYDIFLUMETOFEN (SYN545974) for operators applying A19649B to high crops is 3.1% of the AOEL and 8.8% of the AAOEL and for application in low crops the exposure is 0.65% of the AOEL and 3.2% of the AAOEL. Therefore it can be concluded that the risk for the operator using A19649B during high and low crop tractor application is acceptable without requiring the use of PPE (standard workwear considered) .

Bystander and resident

For the worst case scenario (i.e. application in grapes) the predicted levels of exposure based on the EFSA model for residents are all within the AOEL of PYDIFLUMETOFEN (SYN545974) (2.09% and 0.71% of the AOEL for child and adult resident respectively, sum of all pathways). For bystander, the worst case scenario is also an application of A19649B on grapes (high crops) and the predicted levels of exposure based on the EFSA model are below the AAOEL (1.47% and 0.73% for child and adult bystander exposed to spray drift pathway (worst exposure))
Therefore it can be concluded that there is no undue risk to the resident or bystander after incidental exposure to A19649B.

Worker

According to the EFSA model worker, exposure amounts to 1.3% of the AOEL for hand harvesting in low crops, 14% of the AOEL for re-entry activities in grapes and 2.3 % of the AOEL for re-entry activities in tree crops. Using the measured DFR data in grape and pome for A19649B, the re-entry worker exposure is 7.4% and 0.4% of the AOEL for grape and pome respectively. Therefore there is no unacceptable risk anticipated for the worker wearing adequate clothing (but no PPE), when re-entering crops treated with A19649B.

2.7 RESIDUE

2.7.1 Summary of storage stability of residues

Storage stability of PYDIFLUMETOFEN (SYN545974) has been investigated in plant commodities. In livestock tissues, storage stability of parent PYDIFLUMETOFEN (SYN545974) and metabolites SYN508272, SYN548264, SYN547897, SYN548263 and conjugated 2,4,6-TCP have been investigated. Summary of storage stability data submitted are reported in Table 2.7.1-1 and **Error! Reference source not found.**.

Table 2.7.1-1 Summary of storage stability data in plants

Compound	Category	Commodity	Stability up to (months)
PYDIFLUMETOFEN (SYN545974)	High water	Lettuce head	23
	High oil	Rape seed	23
	High protein	Adzuki bean	23
	High starch	Wheat grain	23
		Potato tuber	23
	High acid	Orange fruit	23

Table 2.7.1-2 Summary of storage stability data in livestock tissues

Compound	Commodity	Stability up to (months)
PYDIFLUMETOFEN (SYN545974)	Bovine muscle	12
	Bovine liver	12
	Bovine milk	12
	Bovine fat	12
	Chicken eggs	12

Compound	Commodity	Stability up to (months)
SYN508272	Bovine milk	12
SYN548264	Bovine milk	12
SYN547897	Bovine liver	**6**
	Bovine kidney	12
SYN548263	Bovine kidney	12
conjugated 2,4,6-TCP	Bovine muscle	12
	Bovine liver	12
	Bovine kidney	12
	Bovine milk	12
	Bovine fat	12
	Chicken eggs	12

Storage stability studies were conducted on at least one commodity from each of the five matrices groups "high water", "high starch", "high acid", "high protein" and "high oil" content for plant commodities. In addition, Syngenta submitted studies on animal matrices. Then, according to OECD guideline on stability, it can be concluded that PYDIFLUMETOFEN (SYN545974) was shown to be stable in all plant matrices for at least 23 months and up to 12 months in muscle, liver, milk, fat and eggs.

Metabolites SYN508272 and SYN548264 were shown to be stable for 12 months in milk when stored at -18°C. Both metabolites SYN548263 and SYN547897 are considered to be stable in kidney for 12 months. However, storage stability data on metabolite SYN547897 in bovine liver has shown decline after a freezer storage period of 6 months.
Residues of conjugated 2,4,6-trichlorophenol are expected to be stable in animal commodities when stored deep frozen at typically ≤-18°C for 12 months.

Storage period of samples analysed in residue trials, field rotational crops and feeding studies are covered by storage stability data.

Stability of residues in sample extract
No study was conducted. However, control samples fortified with the test substance were always extracted and analysed concurrently with the untreated and treated samples of the studies. The satisfactory recovery rates obtained from the fortified samples demonstrated the stability of the residues in the sample extracts throughout the analytical procedure, from extraction until chromatographic determination.

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

The primary metabolism in plants was evaluated on cereals (wheat), fruits and fruiting vegetables (tomato) and pulses and oilseeds (oilseed rape) group. Studies were conducted using [phenyl-U-14C] & [pyrazole-5-14C]-PYDIFLUMETOFEN (SYN545974). The characteristics of these studies are summarized in **Table 2.7.2-1**.

Table 2.7.2-1 : Summary of available metabolism studies in plants

Group	Crop	Label	Application and sampling details				
			Method, F or G[(a)]	Application rate	BBCH growth stage at application	Number	Sampling (DAT)
Cereals	Wheat	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Foliar spray application, F	125 g as/ha	BBCH 32-34 and 58	2	Forage : 10d after application 1 Hay : 29d after application 2 Straw and grain: 50d after application 2
Fruits and fruiting vegetables	Tomato	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Foliar spray application, G	200 g as/ha	BBCH 83 and 86	2	1 and 14 days (fruits only)
			Soil application, G	20 mg as/plant	transplanting stage	1	103 days (fruit only)
Oilseed group	Oilseed rape	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Foliar spray application, F	150 g as/ha	BBCH 65	1	62 days (seed and trash)

a: Field or Glasshouse

Metabolism studies conducted with crops representative of three different crop groups based on the commercially recommended use pattern, i.e. post emergence foliar treatment or a single soil application, have provided a detailed understanding of the metabolism of PYDIFLUMETOFEN (SYN545974) in food and feed commodities (see Table 2.7.2-2). The metabolic pathways in the three studies are similar. In all cases, unchanged parent PYDIFLUMETOFEN (SYN545974) was reported to be the major compound.
Levels of PYDIFLUMETOFEN (SYN545974) were greatest in foliar treated commodities: tomato fruit (91.7% to 96.6% TRR), wheat (70.5% to 91.0% TRR), and oil seed rape (30.0% to 62.6% TRR). Following a single soil application 4.1% TRR was detected as PYDIFLUMETOFEN (SYN545974) in tomato fruit.

Metabolism was limited and the principal metabolic transformations of PYDIFLUMETOFEN (SYN545974) in all commodities occurred via reduction of the parent molecule to produce SYN545547 and via demethylation of the pyrazole ring to produce SYN547891. Residues of SYN545547 and SYN547891 accounted for a maximum of 6.1% TRR and 8.3% TRR, respectively, across all commodities. All metabolites identified were found in their free non-conjugated form.

The level of metabolism in foliar applied tomato fruit was the least extensive of the three crop metabolism studies with significant radioactive residue remaining on the surface of the fruit up to 14 days after application (1DAA: 96.8% to 98.4% TRR; 14DAA: 88.9% to 95.0% TRR). Mature tomato fruit grown in soil treated with PYDIFLUMETOFEN (SYN545974) showed minimal up-take from the soil resulting in low residues (≤0.013 mg/kg).

Following foliar application, the level of metabolism of PYDIFLUMETOFEN (SYN545974) was most extensive in oil seed rape.
The principal metabolic transformation of PYDIFLUMETOFEN (SYN545974) in oil seed commodities occurred via reduction of the parent molecule to produce SYN545547 (trash: 2.8-3.7% TRR; seed: ≤6.1% TRR) and via demethylation of the pyrazole ring to produce SYN547891 (trash: 3.3-5.1% TRR; seed: ≤2.7% TRR). Absolute residues of metabolites did not exceed 0.003 mg/kg. Multiple unidentified polar components were

detected in oil seed rape commodities with none individually exceeding 8.4% TRR. Unextracted residues accounted for ≤7.1% TRR (≤0.004 mg/kg) in trash, and ≤28.2% TRR (≤0.005 mg/kg) in grain.

The extent of metabolism in all wheat commodities was less than that observed in oil seed rape with levels of individual metabolites identified always ≤8.3% TRR.
Metabolites via reduction of the parent molecule to produce SYN545547 and via demethylation of the pyrazole ring to produce SYN547891 were the only metabolites identified. Residues levels of SYN545547 accounted for 1.4% to 3.9% TRR with the largest absolute residues detected in wheat straw (0.059 mg kg, 3.9% TRR). Residue levels of SYN547891 accounted for 1.2% to 8.3% TRR with maximum levels detected in wheat grain. The largest absolute residue was detected in straw (0.065 mg/kg, 4.3% TRR). Multiple unidentified polar components were detected in wheat forage, hay and grain with none individually exceeding 3.3% TRR. Unextracted residues accounted for ≤6.1% TRR (≤0.093 mg/kg) in feed items, and ≤15.2% TRR (≤0.009 mg/kg) in grain.

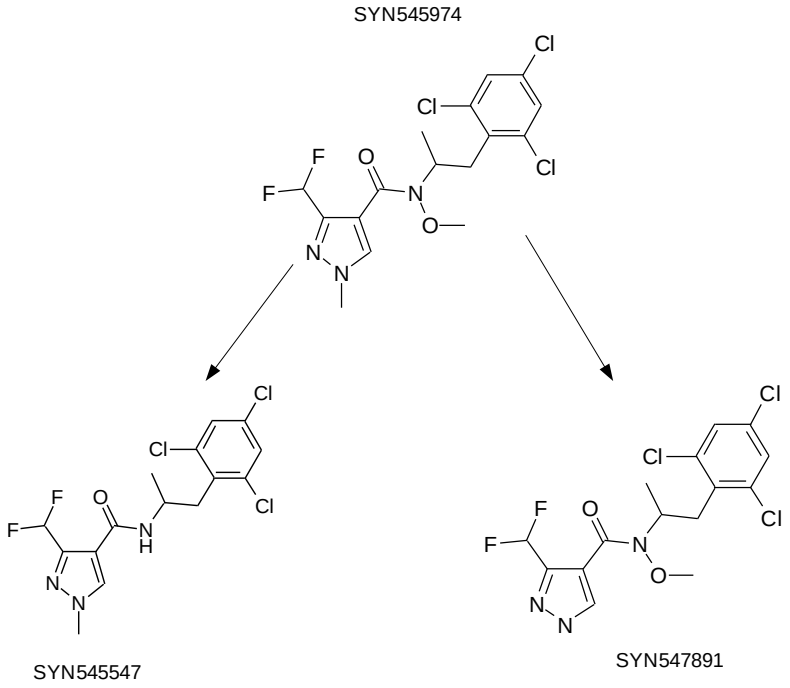
The level of metabolism in tomato fruit was the least extensive of the three crop metabolism studies with individual identified metabolite levels always ≤3.6% TRR.
The surface of the foliar applied tomato fruit samples were washed by immersion into acetonitrile and significant radioactivity was quantified in the surface wash solution. Following analysis of the surface wash solution and extraction of the fruit tissue, metabolite SYN545547 was detected in all fruit samples analysed, up to a maximum level of 3.6% TRR. The metabolite SYN547891 was detected in foliar treated tomato fruit only up to a maximum of 1.6% TRR. Fruit harvested from plants grown in soil treated with PYDIFLUMETOFEN (SYN545974) resulted in minimal translocation of the radioactivity from the soil and demonstrated extensive metabolism of the residue into multiple unidentified low level metabolites, with no individual component exceeding 11.9% TRR (0.002 mg/kg).

Table 2.7.2-2 : Summarized results of available plant metabolism studies of PYDIFLUMETOFEN (SYN545974)

Study	**Spring Wheat (cereals group)**								**Tomatoes (fruiting and vegetables group)**						**Oilseed rape (oilseed group)**			
GAP	foliar spray								foliar spray				soil application		foliar spray			
	2x125 g as/ha, BBCH 32-34 and BBCH 58								2x200 g as/ha, 7d interval, BBCH 83 and BBCH 86 (at least 1N rate compared to intended cGAP)				1x20mg as/plant		1x150 g as/ha, BBCH 65			
Matrix	**forage**		**hay**		**straw**		**grain**		**fruit**				**fruit**		**seed**		**trash**	
PHI (days)	**10d after app.1**		29d after app.2		50 d after app.2		50 d after app.2		1 d		14 d		103 d		62 d		62 d	
BBCH	BBCH39		BBCH 77		BBCH 89		BBCH 89		BBCH 89				BBCH 89		BBCH 89 (maturity)		BBCH 89 (maturity)	
Radiolabels	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*
extractable TRR % *(mg/kg)*	96,5 (0,327)	95,6 (0,445)	94,2 (0,92)	94,2 (1,311)	95,8 (1,232)	94,5 (1,443)	90,4 (0,033)	84,9 (0,048)	100 (0,520)	98,4 (0,473)	99,7 (0,640)	100 (0,632)	NC	97,5 (0,013)	74,5 (0,015)	71,8 (0,014)	81,3 (0,051)	75,6 (0,046)
PYDIFLUMETOFEN (SYN545974)	91 (0,307)	84,3 (0,392)	84,1 (0,821)	70,5 (0,981)	83,6 (1,075)	76,4 (1,167)	81,5 (0,030)	81,6 (0,046)	91,7 (0,477)	95,9 (0,461)	92,2 (0,592)	96,6 (0,611)		4,1 (0,001)	62,6 (0,012)	39,2 (0,007)	50,9 (0,032)	30,0 (0,018)
SYN545547	1,4 (0,005)	2,7 (0,012)	2,4 (0,023)	2,4 (0,034)	2,8 (0,036)	3,9 (0,059)	2,9 (0,001)	2,6 (0,001)	3,6 (0,019)	1,8 (0,009)	3,3 (0,021)	1,4 (0,009)		0,4 (<0,001)	ND	6,1 (0,001)	3,7 (0,002)	2,8 (0,002)
SYN547891	1,2 (0,004)	2,4 (0,011)	3,0 (0,029)	3,6 (0,049)	2,4 (0,032)	4,3 (0,065)	8,3 (0,003)	7,8 (0,004)	1,4 (0,007)	0,6 (0,003)	1,6 (0,011)	1,0 (0,006)		ND	2,7 (0,001)	ND	5,1 (0,003)	3,3 (0,002)
Unassigned % (mg/kg)	NA	2,5 (0,012)	4,2 (0,041)	13,8 (0,189)			NA	3,3 (0,002)	2,1 (0,010)	ND	2,5 (0,015)	1,0 (0,006)		88,9 (0,008)	6,0 (0,001)	8,6 (0,002)	6,2 (0,004)	34,9 (0,022)
Uncharacterised Extract					1,9 (0,024)	1,5 (0,023)								2,6 (<0,001)				
total identified %	93,6	89,4	89,5	76,5	88,8	84,6	92,7	92	96,7	98,3	97,1	99						
non extracted TRR %	3,5 (0.012)	4,4 (0.02)	5,8 (0.057)	5,7 (0.079)	4,6 (0.059)	6,1 (0.093)	9,6 (0.004)	15,2 (0.009)	0,1 (0,001)	1,6 (0,008)	0,3 (0,002)	0,1 (0,001)	NC	2,6 (<0,001)	25,5 (0,005)	28,2 (0,005)	6.5 (0.004)	7.1 (0.004)
Total TRR	100	100	100	99,9	100	101	100	100	100 (0,521)	100 (0,481)	100 (0,642)	100 (0,633)		100 (0,013)	100 (0,02)	100 (0,019)	100 (0,062)	100 (0,061)

According to the results of metabolism in primary crops, reduction of the parent molecule and demethylation of the pyrazole represent the principle metabolic transformations (Figure 2.7.2-1) observed in all three crops with additional metabolism into multiple polar low residue components detected at levels at which identification was not required.

Figure 2.7.2-1 : Proposed metabolic pathway of PYDIFLUMETOFEN (SYN545974) in crops



The metabolism in livestock was evaluated using data on laying hen and lactating goat. Studies were conducted using [phenyl-U-14C] & [pyrazole-5-14C]-SYN545974. The characteristics of these studies are summarized in **Table 2.7.2-3**.

Table 2.7.2-3: Summary of available metabolism studies in animals

Group	Species	Label position	No of animal	Application details		Sample details	
				Rate (mg/kg bw per d)	Duration (days)	Commodity	Time
Laying poultry	Hens	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	6 per radiolabel	3.3 - 3.6 *(876 N compared to layer hen dietary burden intake)*	14	Urine and faeces	Once daily
						Egg yolk	
						Egg white	
						Liver	At sacrifice (11 hours after final dose)
						Muscle	
						Skin and fat	
Lactating ruminants	Goats	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	1 per radiolabel	4.6 *(472 N compared to dairy cattle dietary burden intake)*	7	Urine and faeces	Once daily
						Milk	
						liver	At sacrifice (11 hours after final dose)
						kidneys	
						fat	
						Muscle	

The nature of PYDIFLUMETOFEN (SYN545974) residues in commodities of animal origin was investigated in 2 studies in lactating goats and laying hens using [pyrazole-5-14C]-SYN545974 and [phenyl-U-14C]-SYN545974.

Following the repeated oral administration of radiolabeled PYDIFLUMETOFEN (SYN545974) to goats and laying hens (for 7 and 14 days respectively, at a rate of 4.6 and 3.6 mg a.s. /kg bodyweight), a high proportion of the dose was eliminated in the excreta. There was no evidence of any significant accumulation of radioactivity in eggs or edible tissues in laying hen. In lactating goats, 0.4% of the radioactivity was recovered in liver and no accumulation was detected in milk and other edible tissues.

In metabolism studies in hens and goat, the following compounds have been identified: PYDIFLUMETOFEN (SYN545974), SYN545547, SYN547948, SYN547897, SYN547891, 2,4,6-TCP, SYN508272 and NOA449410. Metabolites [SYN545974]-OH, SYN548263, SYN548264 were identified in goat metabolism only.

In laying hens, a high proportion of the dose was eliminated in the excreta (84.3-99.1%). The major residues in egg whites were unchanged parent (0.014 - 0.025 mg/kg, 26.6 - 46.5 % of the TRR) and SYN508272 (34.3% TRR, 0.018 mg/kg, pyrazole label only). In egg yolk, the same metabolite was observed but at lower level. The major residue in egg yolk were 2,4,6 TCP (0.242 mg/kg, 67.8% of the TRR - phenyl label only) and parent compound (0.011-0.012 mg/kg, 3-12% of the TRR).

2,4,6 TCP and SYN508272 were also the main metabolites identified in muscle with 0.013 mg/kg (48.4% of the TRR for phenyl label) and 0.01 mg/kg (46.3% of the TRR for pyrazole label) respectively. Parent compound was detected below 0.01 mg/kg. The same compounds were identified in skin and fat. 2,4,6 TCP accounted for 0.03 mg/kg (29.3% of the TRR for phenyl label only) and parent compound was identified at 0.01-0.017 mg/kg (30.6-16.6% of the TRR). SYN508272 accounted for less than 0.01 mg/kg in skin and fat.

In liver, almost 50% of the TRR was non-extracted following solvent extraction. After further characterization (sodium dodecyl sulphate solution and protease enzyme hydrolysis), these residues were shown to be a complex mixture of unassigned and highly polar metabolites. Unchanged parent compound was the main recovered compound with 0.021 mg/kg (5.3% of the TRR).

In lactating goat, a high proportion of the dose was eliminated in the excreta too (76.3-84.2%). There was no accumulation of radioactivity in edible tissues except in liver (0.4% of the administered dose i.e. 0.0184 mg/kg). Liver was also the tissue with the highest non extracted radioactivity level (up to 52.6%). After a combination of sodium dodecyl sulphate, and protease enzyme digestion these residues were shown to be a complex mixture of unassigned and highly polar metabolites no single one of which accounting for >7.4% TRR (>0.517 mg/kg).

The major residues in liver were unchanged parent compound (0.179 mg/kg – 0.57 mg/kg, 2.0-8.2% of the TRR), SYN547897 (up to 0.268 mg/kg, 3.0% of the TRR), SYN545547 (up to 0.239 mg/kg, 3.4% of the TRR), NOA449410 (0.248 mg/kg, 2.9 % of the TRR for pyrazole label only), SYN547948 (up to 0.180 mg/kg, 2.6 % of the TRR). Metabolite 2,4,6 TCP was also found at 0.037 mg/kg (0.5% of the TRR for phenyl label only).

In kidney, both metabolites SYN548263 and NOA449410 were detected with levels above 10% of the TRR (0.389 mg/kg and 0.275 mg/kg respectively) for pyrazole label only. All other compounds accounted for less than 10 % of the TRR. SYN545547 were detected in phenyl label at 0.128 mg/kg (7.4% of the TRR)

In milk, 2,4,6 TCP was the main metabolite with 0.052 mg/kg (43.2% of the TRR for phenyl label only). Metabolites SYN548264, SYN548263 and SYN508272 were identified in pyrazole label only at levels ranging from 0.014 to 0.038 mg/kg (>10%TRR).

The major component in muscle was the unchanged parent compound (up to 0.025 mg/kg, 24.4 % of the TRR) followed by metabolite SYN508272 with 0.024 mg/kg (17.7 % of the TRR for pyrazole label only). Other metabolites were detected below 0.01 mg/kg.

In fat, parent PYDIFLUMETOFEN (SYN545974) was the main identified compound (up to 0.206 mg/kg, 73.8% of the TRR)

The metabolism of PYDIFLUMETOFEN (SYN545974) in the rat was consistent with that of the lactating goat (ruminant) and laying hen.

Table 2.7.2-4: Summarized results of available livestock metabolism studies of PYDIFLUMETOFEN (SYN545974)

	laying hen (6 per radiolabelled)										**ruminant-lactating goats**									
	liver		egg yolk		egg white		muscle		skin&fat		milk		liver		kidney		muscle		fat	
time after administration	11h										11h									
	phenyl 14C	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*	*phenyl 14C*	*pyrazole 14C*
TRR % *(mg/kg)*	51,7 (0,209)	52,5 (0,111)	87 (0,311)	81,2 (0,087)	97,7 (0,052)	98,8 (0,051)	84,2 (0,023)	90,1 (0,018)	95,8 (0,096)	91,5 (0,029)	92,3 (0,112)	93,9 (0,124)	50,4 (3,520)	47,4 (4,184)	83,4 (1,443)	90,7 (2,123)	86 (0,087)	94,3 (0,130)	98,8 (0,219)	97,6 (0,272)
PYDIFLUMETOFEN (SYN545974)	5,3 (0,021)	0,5 (0,001)	3,0 (0,011)	11 (0,012)	46,5 (0,025)	26,6 (0,014)	8,7 (0,002)	4,7 (0,001)	16,6 (0,017)	30,6 (0,01)	15,7 (0,019)	8,7 (0,011)	8,2 (0,57)	2,0 (0,179)	0,8 (0,014)	0,5 (0,011)	24,4 (0,025)	13,4 (0,018)	67,2 (0,149)	73,8 (0,206)
SYN545547	1,2 (0,005)	3,3 (0,007)	nd	3,9 (0,004)	nd	nd	nd	nd	nd	nd	nd	nd	3,4 (0,239)	1,8 (0,160)	7,4 (0,128)	nd	nd	nd	nd	nd
SYN547948	0,7 (0,003)	3,2 (0,007)	nd	1,3 (0,001)	7,1 (0,004)	5,5 (0,003)	3,4 (0,001)	1,6 (<0,001)	3 (0,003)	4,1 (0,001)	2,2 (0,003)	0,7 (0,001)	2,6 (0,180)	1,9 (0,170)	0,9 (0,016)	0,7 (0,016)	3,8 (0,004)	1,1 (0,002)	5,3 (0,012)	3,3 (0,009)
SYN547897	2,4 (0,009)	0,9 (0,002)	2,3 (0,008)	6,7 (0,007)	nd	nd	nd	1,1 (<0,001)	1,7 (0,002)	2,6 (0,001)	nd	nd	1,9 (0,136)	3,0 (0,268)	2,9 (0,050)	2,7 (0,063)	1,8 (0,002)	1,2 (0,002)	nd	nd
SYN547891	0,2 (0,001)	nd	nd	2,5 (0,003)	nd	nd	nd	nd	nd	nd	nd	nd	1,4 (0,100)	0,4 (0,038)	nd	nd	nd	nd	nd	nd
[SYN545974]-OH											nd	nd	nd	nd	nd	nd	nd	nd	8,6 (0,019)	10,2 (0,028)
SYN548263												14,2 (0,019)		nd		16,6 (0,389)		4,9 (0,007)		4,3 (0,012)
SYN548264												28,7 (0,038)		nd		0,8 (0,019)		0,6 (0,001)		nd
2,4,6-TCP	nd		67,8 (0,242)		14,5 (0,008)		48,4 (0,013)		29,3 (0,03)		43,2 (0,052)		0,5 (0,037)		1,2 (0,021)		9,0 (0,009)		nd	
SYN508272		2,4 (0,005)		7,2 (0,008)		34,3 (0,018)		46,3 (0,01)		9,6 (0,003)		11,0 (0,014)		nd		1,5 (0,036)		17,7 (0,024)		1,0 (0,003)
NOA449410		nd		6,6 (0,007)		15,4 (0,008)		nd		3,1 (0,001)		2,6 (0,003)		2,9 (0,248)		11,7 (0,275)		3,6 (0,005)		nd
non extracted TRR %	48,3 (0,195)	47,5 (0,100)	13,0 (0,047)	18,7 (0,02)	2,3 (0,001)	1,2 (0,001)	15,8 (0,004)	9,9 (0,002)	4,3 (0,004)	8,4 (0,003)	7,7 (0,009)	6,1 (0,008)	49,7 (3,471)	52,6 (4,643)	16,6 (0,287)	9,2 (0,215)	14 (0,014)	5,7 (0,008)	1,1 (0,002)	2,4 (0,007)
Total TRR	100	100	100	99,9	100	100	100	100	100,1	99,9	100	100	100.1	100	100	99.9	100	100	99.9	100

Figure 2.7.2-2 : Proposed metabolic pathway for PYDIFLUMETOFEN (SYN545974) in laying hens

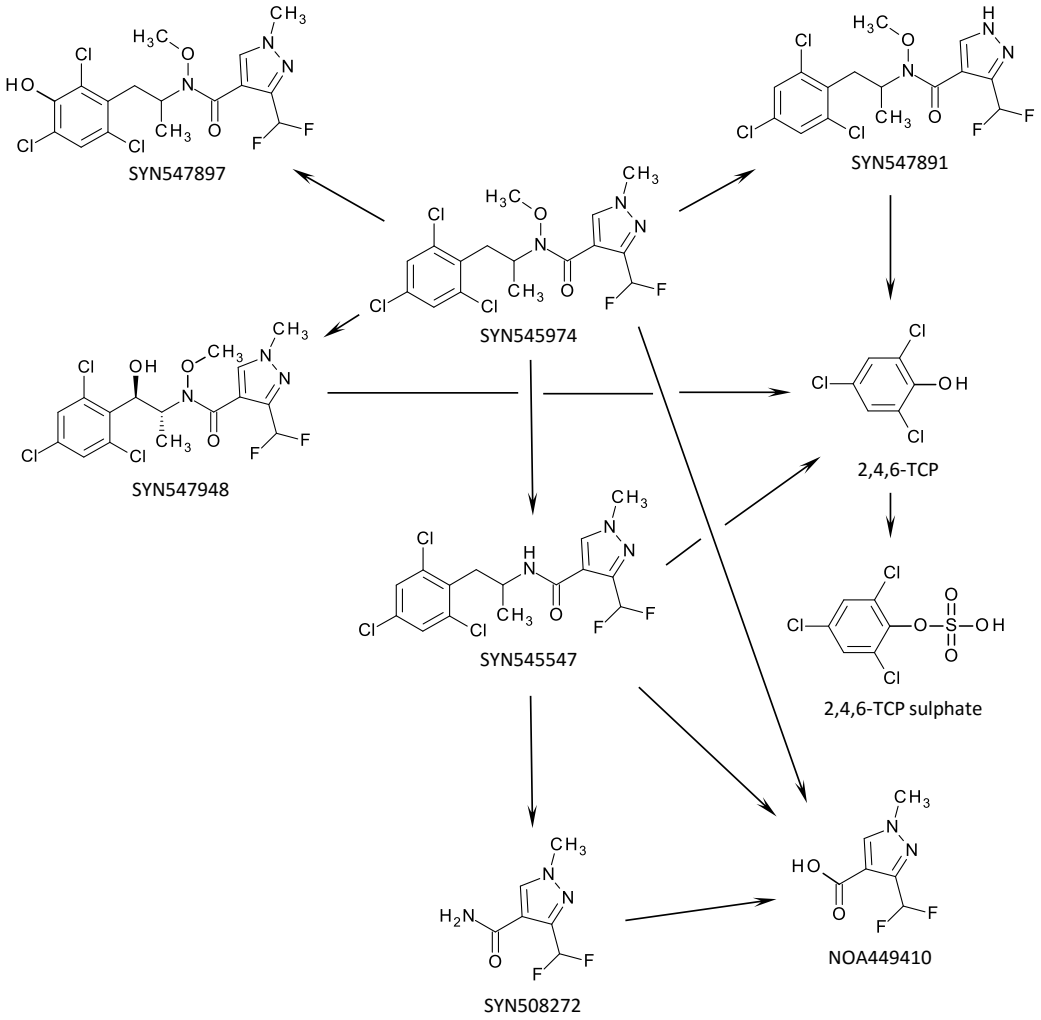
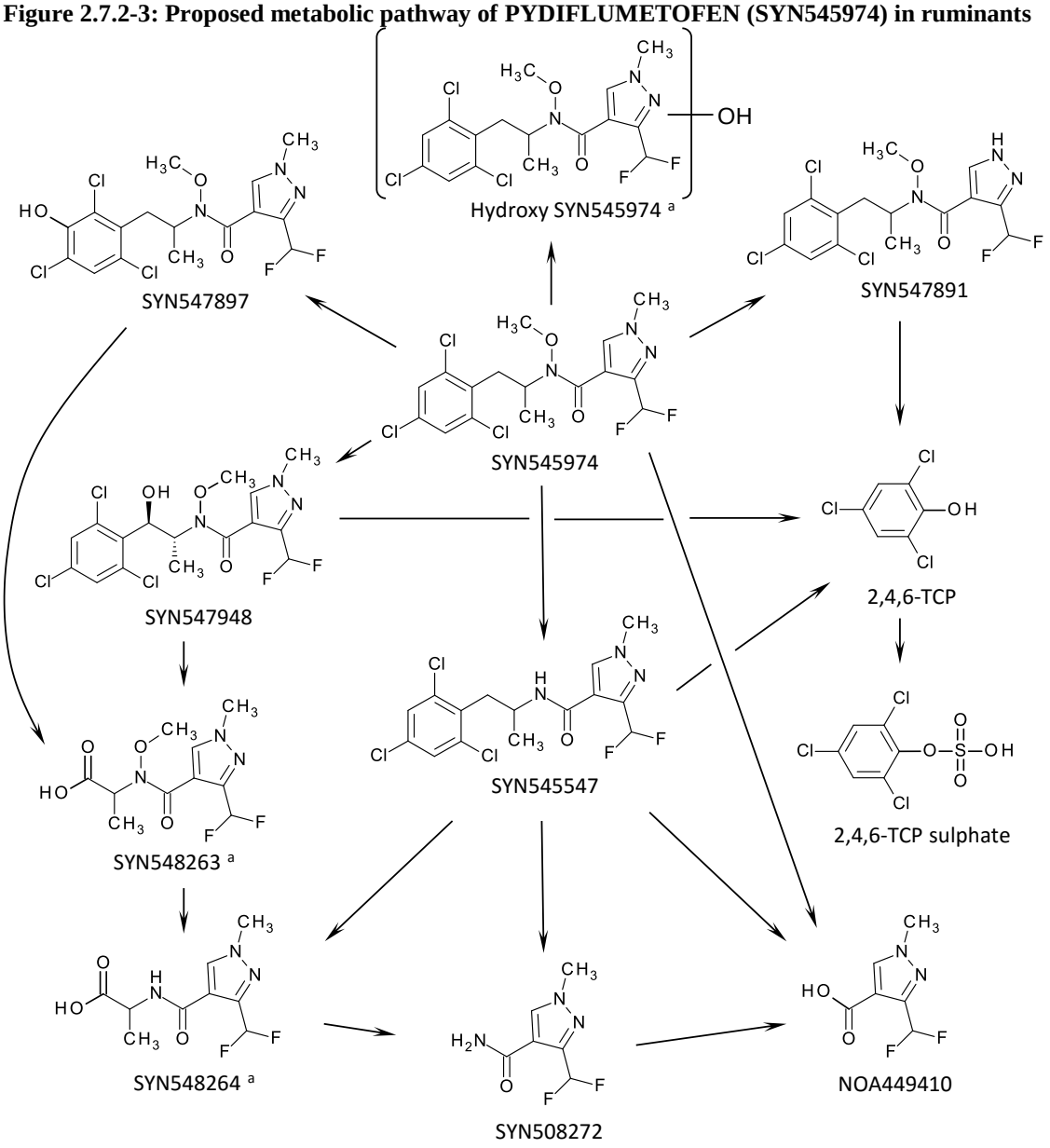


Figure 2.7.2-3: Proposed metabolic pathway of PYDIFLUMETOFEN (SYN545974) in ruminants



2.7.3 Definition of the residue

Residue definition in plants

Proposed residue definition is based on results from primary crop metabolism studies. Results from rat metabolism study as well as toxicology studies were also considered for residue definition proposal.

According to metabolism studies conducted on wheat, tomato and oilseed rape, the parent PYDIFLUMETOFEN (SYN545974) is the major component: 71-91% TRR in wheat commodities, 92 – 97% TRR in tomato fruit and 30-63% TRR in oilseed rape.

It is therefore considered that PYDIFLUMETOFEN (SYN545974) is a good marker and it is proposed that the residue definition for enforcement in plant commodities is PYDIFLUMETOFEN (SYN545974) only.

Metabolism was limited in plant commodities and the metabolites identified were intact molecules. The principal metabolic transformations of PYDIFLUMETOFEN (SYN545974) in all commodities occurred via reduction of the parent molecule to produce SYN545547 and via demethylation of the pyrazole ring to produce SYN547891.

Both metabolites SYN545547 and SYN547891 (glucuronide only) were present in rat metabolism studies. Considering toxicological properties of these metabolites and the amounts recovered in plants commodities, it is not considered necessary to include them in the residue definitions.

In summary, the only compound present at significant levels in crops that is considered relevant to human health risk assessment is unchanged PYDIFLUMETOFEN (SYN545974).

In addition, **metabolism of PYDIFLUMETOFEN (SYN545974) in succeeding crops was investigated on leafy vegetable (lettuce), root and tuber vegetables (turnip) and cereals (wheat). The results confirm the very limited metabolism and the high proportion of parent compound.**

Therefore the proposed residue definitions in commodities of plant origin for risk assessment and monitoring purposes are parent PYDIFLUMETOFEN (SYN545974) only.

Definition of Residue for MRL Setting and Enforcement	PYDIFLUMETOFEN (SYN545974)
Definition of Residue for Risk Assessment	PYDIFLUMETOFEN (SYN545974)

Residue definition in animal commodities

In livestock metabolism, numerous metabolites were identified.

- SYN548264

SYN548264 was a significant component in goat milk at 28.7% TRR (0.038mg/kg, parent equivalents). As a result, bovine whole milk, cream and skimmed milk samples from the dairy cow feeding study were analysed for SYN548264, residue levels were <0.01-0.01 mg/kg in samples from animals dosed at the highest (150 mg/kg DM per day) feeding rate. This metabolite was not recovered in the poultry metabolism study. This compound was found in the rat metabolisms study.

- SYN547891

SYN547891, formed by demethylation of the pyrazole ring in parent, was present at low levels in animal commodities with maximum levels of 2.5% TRR (0.003 mg/kg) in egg yolk and 1.4% of the TRR (0.100 mg/kg) in ruminant liver.

- SYN547948

SYN547948 was present at low levels in most commodities with maximum levels in egg white at 7.1% TRR (0.004 mg/kg) and in goat liver at 2.6% TRR (0.180 mg/kg).

- [SYN545974]-OH

Another hydroxylated form of parent, where the location of hydroxy functionality is uncertain, [SYN545974]-OH was present only in bovine fat up to 10.2% TRR (0.028 mg/kg).

Metabolites SYN548264, SYN547891, SYN547948 and [SYN545974]-OH were found or are precursors of compounds (glucuronide) found in the rat metabolism studies. Their levels are expected to be below the LOQ of 0.01 mg/kg in livestock commodities considering the dietary burdens calculated in framework of this monograph. Thus, these metabolites were not included in the residue definition.

- NOA449410

NOA449410 is a metabolite common to isopyrazam, bixafen, fluxapyroxad and benzovindiflupry and show a very similar structure as the x-fluoromethyl pyrazole structured metabolites of penflufen, penthiopyrad and sedaxane.
NOA449410 was present in several commodities with maximum levels in egg white (15.4% TRR, 0.008 mg/kg), goat kidney (11.7% TRR, 0.275 mg/kg) and goat liver (2.9% TRR, 0.248 mg/kg). NOA449410 was not present in the rat metabolism study. A number of toxicological studies were carried out on this compound (see vol3 B6) showing it to be of low toxicological concern. This metabolite was not sought in the feeding studies.
Considering toxicological properties of this metabolite and its amounts in livestock commodities (lowered to the dietary burden intake calculated), it is not considered necessary to include NOA449410 in the residue definitions.

- SYN545547

SYN545547, formed by methoxylation of parent, was present in hen liver, egg yolk, goat liver and kidney only, with maximum levels of 7.4% TRR (0.128 mg/kg) and 3.4% (0.239 mg/kg) in the kidney and liver, respectively, of phenyl labelled goat. SYN545547 was not seen in the kidney of the corresponding pyrazole labelled goat. SYN545547 was found in egg yolk at low levels and was not present in meat, fat, egg white or milk. This metabolite was recovered in the rat metabolism studies. However its appearance was not investigated in the feeding studies. Considering toxicological properties of this metabolite and its amounts in livestock commodities (lowered to the dietary burden intake calculated), it is not considered necessary to include it in the residue definitions.

- SYN547897

SYN547897 was present as the conjugated metabolite in many animal commodities with maximum levels of 3.0% TRR (0.268 mg/kg) and 2.9% TRR (0.05 mg/kg) in goat liver and goat kidney, respectively.
Liver and kidney samples from the dairy cow feeding study were analysed for SYN547897 (sum of free and conjugated), highest residue levels were 0.06 mg/kg (liver and kidney) in samples from animals dosed at 15 mg/kg DM per day (41.1N compared to the dietary burden intake calculated for dairy cow). Then, SYN547897 level is not expected to be above the LOQ of 0.01 mg/kg in livestock commodities considering the dietary burdens calculated in framework of this monograph. SYN547897 was present in the rat metabolism study but below 10% in urine and blood.

Considering that:

- **it is found at higher levels (max. 0.06/0.06 mg/kg, 0.24/0.36 mg/kg and 0.58/0.59 mg/kg for each dosing level) than parent compound (<LOQ/0.02, <LOQ/0.05, max.0.03/0.12 mg/kg for each dosing level) in the feeding study respectively in kidney/liver,**
- **no toxicological data are available for this compound and it is not sufficiently covered by parent compound (although its structure is very similar),**

RMS considers relevant to include SYN547897 in the residue definition for consumer risk assessment, separately. As SYN547897 is ascribed to 'Cramer Class III' and does not trigger in-silico alerts for genotoxicity or neurotoxicity, a TTC of 1.5 µg/kg bw/d can be used for the assessment of chronic and acute consumer risk in relation to this metabolite species.

- 2,4,6-trichlorophenol

In metabolism studies, 2,4,6-trichlorophenol (predominantly present in conjugated form) was identified in most commodities, being the majority compound in egg yolk with 67.8% TRR (0.242 mg/kg), poultry muscle with 48.4% TRR (0.013mg/kg), fat (29.3% TRR, 0.03 mg/kg) and in milk with 43.2% TRR (0.05 mg/kg). This metabolite was also measured in feeding studies where residue levels of 0.01 mg/kg and 0.02 mg/kg were recovered in bovine kidney and cream at 41.1N dose rate and up to 0.013 mg/kg in egg yolk at 38.9 N dose rate. Conjugated 2,4,6-trichlorophenol was the major metabolite in rats, accounting for 44% of circulating radioactivity at the 300 mg/kg dose level therefore the toxicity of conjugated 2,4,6-trichlorophenol can be considered to have been tested within the parent toxicity studies. Based on these results, this metabolite was included in both residue definitions for monitoring and risk assessment for all livestock commodities.

- SYN508272

SYN508272 is a metabolite common to isopyrazam, bixafen, fluxapyroxad and benzovindiflupry and show a very similar structure as the x-fluoromethyl pyrazole structured metabolites of penflufen, penthiopyrad and sedaxane.
SYN508272 was a significant component in egg white (34.3% TRR, 0.018 mg/kg parent equivalents), poultry muscle (46.3% TRR, 0.01 mg/kg), bovine muscle (17.7, 0.024 mg/kg) and milk (11.0% TRR, 0.014 mg/kg).
In the feeding study, this metabolite was only sought in milk where it was detected (<0.01 mg/kg) in animal dosed at the highest feeding rate (443N).
In the ruminant and hen feeding studies, no parent was recovered at any of the tested doses in muscle. Since the TTRs of SYN508272 were below the TRR of parent compound in metabolism studies, this metabolite is not likely to be found in ruminant or hen muscle in the feeding studies. However, parent compound was recovered above the LOQ (maximum 0.015 mg/kg and 0.038 mg/kg at 3X and 10X dose rate, respectively 121 and 389N considering the dietary burdens calculated) in egg white, then, considering TRRs levels measured in the metabolism study, SYN508272 might have been recovered in the feeding study. Nevertheless, in framework of this monograph this metabolite is not a point of concern given the dietary burden calculations.

- SYN548263

SYN548263, present as the free and conjugated metabolite, was the largest component in goat kidney at 16.6% TRR (0.389 mg/kg, parent equivalents), it was also present in milk at 14.2% (0.019 mg/kg, parent equivalents). Kidney samples from the dairy cow feeding study were analysed for SYN548263 (sum of free and conjugated), maximal residue levels were 0.02 mg/kg and 0.10 mg/kg in samples from animals dosed at 45 mg/kg DM (111.9N) per day and at the highest (150 mg/kg DM per day – 443.4N) feeding rate respectively. The toxicity of metabolite SYN548263 is covered by parent compound toxicity. This metabolite was included in residue definition for risk assessment for ruminant kidney matrice. In feeding study, no parent compound was detected in milk at 1X dose (41.1N). Then, no residue of SYN548263 is expected in milk.

Definition of Residue for MRL Setting and Enforcement	Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974)
Definition of Residue for Risk Assessment	**All matrices:** Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) **Ruminant liver:** Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897 **ruminant kidney:** Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-trichlorophenol (free and conjugated) and SYN548263 expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897

A common problematic with other active substances

Both pyrazole compounds SYN508272 and NOA449410 are metabolites common to isopyrazam, bixafen, fluxapyroxad and benzovindiflupry and show a very similar structure as the x-fluoromethyl pyrazole structured metabolites of penflufen, penthiopyrad and sedaxane.

In the framework of this monograph, these two metabolites were not recovered in plants. However, they can be found in different animal tissues. Nevertheless, their levels are expected to be below the LOQ of 0.01 mg/kg in livestock commodities considering the dietary burdens calculated in framework of this monograph.

2.7.4 Summary of residue trials in plants and identification of critical GAP

Critical GAPs for representative uses of **PYDIFLUMETOFEN (SYN545974)** are presented in Table 2.7.4-1.

Table 2.7.4-1: Critical GAP for representative uses of PYDIFLUMETOFEN (SYN545974)

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	Member state (s)	Crop and/or situation (crop destination/ purpose of crop)		F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season	g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	PHI (days)	Remarks: e.g. safener/synergist per ha
1	EU	Pome fruit	Apple	F	Powdery mildew *(Podosphaera leucotricha)* + scab *(Venturia inaequalis)*	Foliar	BBCH 56-79	a) 3 b) 3	7	a) 0.25 b) 0.75	a) 50 b) 150	400-1500	65	0.14l/Ha LWA in 18000m² LWA/ha = 0.25 l/ha (17ml/hl)
2	EU		Pear	F	scab *(Venturia pyrina)*	Foliar	BBCH 56-79	a) 3 b) 3	7	a) 0.25 b) 0.75	a) 50 b) 150	400-1500	65	0.14l/Ha LWA in 18000m² LWA/ha = 0.25 l/ha (17ml/hl)
3	EU	Grapes (wine & table)		F	Grey mould *(Botrytis cinerea)*	Foliar	BBCH 67-89	a) 2 b) 2	14	a) 1 b) 2	a) 200 b) 400	500-1400	21	
4	EU	Grapes (wine & table)		F	Powdery mildew (*Uncinula necator*)	Foliar	BBCH 13-77	a) 2 b) 2	10	a) 0.2 b) 0.4	a) 40 b) 80	150-1000	21	
5	EU	Potato		F	Early blight *(Alternaria solani*)	Foliar	BBCH 31-89	a) 3 b) 3	14	a) 0.20 b) 0.60	a) 40 b) 120	200-500	7	
6	EU	Fruiting vegetables	Tomato	F	Early blight *(Alternaria solani*)	Foliar	BBCH 51-89	a) 2 b) 2	7	a) 0.35 b) 0.70	a) 70 b) 140	300-1000	1	

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	Member state (s)	Crop and/or situation (crop destination/ purpose of crop)		F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season	g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	PHI (days)	Remarks: e.g. safener/synergist per ha
7	EU	Edible cucurbit	Cucumber	F	Powdery mildew *(Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
8	EU		Courgette/ zucchini	F	Powdery mildew *(Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
9	EU	Inedible cucurbit	Melon	F	Powdery mildew *(Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
10	EU		Watermelon	F	Powdery mildew *(Sphaerotheca fuliginea* and *Erysiphe sp)*	Foliar	BBCH 20-89	a) 2 b) 2	7	a) 0.25 b) 0.50	a) 50 b) 100	300-1000	1	Equivalent to 25 mL/hL
11	EU	Flowering brassica	Broccoli	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	

1	2	3		4	5	6	7	8	9	10	11	12	13	14
						Application				Application rate				
Use No.	Member state (s)	Crop and/or situation (crop destination/ purpose of crop)		F G or I	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Method/ Kind	Timing/Growth stage of crop & season	Max. Number a) per use b) per crop/ season	Minimum interval between applications (days)	L A19649B / ha a) max. rate per appl. b) max. total rate per crop/season	g PYDIFLUMETOFEN (SYN545974) / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	PHI (days)	Remarks: e.g. safener/synergist per ha
12	EU		Cauliflower	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
13	EU	Leafy brassica	Kale	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
14	N-EU	Head brassica	Brussels sprouts	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
15	EU		Cabbage	F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	
16	N-EU	Kohlrabi		F	*Alternaria sp.* and *Mycosphaerella sp.*	Foliar	BBCH 21-49	a) 2 b) 2	14	a) 0.35 b) 0.70	a) 70 b) 140	200-600	14	

2.7.4.1 Apple and pear

9 Northern and 9 Southern trials set up in Europe on apple were submitted in order to support the critical EU Good Agricultural Practice (GAP) for PYDIFLUMETOFEN (SYN545974) on pome-fruit.

One trial was conducted with a total application rate below the range of -25%, therefore it was considered as not compliant with intended GAP. Since dataset was sufficient to propose an MRL, proportionality approach was not applied and this residue trial was not taken into account.

One Northern and one Southern trials were conducted with less critical applications rate (ranging from 37,1 to 40.5 g a.s/ha) but still comprised in the -25% interval when considering the total application rate. Thus, these trials were taken into account.

Residue levels of trials complying with the GAPs are reported in Table 2.7.4-2.

Apple is a major crop both in Northern and Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). 8 trials from Northern Europe and 9 trials from Southern Europe conducted according to the intended GAP are currently available. **Sufficient residue trial data are therefore available for apple and the calculations result in the proposal of an MRL of 0.2 mg/kg.** According to the EU Guideline Document SANCO 7525/VI/95 Rev. 10.2, an extrapolation from apple to pear is possible.

2.7.4.2 Grape

12 Northern and 12 Southern trials set up in Europe on grape were submitted by the applicant to support its proposed GAPs. 8 Northern and 8 Southern trials were conducted according to the intended critical GAP (2 application of 200 g a.s./ha ±25%, PHI 21 d) and their residue levels are reported in Table 2.7.4-2.

Grape is a major crop both in Northern and Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for grape and the calculations result in the proposal of an MRL of 2 mg/kg.**

2.7.4.3 Potato

4 Northern and 3 Southern trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on potato.

All the submitted trials were conducted with a more critical dose (70 g a.s/ha instead of 40 g a.s./ha) which is not included in the ± 25% range. However, since residue levels were all below the LOQ, these trials were considered as acceptable to support the intended GAP. Residue levels of trials complying with the GAPs are reported in **Error! Reference source not found.**.

Potato is a major crop both in Northern and Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). No metabolism study was conducted on potato. According to metabolism studies conducted on tomato, it is not possible to conclude on a zero residue situation. Then, the number of trials shall not be below the minimum of four per zone. One additional residue trials conducted in the South of Europe is required to confirm the non-residue situation.

One additional potato trial in South of Europe is planned in 2017 to confirm the non-residue situation. The additional trial will be provided to RMS once the final report is available (December 2017).

Based on Northern trials, a MRL of 0.01*mg/kg was proposed.

2.7.4.4 Tomato

9 Northern and 8 Southern residue trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on tomato. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 51-89, PHI 1 d).

Tomato is a major crop both in Northern and Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for tomato and the calculations result in the proposal of an MRL of 0.15 mg/kg.**

2.7.4.5 Cucumber, courgette, zucchini

9 Northern and 8 Southern residue trials on cucumber set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on cucurbits with edible peel. All submitted trials were conducted according to the intended GAP (2x50 ga.s./ha, BBCH 20-89, PHI 1 d).

Cucumber is a major crop in Northern Europe and courgette is a major crop in Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for cucurbits with edible peel and the calculations result in the proposal of an MRL of 0.15 mg/kg**. According to the EU Guideline Document SANCO 7525/VI/95 Rev. 10.2, an extrapolation from cucumber to courgette and zucchini is possible when the active substance is used up to or close to harvest. As the intended GAPs on these crops are identical, extrapolation can be made to courgette and zucchini and the same MRL that proposed on cucumber can be proposed.

2.7.4.6 Melon and watermelon

9 Northern and 8 Southern residue trials on melon set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on melon and watermelon. All submitted trials were conducted according to the intended GAP (2x50 ga.s./ha, BBCH 20-89, PHI 1 d).

Melon is a major crop in Southern Europe and watermelon is a major crop both in Southern and Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for melon and the calculations result in the proposal of an MRL of 0.1 mg/kg**. According to the EU Guideline Document SANCO 7525/VI/95 Rev. 10.2, an extrapolation from melon to watermelon is possible when the active substance is used up to or close to harvest. As the intended GAPs on these crops are identical, extrapolation can be made and the same MRL that proposed on melon can be proposed for watermelon.

2.7.4.7 Flowering brassica – Broccoli

8 Northern and 8 Southern residue trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on broccoli. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Broccoli is a minor crop both in Southern and Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for broccoli and the calculations result in the proposal of an MRL of 0.15 mg/kg**.

2.7.4.8 Flowering brassica – cauliflower

8 Northern and 8 Southern residue trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on cauliflower. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Cauliflower is a major crop both in Southern and Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for cauliflower and the calculations result in the proposal of an MRL of 0.07 mg/kg**.

2.7.4.9 Leafy brassica – Kale

8 Northern and 8 Southern residue trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on kale. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Kale is a minor crop both in Southern and Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for kale and the calculations result in the proposal of an MRL of 4 mg/kg**. However, an acute risk has been identified for kale (138.6% ARfD).

2.7.4.10 Head brassica – Brussel sprouts

4 Northern trials set up in Europe were submitted in order to support the critical Northern EU GAP for PYDIFLUMETOFEN (SYN545974) on Brussel sprouts. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Brussel sprout is a minor crop in Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for Brussel sprouts and the calculations result in the proposal of a MRL of 0.3 mg/kg for the Northern of Europe**.

2.7.4.11 Head brassica – cabbage

8 Northern trials and 4 Southern trials set up in Europe were submitted in order to support the critical EU GAP for PYDIFLUMETOFEN (SYN545974) on head cabbage. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Head cabbage is a major crop in Northern Europe and a minor crop in Southern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for head cabbage and the calculations result in the proposal of an MRL of 0.2 mg/kg.**

2.7.4.12 Kohlrabi

4 Northern trials set up in Europe were submitted in order to support the critical Northern EU GAP for PYDIFLUMETOFEN (SYN545974) on kohlrabi. All submitted trials were conducted according to the intended GAP (2x70 ga.s./ha, BBCH 21-49, PHI 14 d).

Kohlrabi is a minor crop in Northern Europe (EU Guideline Document SANCO 7525/VI/95 Rev. 10.2). **Sufficient residue trial data are available for Kohlrabi and the calculations result in the proposal of an MRL of 0.2 mg/kg for the Northern of Europe.**

Table 2.7.4-2: Summary of residue data from the supervised residue trials

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
Representative uses : **Residue definition for monitoring (M):** PYDIFLUMETOFEN (SYN545974) **Residue definition for risk assessment (RA):** PYDIFLUMETOFEN (SYN545974)						
Apple and pear	NEU (8)	<0.01, 4x0.02, 0.03, 0.05, 0.14	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.	0.2	M: 0.14 RA: 0.14	M: 0.02 RA: 0.02
	SEU (9)	<0.01, 3x0.01, 2x0.02, 2x0.04, 0.08				
Grape	NEU (8)	2x0.1, 0.17, 0.21, 0.26, 0.28, 0.48, 1.19	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.	2	M: 1.19 RA: 1.19	M: 0.265 RA: 0.265
	SEU (8)	0.15, 0.19, 0.23, 0.27, 2x0.28, 0.40, 1.17				
Potato	NEU (4)	4x<0.01		0.01*	M: <0.01 RA: <0.01	M: <0.01 RA: <0.01
	SEU (3)	3x<0.01	Insufficient data to derive an MRL	-	-	-
Tomato	NEU (9)	0.01, 0.02, 0.03, 2x0.04, 2x0.05, 0.06, 0.07	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.	0.15	M: 0.07 RA: 0.07	M:0.04 RA: 0.04
	SEU (8)	2x0.03, 3x0.04, 3x0.05				
Cucumber →	NEU (9)	3<0.01, 2x0.02, 0.03, 2x0.04, 0.05	According to the Mann-Withney U-test	0.15	M: 0.07	M: 0.03

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
courgette,	SEU (8)	2x0.02, 0.03, 0.04, 0.05, 2x0.06, 0.07	(α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.		RA: 0.07	RA: 0.03
Melon → watermelon	NEU (9)	3x0.02, 2x0.03, 2x0.04, 2x0.06	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.	0.1	M: 0.06 RA: 0.06	M: 0.03 RA: 0.03
	SEU (8)	0.01, 3x0.02, 2x0.03, 2x0.04				
Broccoli	NEU (8)	<0.01, 0.01, 3x0.02, 2x0.03, 0.07	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.	0.15	M: 0.120 RA: 0.120	M: 0.02 RA: 0.02
	SEU (8)	3x<0.01, 0.01, 2x0.03, 0.07, 0.12				
Cauliflower	NEU	3x<0.01, 3x0.02, 0.03, 0.04	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are different from the northern ones. MRL, HR and STMR derived from each dataset.	0.07	M: 0.04 RA: 0.04	M: 0.02 RA: 0.02
	SEU	7x<0.01, 0.01		0.015	M: 0.01 RA: 0.01	M: 0.01 RA: 0.01
Kale	NEU	0.16, 0.24, 0.72, 0.90, 1.22, 1.51, 1.87, 2.05	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue levels in southern trials are different from the northern ones. MRL, HR and STMR derived from each dataset.	4	M: 2.05 RA: 2.05	M: 1.06 RA: 1.06
	SEU	0.1, 0.11, 0.13, 0.18, 2x0.22, 0.26, 0.32		0.6	M: 0.320 RA: 0.320	M: 0.2 RA: 0.2
Head brassica, Brussels sprouts	NEU	0.05, 0.10, 0.12, 0.13	MRL_{OECD}= 0.3	0.3	M: 0.13 RA: 0.13	M: 0.11 RA: 0.11
Head brassica, cabbage	NEU	7x<0.01, 0.16	According to the Student test, 5% and Mann-Withney U-test (α=5%), residue	0.2	M: 0.16 RA: 0.16	M: 0.01 RA: 0.01

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
	SEU	3x<0.01, 0.04	levels in southern trials are not different from the northern ones. MRL, HR and STMR derived from the merged dataset.			
Kohlrabi	NEU	0.02, 0.05, 2x0.08	MRL_{OECD}= 0.173	0.2	M: 0.04 RA: 0.04	M: 0.065 RA: 0.065
Summary of the data on formulation equivalence OECD Guideline 509						
Crop	**Region**	**Residue data** (mg/kg)	**Recommendations/comments**			
No information provided and not requested						
Summary of data on residues in pollen and bee products (Regulation (EU) No 283/2013, Annex Part A, point 6.10.1)						
Product(s)	**Region**	**Residue data** (mg/kg)	**Recommendations/comments**			
Winter oilseed rape	Indoor (3)	Analyses in honey: 3x<0.01	See 2.7.8	**In honey:** 0.01*	<0.01	<0.01

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Dietary burden calculation

Among the representative uses intended for the inscription of PYDIFLUMETOFEN (SYN545974), products from apples pomace, potatoes (as culls, process waste and dried pulp), cabbage leaves and kale leaves might be fed to livestock. As an acute risk has been identified for kale, it was not considered for the dietary burden calculation.The median and maximum dietary burdens were therefore calculated for different groups of livestock using the OECD Guidance documents n° 64/32 and 73. The input values for all relevant commodities are summarised in Table 2.7.5-1.

Table 2.7.5-1: Input values for the dietary burden calculation for PYDIFLUMETOFEN (SYN545974)

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Risk assessment residue definition: PYDIFLUMETOFEN (SYN545974)				
EU representative uses				
Apple pomace	0.08 (0.02x 3.77)	STMRp (STMRxPF)	0.08 (0.02x 3.77)	STMRp (STMRxPF)
Potato culls	0.01	STMR	0.01	HR
Potato, process waste[1]	0.01	STMR	0.01	STMR
Potato, dried pulp[1]	0.01	STMR	0.01	STMR
Cabbage leaves	0.01	STMR	0.16	HR
Cereal straw[2]	0.09	HR from field rotational crops	0.09	HR from field rotational crops

[1] No default processing factor was used for these potato by-products since residue level in the raw product was below the LOQ.
[2] Since significant residue levels cannot be excluded in barley straw from field rotational crops, highest residue levels from barley straw was taken into account in the dietary burden calculation. Extrapolation was made to all cereal straw (except rice) as a worst case.
Remark: as a worst case, transfer factor on apple pomace (excluding pear pomace) was used for dietary burden calculation.

The results of the calculation are reported in Table 2.7.5-2. The calculated dietary burdens were found to be below the trigger value of 0.004 mg/kg bw for turkey and at the trigger value for poultry layer. For ruminants (except finishing swine), the calculated dietary burdens were found to exceed the trigger value.

Table 2.7.5-2: Results of the dietary burden calculation

Animals	**Median burden** (mg/kg bw)	**Maximum burden** (mg/kg bw)	**Above 0.004 mg /kg bw**	**Maximum burden** (mg/kg DM)	**Highest contributing commodities**	
Beef cattle	0,002	0,006	**Yes**	0,27	Cabbage, heads	leaves
Dairy cattle	0,002	0,010	**Yes**	0,25	Cabbage, heads	leaves
Ram/Ewe	0,002	0,005	**Yes**	0,16	Cabbage, heads	leaves
Lamb	0,002	0,006	**Yes**	0,14	Cabbage, heads	leaves
Pig (breeding)	0,001	0,003	No	0,15	Cabbage, heads	leaves
Pig (finishing)	0,001	0,001	No	0,03	Potato	culls
Poultry broiler	0,001	0,001	No	0,01	Potato	culls
Poultry layer	0,001	0,004	**Yes**	0,06	Cabbage, heads	leaves
Turkey	0,001	0,001	No	0,01	Potato	culls

Feeding studies

The magnitude of residue in egg and tissue samples from poultry was investigated in a feeding study with laying hens. Laying hens were fed diets containing PYDIFLUMETOFEN (SYN545974) at three feeding levels: 0.16, 0.5 and 1.6 mg/kg bw/day. The samples were analyzed for PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP and residues for both compounds were found to be below the LOQs in all tissues and eggs (expect eggs yolk) at the lower tested dose. For the setting of MRLs, residue levels found in feeding study were calculated as the sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP expressed in PYDIFLUMETOFEN (SYN545974). At the level of exposure calculated with representative uses which is below the lowest dose rate of the feeding study, no residues are expected in any poultry commodity. Therefore proposed MRL in these commodities are set at the LOQ.

Table 2.7.5-3: Calculated MRLs for poultry (sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP expressed in PYDIFLUMETOFEN (SYN545974))

Animal	**Residues at closet feeding level** (mg/kg)		**Estimated value at 1N level**		**MRL proposal** (mg/kg)
	Mean	Highest	STMR (mg/kg)	HR (mg/kg)	
Poultry	Closest feeding level		0,16	mg/kg bw	
	38,9	N Layer	224,0	N Turkey	
Meat	-	-	0,000	0,001	-
Muscle	0,032	0,032	0,000	0,001	**0,02***
Fat	0,032	0,032	0,000	0,001	**0,02***
Liver	0,032	0,032	0,000	0,001	**0,02***
Kidney	0,032	0,032	0,000	0,001	**0,02***
Eggs	0,032	0,032	0,000	0,001	**0,02***

(*): Indicates that the MRL is set at the limit of analytical quantification.

The magnitude of residue in milk and tissue samples from ruminants was investigated in a feeding study with lactating cows. Lactating cows were fed diets containing PYDIFLUMETOFEN (SYN545974) at three feeding levels: 0.40, 1.09 and 4.32 mg/kg bw/day. Samples of milk and tissues were analysed for residues of PYDIFLUMETOFEN (SYN545974) (parent) and the 2,4,6-trichlorophenol (TCP) metabolite. At the level of exposure calculated with representative uses which is below the lowest dose rate of the feeding study, no residues are expected in any ruminant tissue or milk.
As PYDIFLUMETOFEN (SYN545974) in pig is expected to have a similar metabolic pathway to the one in ruminant, the feeding study on ruminant (representing 116.9, 318 and 1262 N of pig maximum dietary burden) is used to derive STMR, HR and MRL.

Table 2.7.5-4: Calculated MRLs for poultry (sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP expressed in PYDIFLUMETOFEN (SYN545974))

Animal	**Residues at closet feeding level** (mg/kg)		**Estimated value at 1N level**		**MRL proposal** (mg/kg)
	Mean	Highest	STMR[(b)] (mg/kg)	HR (mg/kg)	
Bovine	Closest feeding level[(a)]:		0,4	mg/kg bw	
	41,1	N Dairy c.	62,6	N Beef c.	
Meat	-	-	0,000	0,001	-
Muscle	0,032	0,032	0,000	0,001	**0,02***
Fat	0,013	0,020	0,000	0,000	**0,02***
Liver	0,035	0,042	0,000	0,001	**0,02***
Kidney	0,022	0,022	0,000	0,001	**0,02***
Milk[(c)]	0,022	0,022	0,000	0,001	**0,02***

Sheep	Closest feeding level[(a)]:		0,4	mg/kg bw	
	69,5	N Lamb	77,4	N Ram/Ewe	
Meat	-	-	0,000	0,000	-
Muscle	0,032	0,032	0,000	0,000	**0,02***
Fat	0,013	0,020	0,000	0,000	**0,02***
Liver	0,035	0,042	0,000	0,001	**0,02***
Kidney	0,022	0,022	0,000	0,000	**0,02***
Milk[(c)]	0,022	0,022	0,000	0,000	**0,02***
Swine	Closest feeding level[(a)]:		0,4	mg/kg bw	
	116,9	N Breeding	488,9	N Finishing	
Meat	-	-	0,000	0,000	-
Muscle	0,032	0,032	0,000	0,000	**0,02***
Fat	0,013	0,020	0,000	0,000	**0,02***
Liver	0,035	0,042	0,000	0,000	**0,02***
Kidney	0,022	0,022	0,000	0,000	**0,02***

(*): Indicates that the MRL is set at the limit of analytical quantification.

2.7.6 Summary of effects of processing

Nature of residue

A study was conducted with [pyrazole-5-^{14}C]-SYN545974 simulating representative hydrolytic conditions for pasteurisation (20 minutes at 90°C, pH 4), boiling/brewing/baking (60 minutes at 100°C, pH 5) and sterilisation (20 minutes at 120°C, pH 6).

No hydrolysis of PYDIFLUMETOFEN (SYN545974) was observed under any of the processing conditions. PYDIFLUMETOFEN (SYN545974) is therefore considered to be hydrolytically stable under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation. According to the OCDE guidelines 507, separate studies should have been performed reflecting labelling of each ring. However, since more than 95% of the recovered radioactivity was identified as parent, no cleavage of the molecule is anticipated, additional study with phenyl ring is not required.

Distribution of residue in peel and pulp

Data on distribution of residues in peel/pulp are available for melon and are detailed in the table hereafter.

commodity	**Individual transfer factor peel/pulp[1]**	**Median TF**
PYDIFLUMETOFEN (SYN545974)		
Melon	2x<0.17 4x <0.25, 4x<0.33, 6x<0.5, 1	<0.33

[1]Transfer factor = residue in pulp (mg/kg)/ residue whole fruit (mg/kg).

Magnitude of residue

Several studies with data on processing are available on grape, tomato, pome-fruits and kale.

Table 2.7.6-1: Crops and commodities obtained from industrial or domestic processing

Raw agricultural commodity	Industrial or household processed products
Grape, fruit	Wet and dry pomace, juice, raisins, grapeseed oil, red and white wine
Tomato, fruit	Paste, puree, washed and peeled fruit, canned fruit, sun-dried fruit, juice, wet and dried pomace
Pome fruit, fruit	Dried fruit, canned fruit, juice, wet pomace and apple sauce
Kale, leaves	Washed leaves and cooked leaves

Based on these studies, processing factors can be calculated and are presented in Table 2.7.6-2.

Table 2.7.6-2: Overview of processing studies

Processed commodity	Individual PF	Median PF
PYDIFLUMETOFEN (SYN545974)		
Grape, pasteurized juice	0.02, 0.02, 0.05, 0.07	0.035
Grape, white aged wine	0.08, 0.11, 0.52, 0.60	0.32
Grape, red wine	0.10, 0.17, 0.20, 0.24	0.19
Grape, raisin	1.71, 2.37, 2.48, 4.75	2.43
Grape, refined seed oil	0.71, 1.02, 1.08, 1.12, 0.71	1.05
Tomato, paste	0.55, 0.82	0.69
Tomato, puree	0.26, 0.41	0.33
Tomato, washed and peel	<0.05, <0.08	n.a.[1]
Tomato, canned	<0.05, <0.08	n.a.[1]
Tomato, sun-dried	9.9, 10.7	10
Tomato, juice	<0.05, <0.08	n.a.[1]
Apple/pear, canned	0.03 (apple) , 0.09 (pear)	0.06
Apple/pear, wet pomace	2.99 (pear), 3.77 (apple)	3.38
Apple/pear, Juice	0.06 (apple), 0.11 (pear)	0.09
Apple, sauce	0.06	0.06 (single value)
Apple/pear, dried	0.41 (apple), 0.62 (pear)	0.52
Kale, washed	1.08, 1.18, 1.58, 1.60	1.38
Kale, cooked	1.2, 1.21, 1.27, 1.73	1.24

[1] Results which were calculated from the LOQ value are not taken into account in the median transfer factor calculation due to high uncertainty.

Results show that PYDIFLUMETOFEN (SYN545974) residues are generally concentrated in dried commodities, apple wet pomace, washed and cooked kale and oil, but diluted in other processed commodities. As regards kales, the domestic preparation employed in the kale processing study (CEMS-6542) involved removing stems and ribs immediately prior to washing. The pre-processed sample comprised leaves with rib and stem attached (raw agricultural commodity) whereas the washed leaves were trimmed kale leaves only (rib and stem removed). It is likely that lower residue levels are present on the stems and ribs compared to the edible part of leaves, resulting in an increased concentration in the edible portion of the leaves that were subsequently washed.

It should be noted that for several processed matrices, the analytical method is not validated (see B.5.1.2).

2.7.7 Summary of residues in rotational crops

Metabolism of PYDIFLUMETOFEN (SYN545974) in soil has been investigated (see B.8). DT50 value of PYDIFLUMETOFEN (SYN545974) was estimated to be 4170 days (based on residues including harsh extracts) (please refer to vol3 B8). As the substance is very persistent in soil, further investigation of residues in rotational crops is required.

Nature of the residue

The metabolism in succeeding crops was evaluated on leafy vegetable (lettuce), root and tuber vegetables (turnip) and cereals (wheat). Studies were conducted using [phenyl-U-14C] & [pyrazole-5-14C]-SYN545974. The characteristics of these studies are summarized in **Table 2.7.7-1Table 2.7.2-1**.

Table 2.7.7-1: Summary of available metabolism studies on rotational crops

Crop group	Crop	Label position	Application and sampling details			
			Method, F or G[a]	Rate (kg a.s./ha)	Sowing intervals (days)	Harvest time
Leafy vegetables	Lettuce	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Bare soil, F	1 x 0.4	30, 120, 270	growth stage (BBCH 41-43, BBCH 45 for 120DAA lettuce) and at maturity (BBCH 49).
Root and tuber vegetables	Turnip	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Bare soil, F	1 x 0.4	30, 120, 270	BBCH 49
Cereals	Wheat	[phenyl-U-14C] & [pyrazole-5-14C]-SYN545974	Bare soil, F	1 x 0.4	30, 120, 270	Forage (BBCH 15-30), hay (BBCH 49-60) and maturity (BBCH 89) growth stage

The crops were sown 30, 120 and 270 days after application (DAA) of the test substance to a sandy loam soil. The actual application rates achieved were 387.8 g a.s./ha for the [phenyl-U-14C]-SYN545974 labelled treatment and 408.6 g a.i./ha for the [pyrazole-5-14C]-SYN545974 labelled treatment.

Since TRR observed in wheat grain, mature lettuce and turnip tubers were below 0.01 mg/kg, no further analysis was conducted. The metabolic pathways in the three crops are similar. In all cases, unchanged parent PYDIFLUMETOFEN (SYN545974) was reported to be the major compound with a maximum of 77.8% TRR (0.024 mg/kg) in wheat forage. Maximum residues of PYDIFLUMETOFEN (SYN545974) were detected in 120 DAA wheat straw (0.063 mg/kg for the pyrazole label). Other metabolites identified were SYN547891 and SYN545547 detected respectively with a maximum of 0.012 (5,5% TRR) and 0.005 mg/kg (2.2%TRR) in wheat straw. There were both present at lower levels than parent compound in all commodities at all rotational intervals. Metabolism pathway for PYDIFLUMETOFEN (SYN545974) in primary crop and rotational crops is similar. Then, the same residue definition applies for rotational crops as for primary crops.

Table 2.7.7-2: Summarized results of available rotational metabolism studies of PYDIFLUMETOFEN (SYN545974)

	Wheat																		**immature lettuce**		**turnip**	
	forage						hay						straw								foliage	
PBI (d)	30		120		270		30		120		270		30		120		270		30		30	
	ph	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*	*ph*	*py*
TRR % (mg/kg)	96,8 (0,03)	96,8 (0,027)	89,2 (0,01)	91,1 (0,025)	96,3 (0,013)	95,5 (0,013)	91,9 (0,06)	90 (0,082)	85,2 (0,05)	84,5 (0,091)	94,7 (0,037)	93,5 (0,033)	86,9 (0,151)	88,2 (0,187)	85,9 (0,131)	85,9 (0,188)	87,3 (0,1)	83,2 (0,138)	85,5 (0,010)	86,1 (0,016)	93,6 (0,010)	89,6 (0,012)
PYDIFLUMETOFEN (SYN545974)	**77,8 (0,024)**	**59,1 (0,016)**	37,3 (0,004)	22,5 (0,006)	59,7 (0,008)	21,9 (0,003)	**50,1 (0,032)**	**23,8 (0,022)**	**42,9 (0,025)**	**52,2 (0,056)**	**76,1 (0,03)**	**67,9 (0,024)**	**30 (0,052)**	**26 (0,055)**	**33,7 (0,051)**	**28,7 (0,063)**	**32,2 (0,037)**	**18,6 (0,030)**	69,3 (0,009)	**76,7 (0,015)**	77,2 (0,008)	44,4 (0,005)
SYN547891	12,0 (0,004)	13,3 (0,004)	4,9 (<0,001)	3,0 (0,001)	7,5 (0,001)	4,6 (0,001)	6,2 (0,004)	3,1 (0,003)	4,5 (0,003)	5,5 (0,006)	9,7 (0,004)	12,2 (0,004)	6,1 (0,011)	5,5 (0,012)	5,3 (0,008)	4,4 (0,010)	5,5 (0,006)	4,6 (0,008)	11,6 (0,001)	6,8 (0,001)	3,9 (<0,001)	4,8 (0,001)
SYN545547	2,2 (0,001)	3,5 (0,001)	2,4 (<0,001)	2,3 (0,001)	3,1 (<0,001)	ND	2,5 (0,002)	1,7 (0,002)	1,5 (0,001)	2,3 (0,002)	3,6 (0,001)	5,6 (0,002)	1,8 (0,003)	2,3 (0,005)	1,4 (0,002)	2,2 (0,005)	2,0 (0,002)	1,5 (0,002)	4,0 (<0,001)	2,3 (<0,001)	ND	3,9 (<0,001)
Unassigned % (mg/kg)	10,6 (0,005)	14,3 (0,004)	33,7 (0,001)	63 (0,013)[3]	16,8 (0,001)	62,4 (0,009)	38,9 (0,025)[4]	53,8 (0,051)[5]	28 (0,019)[6]	27,3 (0,029)[7]	7,3 (0,003)	9,3 (0,003)	43,1 (0,076)[8]	54,3 (0,116)[9]	42,8 (0,068)[10]	46,4 (0,103)[11]	44,2 (0,05)[12]	67,0 (0,107)[13]	3,4 (<0,001)	2,1 (<0,001)	10,2 (0,001)	24,8 (0,002)
non extracted TRR %	3,2 (0,001)	3,2 (0,001)	10,7 (0,001)	8,9 (0,002)	3,7 (<0,001)	4,5 (0,001)	8,1 (0,005)	9,9 (0,009)	14,8 (0,009)	15,5 (0,017)	5,3 (0,002)	6,5 (0,002)	12,2 (0,021)	11,3 (0,024)	14,1 (0,022)	14,1 (0,031)	12,7 (0,014)[2]	16,8 (0,028)[2]	14,5 (0,002)	13,9 (0,003)	6,5 (0,001)	10,4 (0,001)
total TRR % (mg/kg)	100 (0,031)	100 (0,028)	99,9 (0,011)	100 (0,027)	100 (0,014)	100 (0,014)	100 (0,065)	99,9 (0,091)	100 (0,059)	100 (0,108)	100 (0,039)	103 (0,035)	99,1 (0,172)	99,5 (0,211)	100 (0,153)	100 (0,219)	100 (0,114)	100 (0,166)	100 (0,012)	100 (0,019)	100 (0,010)	100 (0,012)

1 - mg/kg calculated directly from summation of the radioactivity present in the extracted radioactivity in the debris and specific activity after aqueous acetonitrile extraction.
2 -Straw (270DAA) was extracted further with 1M HCl and a “clean fractionation” technique to assess natural incorporation.
3- at least 29 individual components none individually exceeding >9.2% TRR (>0.002 mg/kg)
4- at least 21 individual components none individually exceeding >5.0% TRR (>0.003 mg/kg)
5- at least 30 individual components none individually exceeding >7.3% TRR(>0.007 mg/kg)
6- at least 20 individual components none individually exceeding >4.7% TRR (>0.003 mg/kg)
7- at least 17 individual components none individually exceeding >4.3% TRR (>0.005 mg/kg)
8- at least 22 individual components none individually exceeding >6.9% TRR (>0.012 mg/kg)
9- at least 38 individual components none individually exceeding >6.7% TRR (>0.014 mg/kg)
10- at least 27 discrete components, no single one of which >6.0% TRR (>0.009 mg/kg)
11- at least 33 discrete components, no single one of which >4.6% TRR (>0.010 mg/kg)
12- at least 17 discrete components, no single one of which >4.6% TRR (>0.005 mg/kg)
13- at least 19 discrete components, no single one of which >5.8% TRR (>0.009 mg/kg)

In this study one application on bare soil is performed at 0.4 kg a.s./ha which is higher than the intended applied doses of the EU representative uses. However, PYDIFLUMETOFEN (SYN545974) compound is highly persistent in soil (DT 50 of 4170 days), then the accumulation of the active substance was taken into account.

Predicted Environmental Concentration (PEC) in soil after accumulation was calculated after 20 and 100 years for all EU representative uses (see Table 2.7.7-3 and vol3 B8 for details).

Table 2.7.7-3: Initial PECsoil and maximum long term PECsoil for PYDIFLUMETOFEN (SYN545974) according to cGAP (see section B8)

Use	Initial PEC soil (mg/kg)	Background concentration after 20 years (mg/kg)	PECaccu after 20 years (mg/kg)	Background concentration after 100 years (mg/kg)	PECaccu after 100 years
Vines, 2x200 g a.s./ha	0.2131	2.402 (5cm)	2.6151	3.412	3.6251
Vines, 2x40 g a.s./ha	0.0533	0.6003 (5cm)	0.6536	0.8527	0.9060
Pome-fruits, 3x50 g a.s./ha	0.0799	0.9007 (5cm)	0.9806	1.279	1.3589
Cucurbits, 2x50 g a.s./ha	0.0400	0.1125 (20 cm)	0.1525	0.1598	0.1998
Tomatoes, 2x70 g a.s./ha	0.0373	0.1050 (20 cm)	0.1423	0.1492	0.1865
Potatoes, 3x40 g a.s./ha	0.0639	0.1804 (20 cm)	0.2443	0.2562	0.3201
Brassicas , 2x70 g a.s./ha	**0.1119**	**0.3153 (20 cm)**	**0.4272**	**0.4478**	**0.5597**

In the confined rotational crops study, considering that the application is conducted on bare soil, that the soil is not plough up and that PYDIFLUMETOFEN (SYN545974) has a very limited mobility in soil (Koc of 1706 L/kg), the expected concentration in soil was calculated considering a distribution in the top 5 cm depth of the soil with the following equation :

Concentration a.s. in soil (mg/kg) = dose of application into bare soil (mg) / [soil depth (cm^2) x soil density (kg/cm^3)]

With:

- dose of application into bare soil : 0.4 kg as/ha
- soil depth considered: 5 cm
- soil density: 1.5 g/cm^3

Concentration a.s. in soil (mg/kg) = $0.4x10^6$ mg / [(5 cm $x10^8$ cm^2) x $1.5x10^{-3}$ kg/cm^3]
Concentration a.s. in soil = 0.53 mg/kg

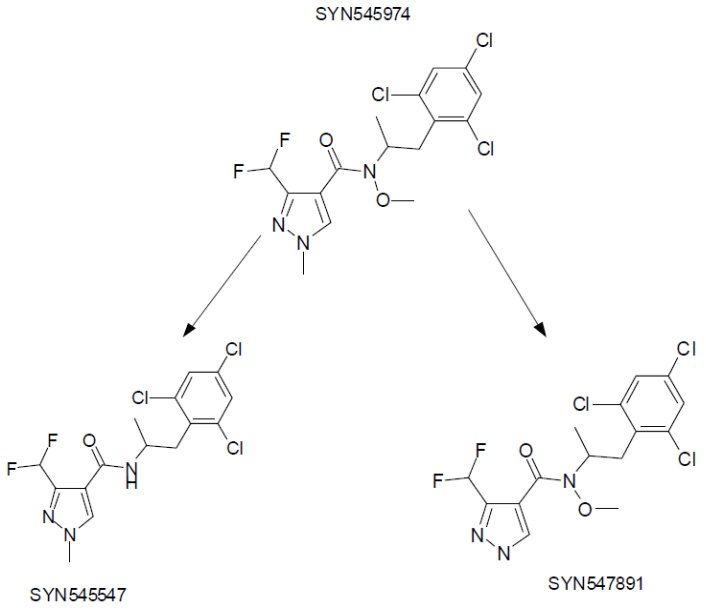
Then, this calculated concentration of active substance in soil has been compared with the value of PEC accumulation obtained for brassicas in order to see if the PEC accumulation in soil was covered by the soil concentration from the submitted confined rotational crop study.

Table 2.7.7-4: Comparison of concentration in soil between confined rotational crop study and PEC at the intended GAP

PECaccu calculated in view of the intended cGAP on brassicas				Concentration in 5 cm of soil calculated based on rotational crop studies (mg/kg)	Ratios			
PECaccu 5 cm after 20 years (mg/kg)	PECaccu 5 cm after 100 years	PECaccu 20 cm after 20 years (mg/kg)	PECaccu 20 cm after 100 years	Metabolism in rotational crops	Ratio concentration 5 cm study/PEC accu 5 cm intended GAP		Ratio concentration 5 cm study/PEC accu 20 cm intended GAP	
					After 20 years	After 100 years	After 20 years	After 100 years
0.4272	0.5597	0.3433	0.4758	0.53	1.25	0.95	1.55	1.12

Ratios have been calculated by dividing the theoretical concentration of PYDIFLUMETOFEN (SYN545974) in the top 5 cm of soil from the confined rotation crop study by the values of PEC accumulation in soil. Both PECaccumulation calculated on 5 and 20 cm were used in order to take into consideration all the agricultural practices (whether the soil is plough or not before sowing succeeding crops). According to the above calculations, theoretical concentration in the top 5 cm of the soil from the confined rotational crop study is equivalent to the PEC accumulation in soil calculated in section B8.
Based on these results, no residue above 0.01 mg/kg is expected in wheat grain, mature lettuce and turnip tubers and foliage at all Plant Back Intervals (PBI) tested. Nevertheless, significant residues of parent compound cannot be excluded in wheat forage and immature lettuce with a PBI of 30 days and in wheat hay and straw for all PBI.

Figure 2.7.7-1: biotransformation pathway for PYDIFLUMETOFEN (SYN545974) in confined rotational crops



Magnitude of residues in rotational crops

Three representative rotated crops (spinach, carrot and barley) were planted back into the treated plots, where PYDIFLUMETOFEN (SYN545974) was applied at 500 g a.s./ha to bare soil, at nominal intervals of 30, 60 and 365 days. Results are summarized in Table 2.7.7-5.

Table 2.7.7-5: residue levels of PYDIFLUMETOFEN (SYN545974) measured in rotational crops

Plant-back Interval (nominal)	Timing and/or Growth Stage (BBCH)	Crop Part	PYDIFLUMETOFEN (SYN545974) Residue Found (mg/kg)			
			Trial S13-01023-01	Trial S13-01023-02	Trial S13-01022-01	Trial S13-01022-02
30	43	Immature spinach	**0.01**	<0.01	<0.01	<0.01
60	43	Immature spinach	**0.02**	<0.01	<0.01	<0.01
365	43	Immature spinach	<0.01	<0.01	<0.01	<0.01
30	49 (NCH)	Spinach leaves	<0.01	<0.01	Not sampled	<0.01
60	49 (NCH)	Spinach leaves	<0.01	<0.01	Not sampled	<0.01
365	49 (NCH)	Spinach leaves	<0.01	<0.01	Not sampled	<0.01
30	49 (NCH)	Carrot roots	**0.02**	<0.01	<0.01	<0.01
30	49 (NCH)	Carrot leaves	<0.01	<0.01	<0.01	<0.01
60	49 (NCH)	Carrot roots	**0.02**	**0.02**	<0.01	<0.01
60	49 (NCH)	Carrot leaves	**0.01**	<0.01	<0.01	<0.01
365	49 (NCH)	Carrot roots	<0.01	<0.01	<0.01	<0.01

Plant-back Interval (nominal)	Timing and/or Growth Stage (BBCH)	Crop Part	PYDIFLUMETOFEN (SYN545974) Residue Found (mg/kg)			
			Trial S13-01023-01	Trial S13-01023-02	Trial S13-01022-01	Trial S13-01022-02
365	49 (NCH)	Carrot leaves	<0.01	<0.01	<0.01	<0.01
30	41	Barley whole plant	<0.01	0.02	<0.01	<0.01
60	41	Barley whole plant	<0.01	<0.01	<0.01	<0.01
365	41	Barley whole plant	<0.01	<0.01	<0.01	<0.01
30	89 (NCH)	Barley grain	<0.01	<0.01	<0.01	<0.01
30	89 (NCH)	Barley straw	**0.06**	**0.02**	**0.02**	<0.01
60	89 (NCH)	Barley grain	<0.01	<0.01	<0.01	<0.01
60	89 (NCH)	Barley straw	**0.09**	**0.02**	**0.03**	<0.01
365	89 (NCH)	Barley grain	<0.01	<0.01	<0.01	<0.01
365	89 (NCH)	Barley straw	**0.01**	**0.01**	**0.01**	<0.01

NCH – normal commercial harvest; No correction of results for either control residues or recovery values has been performed.

The same approach as described above was applied for field rotation crops.

Table 2.7.7-6: Comparison of concentration in soil between rotational crop studies and PEC at the intended GAP

PECaccu calculated in view of the intended cGAP on brassicas				Concentration in 5 cm of soil calculated based on rotational crop studies (mg/kg)	Ratios			
PECaccu 5 cm after 20 years (mg/kg)	PECaccu 5 cm after 100 years	PECaccu 20 cm after 20 years (mg/kg)	PECaccu 20 cm after 100 years	Rotational crops	Ratio concentration 5 cm study/PEC accu 5 cm intended GAP		Ratio concentration 5 cm study/PEC accu 20 cm intended GAP	
					After 20 years	After 100 years	After 20 years	After 100 years
0.4272	0.5597	0.3433	0.4758	0.67	1.56	1.19	1.94	1.4

According to the above calculations, theoretical concentration in the top 5 cm of the soil from the rotational crop studies covers the PEC accumulation in soil calculated in section B8.

In barley grain, residues of PYDIFLUMETOFEN (SYN545974) were below the LOQ at all plant-back intervals. In these conditions, no residue above the LOQ is awaited in cereal grains for human or livestock consumption. However, according to the results, foliar crops (immature spinach and carrot leaves), root crops and cereal straws present a high probability of residues being present at measurable level at plant back interval of 30 and 60 days.

Residue levels measured in rotational crops were taken into account in livestock dietary burden calculation and in risk assessment for human consumer by the applicant. This option does not modify proposed MRL in animal commodities. However, RMS has chosen to propose restrictions instead. Indeed, residue levels measured in roots and tuber crops (carrots, 0.02 mg/kg) raise the need for setting MRLs in succeeding crops. Since no harmonized guidance is available at European level at the present time, RMS is of the opinion that restrictions on succeeding crops are the best option. Nevertheless, since no MRL has to be set on feedstuffs, residue levels measured on straw in the field rotational crops were taken into account in the dietary burden calculation.

Since residue levels above the LOQ cannot be excluded in carrot roots and immature spinach leaves with a PBI of 60 days, a plant back interval of 365 days should be respected for roots and leafy crops.

Certain crop types which could reasonably be rotated with some of the representative uses were not included. Considering the above results in rotational crops and the persistence of the active substance in soil, additional data on an bulb vegetable, a fruiting or a legume vegetable and pulses could be requested. As no data is available, rotation with these groups of crops are not recommended.

It is also noted that no data have been provided to demonstrate the levels of residues found in any of the tested crops at plant back intervals of between 60 and 365 days. Given the lack of data for intermediate plant back intervals and the possibility of accumulation in succeeding crops, further data are considered necessary.

It should be highlighted that no information on whether the soil has been turned before sowing succeeding crops (as it would be the case under usual agricultural practices) is mentioned. Based on the characteristics of the active substance, it is not possible to affirm that the active substance has been uniformly distributed in the top 20 cm depth of the soil where the crops roots will grow and have the opportunity to take up the soil residues.

2.7.8 Summary of other studies

Residue in pollen and bee products

Many of the proposed uses could result in application during flowering (pome fruit, vines, fruiting vegetables) and as a result there is potential for transfer of residues into bee products.

Trials conducted on oilseed rape to assess the potential for the transfer of pydiflumetofen residues to honey were submitted.

Three residue trials, under tunnels, were conducted on winter oilseed rape in Germany in 2016 where honey was sampled following the exposure of bees to treated crop. One application of pydiflumetofen, 62.5 g/L EC as A21857B at a rate of 200 g a.s./ha was made as a tank mix at BBCH 63 (flowering stage) to oilseed rape.

A bee hive was placed in each tunnel in the evening after the application and the bees were allowed to forage freely on the treated crop. Honey was sampled at maturity (from 20 to 27 days after the first exposure of bees) from each of the treatment and control tunnels in the trials, with the exception of trial -02, in which the water content in honey was >20 % at the time of sampling for both treated and control samples.

The residues of pydiflumetofen in all treated honey samples were below the limit of quantification (LOQ, 0.01 mg/kg). No residues of pydiflumetofen at or above the limit of quantification (LOQ, 0.01 mg/kg) were found in the untreated honey samples.

Table 2.7.8-1: Overview of the available residue trials data on honey and MRL proposal

Crop	**Region/ Indoor** (a)	**Residue levels** (mg/kg) **observed in the supervised residue trials relevant to the supported GAPs** (b)	**Recommendations/comments** (OECD calculations)	**MRL** proposals (mg/kg)	**HR** (mg/kg) (c)	**STMR** (mg/kg) (d)
Residue definition for monitoring and enforcement : PYDIFLUMETOFEN (SYN545974) **Residue definition for risk assessment (RA):** PYDIFLUMETOFEN (SYN545974)						
Winter oilseed rape	Indoor (3)	3x<0.01		In honey: 0.01*	<0.01	<0.01

2.7.9 Estimation of the potential and actual exposure through diet and other sources

End-Point	Value	Study	Safety Factor	Reference
PYDIFLUMETOFEN (SYN545974)-				
Acceptable Daily Intake (ADI)	0.092 mg/kg bw/d	Mouse, 80 week	100	-
Acute Reference Dose (ARfD)	0.1 mg/kg bw/d	rabbit, developmental	100	-
SYN547897				
Acceptable Daily Intake (ADI) and accute reference dose (ARfD)	0.0015 mg/kgbw/d	TTC value for non-genotoxic Cramer class III substances	-	-

The consumer risk assessment was performed using revision 2 of the EFSA PRIMo (Pesticide Residue Intake Model). For the chronic and acute intake assessment the proposed MRL, STMR and HR derived from residue trials were considered for plant and animal commodities.

For kidney, residue levels for risk assessment were calculated by crossing measured residue levels of parent, 2,4,6 TCP and SYN548263 (sum expressed in parent compound) in feeding studies with estimated residue levels of the dietary burden. Since residue levels for monitoring and risk assessment were below the LOQ, no conversion factor (monitoring to risk assessment) has been proposed for kidney.

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Residue definition for risk assessment (RA): **PYDIFLUMETOFEN (SYN545974)**				
EU representative uses				
Apple	0.02	STMR	0.14	HR
Pear	0.02	STMR	0.14	HR
Table grape	0.265	STMR	1.19	HR
Wine grape	0.265	STMR	0.265	STMR
Potato	<0.01	STMR	<0.01	HR
Tomato	0.04	STMR	0.07	HR
Cucumber, courgette	0.03	STMR	0.07	HR
Melon, watermelon	0.03	STMR	0.06	HR
Broccoli	0.02	STMR	0.12	HR
Cauliflower	0.02	STMR	0.04	HR
Kale	1.06	STMR	2.05	HR
Brussels sprouts	0.11	STMR	0.13	HR
Head cabbage	0.01	STMR	0.16	HR

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
kohlrabi	0.065	STMR	0.04	HR
Honey	<0.01	MRL	<0.01	HR
Residue definition for risk assessment (RA): **Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP expressed as PYDIFLUMETOFEN (SYN545974)**				
Milk	<0.02	MRL	<0.02	HR
Ruminant meat	<0.02	MRL	<0.02	HR
Ruminant fat	<0.02	MRL	<0.02	HR
Poultry meat	<0.02	MRL	<0.02	HR
Poultry liver	<0.02	MRL	<0.02	HR
Poultry kidney	<0.02	MRL	<0.02	HR
Eggs	<0.02	MRL	<0.02	HR
First residue definition for risk assessment (RA): **Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6 TCP expressed as PYDIFLUMETOFEN (SYN545974)**				
Ruminant liver	<0.02	STMR	<0.02	HR
First residue definition for risk assessment (RA): **Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6 TCP and SYN548263 expressed as PYDIFLUMETOFEN (SYN545974)**				
Ruminant kidney	<0.03	STMR	<0.03	HR

Separate consumer risk assessment for metabolite SYN547897

No toxicological data are available for this compound and it is not sufficiently covered by parent compound based on rat metabolism (although its structure is very similar). As SYN547897 is ascribed to 'Cramer Class III' and does not trigger in-silico alerts for genotoxicity or neurotoxicity, a TTC of respectively 1.5 µg/kg bw/d and 5 µg/kg bw/d can be used for the assessment of chronic and acute consumer risk in relation to this metabolite species.

Residue levels of SYN547897 were obtained by crossing measured residue levels in feeding studies with estimated residue levels of the dietary burden.

Commodity	Chronic risk assessment		Acute risk assessment	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Second residue definition for risk assessment (RA): **SYN547897**				
Bovine liver	<0.01	STMR	0.016	HR
Sheep liver	<0.01	STMR	<0.01	HR

Commodity	**Chronic risk assessment**		**Acute risk assessment**	
	Input value (mg/kg)	**Comment**	**Input value (mg/kg)**	**Comment**
Swine liver	<0.01	STMR	<0.01	HR
Second residue definition for risk assessment (RA): **SYN547897**				
Bovine kidney	<0.01	STMR	0.016	HR
Sheep kidney	<0.01	STMR	<0.01	HR
Swine kidney	<0.01	STMR	<0.01	HR

Table 2.7.9-1: TMDI calculation linked to EU representative uses

Chronic risk assessment - refined calculations								
			TMDI (range) in % of ADI minimum - maximum					
					0	1		
			No of diets exceeding ADI:		---			
Highest calculated TMDI values in % of ADI	MS Diet		Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
1,4	NL child		0,6	Milk and milk products: Cattle	0,2	Table grapes	0,2	Kale
1,3	FR all population		1,2	Wine grapes	0,1	Milk and milk products: Cattle	0,0	Table grapes
1,1	DE child		0,4	Table grapes	0,3	Milk and milk products: Cattle	0,3	Apples
1,0	WHO Cluster diet B		0,5	Wine grapes	0,1	Tomatoes	0,1	Table grapes
0,9	PT General population		0,7	Wine grapes	0,1	Table grapes	0,1	Potatoes
0,8	WHO cluster diet E		0,5	Wine grapes	0,1	Milk and milk products: Cattle	0,0	Table grapes
0,8	FR infant		0,6	Milk and milk products: Cattle	0,1	Apples	0,0	Potatoes
0,7	IE adult		0,4	Wine grapes	0,1	Table grapes	0,1	Milk and milk products: Cattle
0,7	NL general		0,2	Wine grapes	0,1	Milk and milk products: Cattle	0,1	Kale
0,6	WHO cluster diet D		0,1	Kale	0,1	Wine grapes	0,1	Milk and milk products: Cattle
0,5	DK adult		0,4	Wine grapes	0,0	Table grapes	0,0	Tomatoes
0,5	ES child		0,3	Milk and milk products: Cattle	0,0	Tomatoes	0,0	Bovine: Meat
0,5	WHO Cluster diet F		0,2	Wine grapes	0,1	Milk and milk products: Cattle	0,0	Potatoes
0,5	SE general population 90th percentile		0,3	Milk and milk products: Cattle	0,1	Kale	0,0	Potatoes
0,5	WHO regional European diet		0,1	Milk and milk products: Cattle	0,1	Wine grapes	0,0	Tomatoes
0,4	ES adult		0,1	Wine grapes	0,1	Milk and milk products: Cattle	0,0	Tomatoes
0,4	UK Adult		0,3	Wine grapes	0,0	Tomatoes	0,0	Potatoes
0,3	UK vegetarian		0,2	Wine grapes	0,0	Tomatoes	0,0	Table grapes
0,3	FR toddler		0,1	Table grapes	0,1	Apples	0,1	Potatoes
0,3	LT adult		0,1	Milk and milk products: Cattle	0,0	Apples	0,0	Potatoes
0,2	DK child		0,1	Cucumbers	0,1	Table grapes	0,1	Apples
0,2	PL general population		0,1	Table grapes	0,0	Apples	0,0	Tomatoes
0,2	UK Toddler		0,1	Table grapes	0,0	Potatoes	0,0	Apples
0,1	FI adult		0,1	Wine grapes	0,0	Tomatoes	0,0	Potatoes
0,1	IT kids/toddler		0,1	Tomatoes	0,0	Table grapes	0,0	Apples
0,1	IT adult		0,1	Tomatoes	0,0	Table grapes	0,0	Apples
0,1	UK Infant		0,0	Potatoes	0,0	Apples	0,0	Brussels sprouts

Separate risk assessment for metabolite SYN547897 (TTC approach)

Chronic risk assessment

		TMDI (range) in % of ADI minimum - maximum					
		No of diets exceeding ADI:	---				
Highest calculated TMDI values in % of ADI	MS Diet	Highest contributor to MS diet (in % of ADI)	Commodity / group of commodities	2nd contributor to MS diet (in % of ADI)	Commodity / group of commodities	3rd contributor to MS diet (in % of ADI)	Commodity / group of commodities
0,2	WHO Cluster diet B	0,1	Bovine: Liver	0,1	Bovine: Liver		FRUIT (FRESH OR FROZEN)
0,2	IE adult	0,2	Sheep: Liver		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)
0,2	DK child	0,2	Bovine: Liver		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)
0,2	NL child	0,1	Bovine: Liver	0,0	Swine: Liver	0,0	Bovine: Kidney
0,2	UK Infant	0,1	Bovine: Liver	0,0	Bovine: Kidney		FRUIT (FRESH OR FROZEN)
0,1	DK adult	0,1	Bovine: Liver		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)
0,1	ES child	0,0	Swine: Liver	0,0	Bovine: Liver	0,0	Swine: Kidney
0,0	WHO cluster diet D	0,0	Bovine: Liver	0,0	Bovine: Liver		FRUIT (FRESH OR FROZEN)
0,0	NL general	0,0	Bovine: Liver	0,0	Swine: Liver		FRUIT (FRESH OR FROZEN)
0,0	UK Toddler	0,0	Bovine: Liver	0,0	Bovine: Kidney		FRUIT (FRESH OR FROZEN)
0,0	LT adult	0,0	Bovine: Liver	0,0	Swine: Liver		FRUIT (FRESH OR FROZEN)
0,0	WHO Cluster diet F	0,0	Bovine: Liver	0,0	Bovine: Liver		FRUIT (FRESH OR FROZEN)
0,0	ES adult	0,0	Bovine: Liver	0,0	Swine: Liver	0,0	Swine: Kidney
0,0	WHO cluster diet E	0,0	Bovine: Liver		FRUIT (FRESH OR FROZEN)		FRUIT (FRESH OR FROZEN)
0,0	UK Adult	0,0	Bovine: Liver	0,0	Bovine: Kidney		FRUIT (FRESH OR FROZEN)
0,0	WHO regional European diet	0,0	Bovine: Liver	0,0	Bovine: Kidney		FRUIT (FRESH OR FROZEN)

Table 2.7.9-2: IESTI calculation linked to EU representative uses

Acute risk assessment /children - refined calculations						**Acute risk assessment / adults / general population - refined calculations**					
The acute risk assessment is based on the ARfD.											
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
No of commodities for which ARfD/ADI is exceeded (IESTI 1):		**1**	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		**---**	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		**---**	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		**---**
IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
138,6	Kale	2,05 / 1,47	99,0	Kale	2,05 / -	41,8	Kale	2,05 / -	37,8	Table grapes	1,19 / -
77,9	Table grapes	1,19 / -	77,9	Table grapes	1,19 / -	37,8	Table grapes	1,19 / -	31,0	Kale	2,05 / -
13,7	Apples	0,14 / -	10,1	Apples	0,14 / -	6,3	Wine grapes	0,265 / -	6,3	Wine grapes	0,265 / -
12,7	Pears	0,14 / -	9,2	Pears	0,14 / -	5,1	Head cabbage	0,16 / -	3,0	Head cabbage	0,16 / -
9,1	Melons	0,06 / -	9,1	Melons	0,06 / -	3,1	Apples	0,14 / -	2,6	Apples	0,14 / -

Processed commodities											
	No of commodities for which ARfD/ADI is exceeded:		**---**					**No of commodities for which ARfD/ADI is exceeded:**		**---**	
			*****)**							*****)**	
	Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)					Highest % of ARfD/ADI	Processed commodities	pTMRL/ threshold MRL (mg/kg)	
	0,9	Apple juice	0,018 / -					0,2	Quince jelly	0,2 / -	
	0,7	Tomato juice	0,04 / -					0,1	Apple juice	0,018 / -	
	0,3	Pear juice	0,018 / -					0,1	Tomato (preserved-	0,04 / -	
	0,3	Grape juice	0,009275 / -					0,0	Wine	0,009275 / -	
	0,1	Potato puree (flakes)	0,01 / -					0,0	Raisins	0,0486 / -	

Separate risk assessment for metabolite SYN547897 (TTC approach)

Acute risk assessment /children						Acute risk assessment / adults / general population					
The acute risk assessment is based on the ARfD.											
For each commodity the calculation is based on the highest reported MS consumption per kg bw and the corresponding unit weight from the MS with the critical consumption. If no data on the unit weight was available from that MS an average European unit weight was used for the IESTI calculation.											
In the **IESTI 1** calculation, the variability factors were 10, 7 or 5 (according to JMPR manual 2002), for lettuce a variability factor of 5 was used. In the **IESTI 2** calculations, the variability factors of 10 and 7 were replaced by 5. For lettuce the calculation was performed with a variabilty factor of 3.											
Threshold MRL is the calculated residue level which would leads to an exposure equivalent to 100 % of the ARfD.											
No of commodities for which ARfD/ADI is exceeded (IESTI 1):		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 1):**		---	**No of commodities for which ARfD/ADI is exceeded (IESTI 2):**		---
IESTI 1	***)**	****)**	**IESTI 2**	***)**	****)**	**IESTI 1**	***)**	****)**	**IESTI 2**	***)**	**)
Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)	Highest % of ARfD/ADI	Commodities	pTMRL/ threshold MRL (mg/kg)
2,6	Bovine: Liver	0,016 / -	2,6	Bovine: Liver	0,016 / -	0,9	Bovine: Liver	0,016 / -	0,9	Bovine: Liver	0,016 / -
1,2	Bovine: Kidney	0,016 / -	1,2	Bovine: Kidney	0,016 / -	0,5	Bovine: Kidney	0,016 / -	0,5	Bovine: Kidney	0,016 / -
0,3	Swine: Kidney	0,01 / -	0,3	Swine: Kidney	0,01 / -	0,3	Swine: Kidney	0,01 / -	0,3	Swine: Kidney	0,01 / -
0,2	Swine: Liver	0,01 / -	0,2	Swine: Liver	0,01 / -	0,1	Sheep: Liver	0,01 / -	0,1	Sheep: Liver	0,01 / -
						0,1	Swine: Liver	0,01 / -	0,1	Swine: Liver	0,01 / -

2.7.10 Proposed MRLs and compliance with existing MRLs

Considering available residue trials and livestock feeding studies, MRLs related to EU intended uses can be proposed and are summarized below.

Table 2.7.10-1: proposed MRL for EU representative uses

Commodities	Proposed MRL	Comments
Apple	0,20 mg/kg	
Pear	0,20 mg/kg	
Table grape	2 mg/kg	
Wine grape	2 mg/kg	
Potatoes	0,01* mg/kg	MRL derived based on Northern trials
Tomato	0,15 mg/kg	
Cucumber	0,15 mg/kg	
Courgette	0,15 mg/kg	
Melon	0,10 mg/kg	
Watermelon	0,10 mg/kg	
Broccoli	0,15 mg/kg	
Cauliflower	0,07 mg/kg	
Head cabbage	0,20 mg/kg	
Brussels sprout	0,30 mg/kg	
Kales	An acute risk has been identified on kales	
Kohlrabi	0,20 mg/kg	
Ruminant muscle	0.02* mg/kg	
Ruminant fat	0.02* mg/kg	
Ruminant liver	0.02* mg/kg	
Ruminant kidney	0.02* mg/kg	
Milk	0.02* mg/kg	
Poultry muscle	0.02* mg/kg	
Poultry fat	0.02* mg/kg	
Poultry liver	0.02* mg/kg	
Poultry kidney	0.02* mg/kg	
Eggs	0.02* mg/kg	

2.7.11 Proposed import tolerances and compliance with existing import tolerances

See Appendix to the Volume 3 B7-CA of the DAR.

2.8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

2.8.1 Summary of fate and behaviour in soil

Route of degradation

The fate and behaviour of PYDIFLUMETOFEN (SYN545974) in soils was investigated using both [^{14}C]-phenyl labelled and [^{14}C]-pyrazole labelled test substance.

The degradation of PYDIFLUMETOFEN (SYN545974) under dark, aerobic laboratory soil was investigated in five soils. Degradation was slow and no metabolites were observed at levels ≥5% of applied radioactivity. Levels of evolved carbon dioxide ($^{14}CO_2$) reached 0.2% to 16.5% AR by the end of the aerobic soil incubations at 365 DAT and unextracted residues increased slowly to between 5.2% AR and 14.9% AR at 365 DAT.

The degradation of PYDIFLUMETOFEN (SYN545974) under anaerobic laboratory soil conditions was also very slow. The study was conducted with four soils, with a preliminary aerobic incubation of 30 days before flooding the test soil samples. No novel metabolites were identified or formed at ≥5% AR during the anaerobic incubation. Mineralisation to carbon dioxide ($^{14}CO_2$) was negligible in all soils, reaching a maximum of <1% AR by the end of soil incubations (120 days). Unextracted residues increased slowly to between 4.8% AR and 10.0% AR at 120 DAT.

In a laboratory soil photolysis study PYDIFLUMETOFEN (SYN545974) degraded relatively slowly in both dry and moist soil. No novel metabolites were identified or formed at ≥5% AR.

The enantiomeric composition of PYDIFLUMETOFEN (SYN545974) in soils was determined at the end of the aerobic and anaerobic incubations and at the end of the irradiation period in the soil photolysis study compared to the ratio in the PYDIFLUMETOFEN (SYN545974) application solutions. The PYDIFLUMETOFEN (SYN545974) enantiomer did not change significantly over the course of these degradation studies.

Rate of degradation

The rate of degradation of PYDIFLUMETOFEN (SYN545974) in standard dark aerobic laboratory studies has been determined in five different soil types at 20°C, pF2. DT50 values were calculated both based on residues from non-harsh extractions (option supported by the applicant) and based on residues including harsh extractions (following RMS request). Only the latter ones are presented below, however Member States and EFSA are kindly invited to comment on this point (please refer to Vol. 3 B8 (AS) for further details). An expert discussion on the appropriate solvent extraction systems for determining route and rate of degradation in soil is proposed.

SYN545192 does not degrade significantly, and trigger DT50 values (based on residues including harsh extractions) range from 469 to 4170 days, with DT_{90} values ranging from 1560 to >10000 days. Modelling $DegT_{50}$ values range from 469 to 4170 days, with a geometric mean of 1440 days.

Field soil studies were performed at six European locations across north and south Europe. PYDIFLUMETOFEN (SYN545974), as the SC formulation A19649B, was applied at 204 g a.s./ha to bare soil. The treated plots were covered with a thin layer of sand immediately after application to minimise the potential impact of surface processes on dissipation. Soil core samples were taken to a depth of up to 100 cm and analysed for residues of PYDIFLUMETOFEN (SYN545974). At the end of the sampling period, after approximately two years, total soil residues of PYDIFLUMETOFEN (SYN545974) at the six trial locations had dissipated by 38% to 76%, based on the nominal application rate. The enantiomeric composition of PYDIFLUMETOFEN (SYN545974) did not change significantly during the field soil dissipation studies.

Trigger field DT_{50} values for PYDIFLUMETOFEN (SYN545974) range from 29 to 8540 days, with $DegT_{90}$ values ranging from 1820 to >10000 days. Although the results of this kinetic analysis indicate that dissipation of PYDIFLUMETOFEN (SYN545974) from soil was slow it should be noted that these studies were designed according to the guidance of EFSA (2014) and as such losses *via* surface processes such as photolysis and volatilisation were minimised and the dissipation of PYDIFLUMETOFEN (SYN545974) occurred solely as a result of microbial degradation. The field soil DegT50matrix values for PYDIFLUMETOFEN (SYN545974) corrected to the standard conditions of 20ºC and moisture at 10 kPa (pF2) range from 654 to 3210 days, with a geometric mean of 1334 days.

According to EFSA (2014), since geomean laboratory DT50 is longer than 240 days and since at least 4 field $DegT50_{matrix}$ are available, the geomean of field DegT50matrix values should be used for environmental exposure modelling.

Mobility

Adsorption coefficients for PYDIFLUMETOFEN (SYN545974) were determined in 6 soils using the batch equilibrium method. K_{FOC} values ranged from 1165 to 2206 mL/g (geomean: 1706 mL/g) and 1/n ranged from 0.84 to 0.90 (arithmetic mean: 0.88). There is no indication of a relationship between soil adsorption of PYDIFLUMETOFEN (SYN545974) and soil pH. Using the McCall Classification scale, PYDIFLUMETOFEN (SYN545974) can be classified as having a low to slight potential mobility in soil.

Adsorption coefficients were also determined for the 2 water metabolites SYN545547 and NOA449410 in 5 soils, using the batch equilibrium method.
For SYN545547, K_{FOC} values ranged from 323 to 759 mL/g (geomean: 608 mL/g) and 1/n ranged from 0.84 to 0.90 (arithmetic mean: 0.86). There is no indication of a relationship between soil adsorption of SYN545547 and soil pH. Using the McCall Classification scale, SYN545547 can be classified as having a low to medium potential mobility in soil.
For NOA449410, K_{FOC} values ranged from 0.3 to 6.1 mL/g (geomean: 2.1 mL/g) and 1/n ranged from 0.78 to 1.02 (arithmetic mean: 0.90). There is no indication of a relationship between soil adsorption of NOA449410 and soil pH. Using the McCall Classification scale, NOA449410 can be classified as having a very high potential mobility in soil.

Column leaching studies, aged residue column leaching studies and lysimeter studies were not conducted since reliable adsorption coefficient values could be obtained from the adsorption/desorption studies reported. No studies are required.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

The fate and behaviour of PYDIFLUMETOFEN (SYN545974) in water was investigated using both [^{14}C]-phenyl labelled and [^{14}C]-pyrazole labelled test substance, except for hydrolysis which was studied with [^{14}C]-pyrazole labelled test substance only.

PYDIFLUMETOFEN (SYN545974) was stable to hydrolysis under acidic, neutral and alkaline conditions at 50°C. It is therefore expected to be stable at 25°C.
Aqueous photolysis of PYDIFLUMETOFEN (SYN545974) was studied in pH7 buffer (direct photolysis) and in natural water (indirect photolysis). PYDIFLUMETOFEN (SYN545974) was degraded, primarily by dechlorination and phenyl ring degradation to produce phenyl-hydroxylated metabolites, carboxylic acid metabolites and carbon dioxide. Estimated DT50 were 93 and 35 days (summer sunlight 30-50°N) in pH 7 buffer and natural water, respectively. No photo-degradates reached levels ≥ 5% AR via direct photolysis. Photolysis in natural water led to the formation of SYN548261 at ≥ 5% AR at two consecutive sampling intervals (maximum 7.3% AR after 21 days) and NOA449410 at a maximum level of 5.8% AR by the end of the experimental period (30 days).

PYDIFLUMETOFEN (SYN545974) was not considered readily biodegradable under the conditions of the available 28-day ready biodegradability test. In addition, results from hydrolysis and water/sediment studies show that PYDIFLUMETOFEN (SYN545974) is not degraded in the aquatic environment to a level > 70 % within a 28-day period. As a consequence, PYDIFLUMETOFEN (SYN545974) is considered not rapidly degradable.

The aerobic mineralisation and degradation of PYDIFLUMETOFEN (SYN545974) in surface water was determined in the laboratory under dark conditions and light/dark conditions. No significant degradation of PYDIFLUMETOFEN (SYN545974) was observed throughout the study. Mineralization was low (< 1%) in all systems tested. DT50 were extrapolated beyond the study period in all incubation groups and ranged from 637 to >1000 days for dark incubation and from 402 to 662 days for light/dark incubation.

The rate and route of degradation of [^{14}C]-SYN545974 has been investigated in two water-sediment systems under laboratory aerobic and anaerobic conditions in the dark.
In the aerobic systems 70-74% of applied PYDIFLUMETOFEN (SYN545974) remained in the total systems after 100 days (end of study). Only one metabolite was observed at levels above 5% AR and this was identified as

SYN545547. It increased throughout the duration of the study and accounted for up to 12.3% AR in sediment extracts and 12.8% AR in the total system after 100 days.
In the anaerobic systems, 54-64% of applied PYDIFLUMETOFEN (SYN545974) remained in the total systems after 100 days. As in the aerobic systems, the only metabolite exceeding 5% of applied radioactivity was SYN545547. It increased throughout the duration of the study and accounted for up to 26.5% AR in sediment extracts, 10.8% in water and 32.4% AR in the total system after 100 days.

The rate of degradation of PYDIFLUMETOFEN (SYN545974) and its metabolite SYN545547 in aquatic systems were assessed from the data from the aerobic water-sediment study according to FOCUS guidance on degradation kinetics (FOCUS 2006, 2011). The persistence endpoints for PYDIFLUMETOFEN (SYN545974) were $DegT_{50}$ 270-299 days ($DegT_{90}$ 976-1100 days) for degradation in the whole system and DT_{50} 0.74-8.03 days ($DegT_{90}$ 33.1-86.9 days) for dissipation in the water column. The modelling endpoints for PYDIFLUMETOFEN (SYN545974) ranged from 244 to 252 days (geometric mean $DegT_{50}$ 248 days) for degradation in the whole system. For the metabolite SYN545547, persistence endpoints were $DegT_{50}$ 18.6-455 days ($DegT_{90}$ 61.9-1510 days). The modelling whole system degradation endpoints ranged from 18.6 to 455 days (geometric mean $DegT_{50}$ 92.0 days).

The enantiomeric composition of PYDIFLUMETOFEN (SYN545974) in water was determined at the end of the aerobic mineralization study, at the end of the aerobic and anaerobic incubations in water/sediment studies, and at the end of the irradiation period in the water photolysis study compared to the ratio in the PYDIFLUMETOFEN (SYN545974) application solutions. The PYDIFLUMETOFEN (SYN545974) enantiomer did not change significantly over the course of these degradation studies.

Satisfactory information was not available to address the effect of water treatment processes on the nature of the residues that might be present in water when it is abstracted for drinking water. A data gap has been identified.

2.8.2.1 Rapid degradability of organic substances

Table 53: Summary of relevant information on rapid degradability

Method	Results	Key or Supportive study	Remarks	Reference
OECD 301 F	After 28 days: $ThOD_{NO3}$ = 3.4% $ThOD_{NH3}$ = 4.7%	Key study	-	Simon, M., (2015)

2.8.2.1.1 Ready biodegradability

The ready biodegradability of PYDIFLUMETOFEN (SYN545974) was determined in Simons 2015 by observing the BOD (biochemical oxygen demand, OECD 301F) using manometric methods over 28 days at 22°C in the dark. An inoculum control and a procedure control as well as toxicity controls were incubated for 28 days in the darkness at 22°C. Aerobic activated sludge from a waste water treatment plant treating predominantly domestic wastewater was used as the inoculum. As a procedure control, the reference item sodium benzoate was tested. The toxicity control contained both test material and the reference item sodium benzoate.

The percentage biodegradation of test material and of the reference item sodium benzoate was calculated based on their biochemical oxygen demand (BOD) and theoretical oxygen demand (ThOD). Since the test item contains nitrogen, the % biodegradation was calculated based on the $ThOD_{NH4}$ (considering that nitrification is absent) and $ThOD_{NO3}$ (considering that nitrification is complete). No significant biological oxygen demand was observed and consequently the effects of nitrification did not need to be considered.

Biodegradation in sludge exposed to the test item
The biochemical oxygen demand (BOD) of the test item PYDIFLUMETOFEN (SYN545974) in the test media was in the range of the inoculum controls throughout the study period of 28 days. Consequently, PYDIFLUMETOFEN (SYN545974) was not biodegradable under the test conditions within 28 days.

Biodegradation of the reference item in the procedure controls
In the procedural controls, the reference item was degraded by an average of 81% by Exposure Day 14, thus confirming suitability of the activated sludge. At the end of the test (Day 28), the reference item was degraded by an average of 84%.

Biodegradation in the toxicity control
In the toxicity control containing both the test item PYDIFLUMETOFEN (SYN545974) and the reference item the course of oxygen consumption over the 28 day exposure period was similar to the two procedure controls, containing only the reference item. Within 14 days of exposure, biodegradation amounted to 58% based on the $ThOD_{NO3}$ and to 64% based on the $ThOD_{NH3}$.
Thus, according to the test guidelines, the test item had no inhibitory effect on activated sludge microorganisms at the tested concentration of 44 mg/L because biodegradation in the toxicity control was >25% within 14 days.

2.8.2.1.2 BOD5/COD

No data available.

2.8.2.2 Other convincing scientific evidence

2.8.2.2.1 Aquatic simulation tests

Please refer to 2.8.2.

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

No data available.

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Please refer to 2.8.2.1.1.

2.8.2.2.4 Soil and sediment degradation data

Please refer to 2.8.1 for soil degradation and to 2.8.2 for sediment degradation (water/sediment systems).

2.8.2.2.5 Hydrolysis

Please refer to 2.8.2.

2.8.2.2.6 Photochemical degradation

Please refer to 2.8.2.

2.8.2.2.7 Other / Weight of evidence

No additional data available.

2.8.3 Summary of fate and behaviour in air

PYDIFLUMETOFEN (SYN545974) has a vapour pressure of 1.84×10^{-7} Pa at 20°C. According to FOCUS Air guidance criteria, significant volatilisation of PYDIFLUMETOFEN (SYN545974) is therefore unlikely to occur.

The reaction of PYDIFLUMETOFEN (SYN545974) in the atmosphere with hydroxyl radicals has been estimated using the method of Atkinson as developed in the Atmospheric Oxidation Program v1.91.
The estimated half-life of PYDIFLUMETOFEN (SYN545974) in the atmosphere (by hydroxyl radical oxidation) is 5.85 hours, based on OH (12h) concentration of 1.5×10^6 radicals/cm^3 as recommended in FOCUS Air guidance document. PYDIFLUMETOFEN (SYN545974) is therefore not expected to be persistent in air and is unlikely to be subject to significant concerns relating to long range atmospheric transport and atmospheric accumulation.

Based on the available data, PYDIFLUMETOFEN (SYN545974) is unlikely to undergo significant volatilisation and any residues reaching air will be rapidly degraded. Therefore the compound will not be subject to significant concerns related to long range atmospheric transport and atmospheric accumulation.

2.8.3.1 Hazardous to the ozone layer

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone

layer

Based on the available data presented under 2.8.3, there is no evidence that PYDIFLUMETOFEN (SYN545974) may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

2.8.3.1.2 Comparison with the CLP criteria

Based on the available data presented under 2.8.3, there is no evidence that PYDIFLUMETOFEN (SYN545974) may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Based on the available data presented under 2.8.3, there is no evidence that PYDIFLUMETOFEN (SYN545974) may present a danger to the structure and/or the functioning of the stratospheric ozone layer.

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

PYDIFLUMETOFEN (SYN545974) is a new active substance and therefore monitoring data are not available. No monitoring data relative to metabolites was provided.

2.8.5 Definition of the residues in the environment requiring further assessment

The following residue definition for risk assessment in environmental compartments is proposed:

Soil: PYDIFLUMETOFEN (SYN545974)

Groundwater: PYDIFLUMETOFEN (SYN545974)

Surface water: PYDIFLUMETOFEN (SYN545974), SYN548261 and NOA449410

Sediment: PYDIFLUMETOFEN (SYN545974), SYN545547

Air: PYDIFLUMETOFEN (SYN545974)

2.8.6 Summary of exposure calculations and product assessment

Exposure calculations were performed for the intended uses of A19649B, a suspension concentrate (SC) containing 200 g/L PYDIFLUMETOFEN (SYN545974).

Soil

PECsoil were calculated for the formulation A19649B and the active substance PYDIFLUMETOFEN (SYN545974) according to FOCUS recommendations (FOCUS 1997, FOCUS 2014) for all the intended uses. For PYDIFLUMETOFEN (SYN545974), the longest laboratory best-fit DT_{50} at 20°C was used. Since PYDIFLUMETOFEN (SYN545974) is persistent in soil, PECplateau values were also calculated.

PECsoil are available in Volume 3 B.8 (PPP) under B.8.2.

Groundwater

PECgw were calculated for the intended uses of A19649B for PYDIFLUMETOFEN (SYN545974) according to FOCUS recommendations (FOCUS 2000, FOCUS 2014, European Commission 2014) at Tier 1 of the tiered assessment scheme proposed by the FOCUS groundwater higher tier working group. The models FOCUS PELMO 5.5.3, FOCUS PEARL 4.4.4 and FOCUS MACRO 5.5.4 were used. Annual applications of A19649B were considered.

PECgw for PYDIFLUMETOFEN (SYN545974) were calculated using both laboratory and field DT50. However according to EFSA (2014), the geomean of field DegT50matrix is suitable for environmental exposure modelling. Only results from the simulations performed with the field DT50 are presented below.

PECgw for PYDIFLUMETOFEN (SYN545974) are < 0.1 µg/L in all scenarios for all intended uses (please refer to Volume 3 B.8 (PPP) under B.8.3). Therefore the potential for groundwater exposure by PYDIFLUMETOFEN (SYN545974) above the parametric drinking water limit of 0.1 µg/L from the representative uses is expected to be low in geoclimatic situations that are represented by the relevant FOCUS groundwater scenarios.

Surface water and sediment

PECsw and PECsed for the intended uses of A19649B were calculated for PYDIFLUMETOFEN (SYN545974) and its metabolites according to FOCUS recommendations (FOCUS 2001, FOCUS 2015), considering the entry routes spray drift, drainage and runoff.

FOCUS Step 1-2 calculations were performed for PYDIFLUMETOFEN (SYN545974) and its metabolites SYN548261, NOA449410 and SYN545547 using the tool STEPS 1-2 in FOCUS version 3.2.

Further calculations were performed in FOCUS Step 3 for PYDIFLUMETOFEN (SYN545974) using the software package FOCUS SWASH 5.3, including FOCUS MACRO 5.5.4, FOCUS PRZM 4.3.1 and FOCUS TOXSWA 4.4.

No mitigation measures were implemented.

PECsw are available in Volume 3 B.8 (PPP) under B.8.5. It is highlighted that the calculations provided for the use pome fruits cover the application period from BBCH 70. Additional calculations should be provided by the applicant to cover the whole intended application period (BBCH 56-79) for PYDIFLUMETOFEN (SYN545974) and its metabolites in Step 1-2 and for PYDIFLUMETOFEN (SYN545974) in Step 3. For potatoes, for multiple applications on potatoes with late application window, the interval between applications was erroneously set to 1 day in Step 3. Updated PECsw calculations should be provided by the applicant for this use.

Air

Based on the available data, PYDIFLUMETOFEN (SYN545974) is unlikely to undergo significant volatilisation and any residues reaching air will be rapidly degraded. Therefore the compound will not be subject to significant concerns related to long range atmospheric transport and atmospheric accumulation. No PEC calculations are considered necessary.

Other routes of exposure

No other routes of exposure were identified.

2.9 EFFECTS ON NON-TARGET SPECIES

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Table 54: Summary of PYDIFLUMETOFEN (SYN545974) and A19649B toxicity endpoints for birds

Test type	Test substance	Test species	Endpoint	Value (ppm a.s.)	Value (mg a.s./kg bw/d)	Reference (Author, date, Syngenta File No.)
Acute oral	PYDIFLUMETOFEN (SYN545974)	Bobwhite quail (*Colinus virginianus*)	LD_{50}	-	>2 000 [a]	2013 SYN545974_10062
		Canary (*Serinus canaria*)	LD_{50}	-	>2 000 [a]	2013a SYN545974_10065
	A19649B	Bobwhite quail (*Colinus virginianus*)	LD_{50}	>2 000[a]	>372	2014 A19649B_10017
Short-term dietary	PYDIFLUMETOFEN (SYN545974)	Bobwhite quail (*Colinus virginianus*)	LC_{50}	>5 620	>1 258	2013 SYN545974_10063

Test type	Test substance	Test species	Endpoint	Value (ppm a.s.)	Value (mg a.s./kg bw/d)	Reference (Author, date, Syngenta File No.)
		Mallard duck (*Anas platyrhynchos*)	LC_{50}	>5 620	>2 437	2013a SYN545974_10064
Sub-chronic and reproduction	PYDIFLUMETOFEN (SYN545974)	Bobwhite quail (*Colinus virginianus*)	NOEC	1 000	90.1	2015 SYN545974_10130
		Mallard duck (*Anas platyrhynchos*)	NOEC	1 000	141	2014 SYN545974_10134

[a] Conducted following test guideline OECD 223.

Table 55: Summary of PYDIFLUMETOFEN (SYN545974) and A19649B toxicity endpoints to mammals

Test substance	Test type	Test species	Endpoint	Value	Reference (Author, date, Syngenta File No.)
PYDIFLUMETOFEN (SYN545974)	Acute oral	Rat	LD_{50}	>5 000 mg a.s./kg bw	2012 SYN545974_10043
	2 generation	Rat	NOAEL	36 mg a.s./kg bw/d	2015 SYN545974_10177
A19649B	Acute oral	Rat	LD_{50}	2 958 mg A19649B/kg bw	2013 A19649B_10003

The risk assessments for birds and mammals were conducted in accordance with EFSA guidance (European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438).

Endpoints retained for the risk assessment are acute LD50 = 3776 mg/kg b.w. (extrapolation of >2000 x 1.888 factor for studies with no effect of the limit tested concentration), and NOEC = 90.1 mg/kg b.w./d for birds.

In a first screening step risk assessment, the acute TER value is higher than the trigger value of 10 for small omnivorous and insectivorous birds indicating an acceptable acute risk for birds.
In a first screening step risk assessment, the long term TER value is higher than the trigger value of 5 for small omnivorous and insectivorous birds indicating an acceptable long term risk for birds.

As the ratio of effective application rate (400 g/ha) to the long-term endpoint (90.1 mg/kg bw/d) does not exceeds the trigger ratio of 3000 for less sorptive substances ($K_{oc} \geq 500$ L/kg), no unacceptable risks are expected and further calculations are not required.

Due to the log K_{OW} (3.8 > 3) of pydiflumetofen, the risk to birds through secondary poisoning had be assessed.
The TER value for pydiflumetofen exceeds the long-term trigger value of 5, indicating that the risk to earthworm eating birds and to fish-eating birds is acceptable following use of PYDIFLUMETOFEN (SYN545974) according to the proposed use pattern.

Endpoints retained for the risk assessment are acute LD50 >5000 mg/kg b.w. and NOAEL = 36 mg/kg b.w./d for mammals.

In a first screening step risk assessment, the acute TER value is higher than the trigger value of 10 for small herbivorous mammal indicating an acceptable acute risk for mammals.
In a first screening step risk assessment, the long term TER value is higher than the trigger value of 5 for small herbivorous mammal indicating an acceptable long term risk for mammals, except for the use on grapes. For the refined 1st Tier assessment (2 x 200 g a.s./ha on grapes with 14d interval for BBCH 67-89 and 2 x 40 g a.s./ha on grapes with 10d interval for BBCH 13-77), the long term TER value is higher than the trigger value of 5 for small and large herbivorous, small omnivorous, small insectivorous.

As the ratio of effective application rate (400 g/ha) to the long-term endpoint (36 mg/kg bw/d) does not exceeds

the trigger ratio of 3000 for less sorptive substances ($K_{oc} \geq 500$ L/kg), no unacceptable risks are expected and further calculations are not required.

Due to the log K_{OW} (3.8 > 3) of pydiflumetofen, the risk to mammals through secondary poisoning had be assessed.
The TER value for pydiflumetofen exceeds the long-term trigger value of 5, indicating that the risk to earthworm eating mammals and to fish-eating mammals is acceptable following use of PYDIFLUMETOFEN (SYN545974) according to the proposed use pattern.

2.9.2 Summary of effects on aquatic organisms

2.9.2.1 Bioaccumulation

Table 56: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioconcentration in Bluegill sunfish (*Lepomis macrochirus)* Nominal test conc. 4.9 µg/L, flow-through Uptake; 19 days Depuration; 7 days 23°C Test material; [Phenyl-U-^{14}C]SYN545974 (purity; 97.5%, radiochemical purity; 99.1%); Non-radiolabelled test material; PYDIFLUMETOFEN (SYN545974) (purity; 98.5%) Guidelines: OECD 305, OPPTS 850.1730 GLP	BCF_{SS} = 27.7 $BCF_{SS, Lipid Normalised}$ = 31.1 BCF_k = 168 $BCF_{k, lipid normalised}$ = 189	BCF based on measured test concentrations in water and whole fish tissue using LSC and HPLC/RAM Depuration half-life of accumulated residues was 0.52, 0.44 and 0.41 days for edible, non-edible and whole fish respectively	***Anonymous*** (2014)

2.9.2.1.1 Estimated bioaccumulation

The experimentally derived Log Kow of PYDIFLUMETOFEN (SYN545974) is 3.8 at 25°C. For classification and labelling purposes a substance with Log Kow <4 may be considered unlikely to bioaccumulate in aquatic organisms. Therefore, PYDIFLUMETOFEN (SYN545974) has a low potential for biocaccumulation.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

For pesticide registration, a Log Kow >3 triggers the requirement for a bioconcentration study. Since the Log Kow of PYDIFLUMETOFEN (SYN545974) is >3 a bioconcentration study has been conducted (***Anonymous*** 2014).
The bioconcentration factors $BCF_{SS, lipid-normalized}$ and $BCF_{k, lipid-normalized}$ for whole fish were 31.1 and 189, respectively. According to CLP criteria, a measured BCF≥500 indicates a potential for bioaccumulation. Since both BCF_{SS} and BCF_K are <500, PYDIFLUMETOFEN (SYN545974) is not considered to be bioaccumulative for the purpose of classification and labelling. Therefore, PYDIFLUMETOFEN (SYN545974) have a low potential for biocaccumulation

2.9.2.2 Acute aquatic hazard

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA panel on plant protection products and their residues (PPR). European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2013;11(7):3290.

The risk assessment for the active substance, the formulation and the metabolites was conducted with the FOCUS PECsw.

Table 57: Summary of relevant information on acute aquatic toxicity

Substance	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Fish						
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Lepomis macrochirus* (Bluegill Sunfish)	OECD 203, OPPTS 850.1075	96 hour LC_{50}	0.48 mg/L Mean measured	Flow-through Dilution water control used pH 7.1-7.4 21-22°C GLP	***Anonymous***, 2014 SYN545974_10129
			96 hour NOEC	0.2 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Cyprinus carpio* (Common carp)	OECD 203, OPPTS 850.1075	96 hour LC_{50}	0.33 mg/L Mean measured	Flow-through Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.2-7.4 22-23°C GLP	***Anonymous***, 2013a SYN545974_10066
			96 hour NOEC	0.13 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 99.5%)	*Oncorhynchus mykiss* (Rainbow Trout)	OECD 203, EC L142/446 C.1, OPPTS 850.1075	96 hour LC_{50}	0.18 mg/L Mean measured	Flow-through Dilution water and solvent (DMF 0.1 ml/L) control used. pH 6.7 – 7.4 14-16°C GLP	***Anonymous***, 2012 SYN545974_10014
			96 hour NOEC	0.12 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 99.5%)	*Pimephales promelas* (Fathead Minnow)	OECD 203, OPPTS 850.1075	96 hour LC_{50}	0.35 mg/L Mean measured	Flow-through Dilution water and solvent (DMF 0.1 ml/L) control used. pH 6.9 – 7.3 21-23°C GLP	***Anonymous***, 2013 SYN545974_10068
			96 hour NOEC	0.24 mg/L Mean measured		
PYDIFLUMETOFEN (SYN54597	*Cyprinodon variegatus* (Sheepshead	Guidelines: OECD 203,	96 hour LC_{50}	0.66 mg/L Mean measured	Flow-through Dilution	***Anonymous***, 2013b SYN545974

4) tech. (purity 98.5%)	Minnow)	OPPTS 850.1075	96 hour NOEC	0.48 mg/L Mean measured	water and solvent (DMF 0.1 ml/L) control used pH 7.7 – 7.8 22-23°C GLP	_10066
Aquatic invertebrates including sediment						
PYDIFLUMETOFEN (SYN545974) tech. (purity 99.5%)	*Daphnia magna* (water flea)	OECD 202, EC L142/456 C.2, OPPTS 850.1010	48 hour EC_{50}	0.42 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 8.0 – 8.3 20-21°C GLP	Fournier, 2012a SYN545974_10016
			48 hour NOEC	0.057 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Asellus aquaticus*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	4.21 mg/L Mean measured (immobility)	Static Negative and solvent (DMF 0.1 ml/L) control used. pH 8.03 – 8.29 19.8-20.7°C GLP	Pickering, 2015 SYN545974_10305
			48 hour NOEC	3.07 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Chaoborus crystallinius*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	2.49 mg/L Mean measured (mobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used pH 7.7 – 8.38 18.4-20.4°C GLP	Joyce, 2015 SYN545974_10341
			48 hour NOEC	0.333 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Chironomus riparius*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	0.69 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used pH 7.7 – 8.38 19.5 - 21.6°C GLP	Joyce, 2015a SYN545974_10316 Pickering, 2015a SYN545974_10315
			48 hour NOEC	0.351 mg/L Mean measured (immobility)		

PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Cloeon dipterum*	No specific guideline but OECD 202 was consulted	48 hour NOEC	5.01 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. No significant dose response therefore an EC50 could not be determined. pH 7.73 – 8.25 18.1 - 20.6°C GLP	Pickering, 2015a SYN545974_10315
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Crangonx pseudogracilis*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	1.23 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.73 – 8.25 18.1 - 20.6°C GLP	Pickering, 2015b SYN545974_10306
			48 hour NOEC	0.333 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Cyclops agilis speratus*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	4.17 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.66 – 8.49 18.4 – 21.9°C GLP	Joyce, 2015b SYN545974_10347
			48 hour NOEC	1.94 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Lumbriculus variegatus*	No specific guideline but OECD 202 was consulted	48 hour EC_{50}	4.65 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.93 – 8.57 16.2 – 23.3°C GLP	Pickering, 2015c SYN545974_10304
			48 hour NOEC	3.14 mg/L Mean measured (immobility)		

PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Lymnaea stagnalis*	No specific guideline but OECD 202 was consulted	48 hour NOEC	7.3 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.89 – 8.68 19.2 – 20.4°C GLP Only a single concentration tested therefore an EC50 could not be determined	Pickering, 2015d SYN545974_10303
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Hyalella azteca*	OECD 202, OPPTS 850.1010	48 hour LC_{50}	0.12 mg/L Mean measured (immobility)	Static Dilution water control used. pH 8.2 – 8.5 22.6 – 24.7°C GLP	Brougher *et al*. 2015 SYN545974_10354
			48 hour NOEC	0.009 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 99.5%)	*Americamysis bahia* (Mysid)	OPPTS 850.1035, OPPTS 850.1000	96 hour LC_{50}	0.16 mg/L Mean measured (immobility)	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.8 - 8.2 24 - 25°C GLP	Fournier, 2012b SYN545974_10015
			96 hour NOEC	0.11 mg/L Mean measured (immobility)		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Crassostrea virginica* (Eastern Oyster)	OPPTS 850.1025	96 hour EC_{50}	0.31 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.5 - 8.1 21 - 23°C GLP	Fournier, 2014a SYN545974_10099
PYDIFLUMETOFEN (SYN545974) tech.	*Leptocheirus plumulosus* (amphipod)	OPPTS 850.1740	10 day LC_{50}	>92 mg/kg Mean measured	Static Negative and solvent (acetone)	Bradley, 2015b SYN545974_50120

(purity 98.5%)			10 day NOEC	46 mg/kg Mean measured (mortality)	control used. pH (water) 7.8 - 8.4 24 - 26°C GLP	
Algae and aquatic plants						
PYDIFLU METOFEN (SYN545974) tech. (purity 99.5%)	*Pseudokirchneriella subcapitata* (Freshwater Green Alga)	OECD 201, OPPTS 850.5400, EC 761/2009 C.3	72 hour E_rC_{50}	>5.9 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH at start 7.3 – 7.5 pH at end 7.9 – 9.6 23 – 24°C GLP	Kirkwood, 2013 SYN545974 _10013
			72 hour E_yC_{50}	3.6 mg/L Mean measured		
			72 hour E_bC_{50}	4.3 mg/L Mean measured		
			72 hour NOEC 96 hour NOE_rC	0.9 mg/L (for all endpoints) Mean measured 0.093 mg/L		
PYDIFLU METOFEN (SYN545974) tech. (purity 98.5%)	*Skeletonema costatum* (Marine diatom)	OECD 201, OPPTS 850.5400	72 hour E_rC_{50}	2.7 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH at start 7.8 – 8.1 pH at end 7.7 – 8.5 20 – 22°C GLP	Soucy, 2014 SYN545974 _10105
			72 hour E_yC_{50}	2.7 mg/L Mean measured		
			72 hour E_bC_{50}	2.7 mg/L Mean measured		
			72 hour NOE_rC	2.4 mg/L (for all endpoints) Mean measured		
PYDIFLU METOFEN (SYN545974) tech. (purity 98.5%)	*Anabaena flos-aquae* (Freshwater Blue-Green Alga)	OECD 201, OCSPP 850.4550	72 hour E_rC_{50}	3.6 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control	Soucy, 2013 SYN545974 _10091
			72 hour E_yC_{50}	3.5 mg/L Mean measured		

			72 hour E_bC_{50}	3.6 mg/L Mean measured	used. pH at start 7.0 – 7.2 pH at end 7.5 – 9.6 23 – 25°C GLP	
			72 hour NOE_rC	2.7 mg/L (for all endpoints) Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Navicula pelliculosa* (Freshwater Diatom)	OECD 201, OCSPP 850.4550	72 hour E_rC_{50}	1.6 mg/L Mean measured	Static Dilution water and solvent (DMF 0.1 ml/L) control used. pH at start 7.3 – 7.6 pH at end 7.3 – 8.7 24 – 26°C GLP	Soucy, 2015 SYN545974_10097
			72 hour E_yC_{50}	1.5 mg/L Mean measured		
			72 hour E_bC_{50}	1.5 mg/L Mean measured		
			72 hour NOE_rC	0.89 mg/L (for all endpoints) Mean measured		
PYDIFLUMETOFEN (SYN545974) tech. (purity 98.5%)	*Lemna gibba* (Duckweed)	OECD 221 OPPTS 850.4400	7 day E_rC_{50}	>6.3 mg/L (for all endpoints) Mean measured	Semi-static Dilution water and solvent (DMF 0.1 ml/L) control used. pH 7.8 – 8.2 (new solns.) pH 8.4 – 9.0 (aged solns.) 24 – 25°C GLP Results are based on frond no. and dry wt	Soucy, 2015a SYN545974_10088
			7 day E_yC_{50}	>6.3 mg/L (for all endpoints) Mean measured		
			7 day NOE_rC	6.3 mg/L (for all endpoints) Mean measured		

2.9.2.2.1 Acute (short-term) toxicity to fish

Five studies on PYDIFLUMETOFEN (SYN545974) with supporting specific analysis showed short-term (96 hour) acute toxicity to fish across a range of species (see below). 96 hour LC_{50} values were within a factor of 4 with the lowest being for *Oncorhynchus mykiss* (LC_{50} = 0.18 mg/L).

Table 56: Summary of PYDIFLUMETOFEN (SYN545974) acute toxicity endpoints for fish

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Acute toxicity	PYDIFLUMETOFEN (SYN545974)	*Oncorhynchus mykiss* (Rainbow trout)	96 hour LC_{50} (flow-through)	0.18	***Anonymous***, 2012a SYN545974_10014
		Cyprinus carpio (Common carp)	96 hour LC_{50} (flow-through)	0.33	***Anonymous***, 2013a SYN545974_10066
		Pimephales promelas (Fathead minnow)	96 hour LC_{50} (flow-through)	0.35	***Anonymous***, 2013b SYN545974_10068
		Cyprinodon variegatus (Sheepshead minnow)	96 hour LC_{50} (flow-through)	0.66	***Anonymous***, 2013c SYN545974_10067
		Lepomis macrochirus (Bluegill sunfish)	96 hour LC_{50} (flow-through)	0.48	***Anonymous***, 2014a SYN545974_10129

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

Thirteen studies on PYDIFLUMETOFEN (SYN545974) with supporting specific analysis showed short-term (48 hour) acute toxicity to aquatic invertebrates across a range of species, including sediment dwelling species, with EC_{50}'s ranging from 0.12 to > 7.3 mg/L (see below). The lowest EC_{50} was for the freshwater amphipod *Hyalella Aztec* (48 hour EC_{50} = 0.12 mg/L).

Table 57: Summary of PYDIFLUMETOFEN (SYN545974) acute toxicity endpoints for aquatic invertebrates (including sediment dwelling species)

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Acute toxicity	PYDIFLUMETOFEN (SYN545974)	*Daphnia magna* (Water flea)	48 hour EC_{50} (static)	0.42	Fournier, 2012b SYN545974_10016
		Americamysis bahia (Mysid shrimp)	96 hour LC_{50} (static)	0.16	Fournier, 2012c SYN545974_10015
		Asellus aquaticus (Water louse)	48 hour EC_{50} (static)	4.21	Pickering, 2015a SYN545974_10305
		Chaoborus crystallinus (Phantom midge)	48 hour EC_{50} (static)	2.49	Joyce, 2015 SYN545974_10341
		Chironomus riparius (Non-biting midge / Harlequin fly)	48 hour EC_{50} (static)	0.69	Joyce, 2015a SYN545974_10316
		Cloeon dipterum (Mayfly)	48 hour EC_{50} (static)	>5.01	Pickering, 2015a SYN545974_10315
		Crangonx pseudogracilis (Freshwater amphipod)	48 hour EC_{50} (static)	1.23	Pickering, 2015b SYN545974_10306
		Crassostrea virginica (Eastern oyster)	96 hour EC_{50} shell deposition (flow through)	0.31	Fournier, 2014b SYN545974_10099

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
		Cyclops agilis speratus (Copepod)	48 hour EC_{50} (static)	4.17	Joyce, 2015c SYN545974_10347
		Hyalella Azteca (Freshwater amphipod)	48 hour LC_{50} (static)	0.12	Brougher *et al*, 2015 SYN545974_10354
		Lumbriculus variegatus (Blackworm)	48 hour EC_{50} (static)	4.65	Pickering, 2015c SYN545974_10304
		Lymnaea stagnalis (Great pond snail)	48 hour EC_{50} (static)	>7.30	Pickering, 2015e SYN545974_10303
		Leptocheirus plumulosus (Amphipod)	10 day LC_{50} (spiked sediment)	>92	Bradley, 2015b SYN545974_50120

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Four studies on PYDIFLUMETOFEN (SYN545974) with supporting specific analysis showed short-term (72 h) toxicity to a range of algae species, with 72 h EC_{50}'s for growth (E_rC_{50}) ranging from 1.6 to > 5.9 mg/L (see values in bold below). An additional study showed relatively lower short-term toxicity to Duckweed (*Lemna gibba*) (7 day EC_{50} > 6.3 mg/L). The lowest E_rC_{50} was for the freshwater diatom *Naviculla pelliculosa* (72 h E_rC_{50} = 1.6 mg/L).

Table 58: Summary of PYDIFLUMETOFEN (SYN545974) toxicity endpoints for algae and aquatic plants

Test type	Test item	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Algal toxicity	PYDIFLUMETOFEN (SYN545974)	*Pseudokirchneriella subcapitata* (Green alga)	72 hour E_bC_{50}	4.3	Kirkwood, 2013 SYN545974_10013
			72 hour E_yC_{50}	3.6	
			72 hour E_rC_{50}	**> 5.9**	
		Anabaena flos-aquae (Blue-green alga)	72 hour E_bC_{50}	3.6	Soucy, 2013 SYN545974_10091
			72 hour E_yC_{50}	3.5	
			72 hour E_rC_{50}	**3.6**	
		Naviculla pelliculosa (Diatom)	72 hour E_bC_{50}	1.5	Soucy, 2015a SYN545974_10097
			72 hour E_yC_{50}	1.5	
			72 hour E_rC_{50}	**1.6**	
		Skeletonema costatum (Diatom)	72 hour E_bC_{50}	2.7	Soucy, 2014 SYN545974_10105
			72 hour E_yC_{50}	2.7	
			72 hour E_rC_{50}	**2.7**	
Aquatic plant toxicity	PYDIFLUMETOFEN (SYN545974)	*Lemna gibba* (Duckweed)	7 day EC_{50} Fronds	>6.3	Soucy, 2015b SYN545974_10088
			7 day EC_{50} Dry weight	>6.3	

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

No additional data

2.9.2.3 Long-term aquatic hazard

Table 59: Summary of relevant information on chronic aquatic toxicity

Substance (purity)	Species	Test guidelines	Endpoint	Toxicity value	Conditions	Reference
Fish						
PYDIFLUMETOFEN (SYN545974) (purity 99.5%)	*Pimephales promelas* (Fathead Minnow)	OECD 210, OPPTS 850.1400, EC L.142/603, C.15	32 day NOEC (survival, mean length and mean dry weight)	0.025 mg/L Mean measured	Fish early life stage test 32 day (28 day post hatch) Flow-through Dilution water and solvent (DMF 0.004 ml/L) control used. pH 7.1-7.8 24-27°C GLP A statistically significant reduction in larval hatch at 0.064 mg/L was not biologically significant as it did not lead to significant effects on larval survival compared to control	***Anonymous***, 2015a SYN545974_10080
			32 day NOAEC (survival, mean length and mean dry weight)	0.064 mg/L Mean measured		
			32 day EC_{10} 32 day EC_{20} (Body length)	0.15 0.32 mg/L Mean measured		
			32 d EC_{10} (Body weight)	0.13 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) (purity 98.5%)	*Cyprinodon variegatus* (Sheepshead Minnow)	OECD 210, OPPTS 850.1400	32 day NOEC (survival, mean length and mean dry weight)	0.17 mg/L Mean measured	Fish early life stage test 34 day (28 day post hatch)	***Anonymous***, 2015b SYN545974_10293

			EC_{10} (Embryo hatching success)	0.34 mg/L Mean measured	Flow-through Dilution water control used. pH 7.2-8.0 25-26°C GLP	
Aquatic invertebrates including sediment						
PYDIFLUMETOFEN (SYN545974) (purity 99.5%)	*Daphnia Magna*	OECD 211, OPPTS 850.1300, EC L.142/674, C.20	21 day NOEC (survival, reproduction, growth)	0.042 mg/L Mean measured	Reproduction test 21 day Static-renewal pH 7.8-9.0 20-21°C Dilution water control used. GLP	Fournier, 2015 SYN545974_10017
			21 day EC_{10} 21 day EC_{20} (survival)	0.094 >0.31 mg/L Mean measured		
			21 day EC_{10} 21 day EC_{20} (reproduction)	0.085 0.13 mg/L Mean measured		
			21 day EC_{10} 21 day EC_{20} (body length)	0.21 >0.31 mg/L Mean measured		
			21 day EC_{10} 21 day EC_{20} (dry weight)	0.16 0.20 mg/L Mean measured		
PYDIFLUMETOFEN (SYN545974) (purity 98.5%)	*Chironomus dilutus*	OPPTS 850.1760, EPA Test method 100.4 (2000)	20 day EC_{50} (larval survival & growth)	> 93 mg/kg dry wt Mean measured	Life-Cycle Test 59 d Negative and solvent (acetone) control used. pH (water) 7.3-7.7 21-26°C GLP	Sayers, 2015b SYN545974_10293
			20 day EC_{10} 20 day EC_{20} (growth)	>93 >93 mg/kg dry wt Mean measured		
			20 day NOEC	15 mg/kg dry wt Mean measured		

			59 day EC_{50} (emergence & reproduction)	> 47 mg/kg dry wt Mean measured		
			59 day EC_{20} (% emergence)	22 mg/kg dry wt Mean measured		
			59 day EC_{10} 59 day EC_{20} (male/female emergence rate)	>93 >93 mg/kg dry wt Mean measured		
			59 day EC_{10} 59 day EC_{20} (male/female days to death)	>93 >93 mg/kg dry wt Mean measured		
			59 day EC_{10} 59 day EC_{20} (eggs per egg mass)	>93 >93 mg/kg dry wt Mean measured		
			59 day EC_{10} 59 day EC_{20} (% hatch)	30 49 mg/kg dry wt Mean measured		
			59 day NOEC (emergence)	15 mg/kg dry wt Mean measured		
PYDIFLUMETOFEN (SYN545974) (purity 98.5%)	*Hyalella azteca* (Freshwater Amphipods)	OPPTS 850.1770, EPA Test method 100.4	For survival, 28, 35 and 42 days; LC_{50} LC_{20} 42 day NOEC	>88 mg/kg >88 mg/kg 7.6 mg/kg Mean measured	Life cycle test 42 day Negative and solvent (acetone) control used. pH (water)	Bradley, 2015c SYN545974_10094

			For growth; 28 day EC_{50} 28 day EC_{20} 28 day EC_{10} 28 day NOEC 42 day EC_{50} 42 day EC_{20} 42 day EC_{10} 42 day NOEC	 >88mg/kg >88 mg/kg >88 mg/kg 36 mg/kg >88 mg/kg >88 mg/kg >88 mg/kg 36 mg/kg Mean measured	7.0-7.4 22-25°C GLP [1] for reproduction a 35 day EC_{50} = 76 mg/kg was calculated but deemed unreliable as is was lower that the NOEC All results reported as dry wt	
			For reproduction; 35 day EC_{50} 35 day NOEC 42 day EC_{50} 42 day NOEC	 > 88 mg/kg[1] 88 mg/kg >88 mg/kg 88 mg/kg Mean measured		
			For male:female ratio; 42 day NOEC	 88 mg/kg Mean measured		
PYDIFLUMETOFEN (SYN545974) (purity 98.5%)	*Americamysis bahia* (Mysids)	OCSPP 850.1350	28 day NOEC (survival, reproduction, growth) * EC_{10} and EC_{20} values were not able to be calculated.	0.076 mg/L Mean measured	Life-cycle toxicity Dilution water control used. pH 7.6-8.1 25±2°C GLP	Sayers, 2015~~b~~c SYN545974_10167

2.9.2.3.1 Chronic toxicity to fish

Two studies on PYDIFLUMETOFEN (SYN545974) with supporting specific analysis reported long-term chronic NOECs for fish (see below). The lowest NOEC considered for classification purposes was the reported NOEC of 0.025 mg/L.

Table 60: Summary of PYDIFLUMETOFEN (SYN545974) chronic toxicity endpoints for fish

Test type	Test substance	Test species	Endpoint	Value (mg a.s./L)	Reference (Author, date, Syngenta File No.)
Chronic toxicity	PYDIFLUMETOFEN (SYN545974)	*Pimephales promelas* (Fathead minnow)	Early Life Stage (ELS) 32 day NOEC 32 day NO(A)EC	 0.025 0.064	***Anonymous***, 2015a SYN545974_10080
			28 d Body length EC_{10} EC_{20}	 0.15 0.32	
			28 d Body weight EC_{10}	 0.13	
		Cyprinodon variegatus (Sheepshead minnow)	Early Life Stage (ELS) 32 day NOEC	 0.17	***Anonymous***, 2015b SYN545974_10293
			Embryo hatching success EC_{10}	 0.34	

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

Four studies on PYDIFLUMETOFEN (SYN545974) with supporting specific analysis reported long-term chronic NOECs for aquatic invertebrates, including sediment dwelling species (see below). The lowest reported NOEC was for the mysid shrimp *Americamysis bahia* (NOEC = 0.037 mg/L).

Table 93: Summary of PYDIFLUMETOFEN (SYN545974) chronic toxicity endpoints for aquatic invertebrates (includes sediment dwelling species)

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Chronic toxicity	PYDIFLUMETOFEN (SYN545974)	*Daphnia magna* (Water flea)	(static renewal) 21 day NOEC	 0.042 mg/L	Fournier, 2015 SYN545974_10017
			Survival 21 day EC_{10} 21 day EC_{20}	 0.094 mg/L >0.31 mg/L	
			Reproduction 21 day EC_{10} 21 day EC_{20}	 0.085 mg/L 0.13 mg/L	
			Body length 21 day EC_{10} 21 day EC_{20}	 0.21 mg/L >0.31 mg/L	
			Dry weight 21 day EC_{10} 21 day EC_{20}	 0.16 mg/L 0.20 mg/L	

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
		Americamysis bahia[a] (Mysid shrimp)	28 day NOEC (flow through)	0.076 mg/L	Sayers, 2015c SYN545974_10167
		Chironomus dilutus (Dipteran midge)	56 day NOEC (spiked sediment)	15 mg/kg	Bradley, 2015b SYN545974_10095
			20 d growth EC_{10} EC_{20}	>93 mg/kg >93 mg/kg	
			59 d % emergence EC_{20}	22 mg/kg	
			59 d male/female emergence rate EC_{10} EC_{20}	>93 mg/kg >93 mg/kg	
			59 d male/female days to death EC_{10} EC_{20}	>93 mg/kg >93 mg/kg	
			59 d eggs per egg mass EC_{10} EC_{20}	>93 mg/kg >93 mg/kg	
			59 d % hatch EC_{10} EC_{20}	30 mg/kg 49 mg/kg	
		Hyalella Azteca (Freshwater Amphipods)	42 day NOEC (spiked sediment)	7.6 mg/kg	Bradley, 2015c SYN545974_10094
			28 d survival LC_{20}	>88 mg/kg	
			28 d body length EC_{10} EC_{20}	>88 mg/kg >88 mg/kg	

Test type	Test substance	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
			42 d body length EC_{10} EC_{20}	>88 mg/kg >88 g/kg	

[a] EC_{10} and EC_{20} values were not able to be calculated.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

The aquatic plant studies provide chronic endpoints (NOECs) as summarised below. The 72 NOEC values for growth range from 0.89 to 6.3 mg/L (see values in bold below). The lowest NOEC was for the freshwater diatom *Naviculla pelliculosa* (72 hour NOEC = 0.89 mg/L).

Table 100: Summary of chronic PYDIFLUMETOFEN (SYN545974) toxicity endpoints for algae and aquatic plants

Test type	Test item	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Algal toxicity	PYDIFLUMETOFEN (SYN545974)	*Pseudokirchneriella subcapitata* (Green alga)	72 hour NOEC (biomass)	0.9	Kirkwood, 2013 SYN545974_10013
			72 hour NOEC (yield)	0.9	
			72 hour NOEC (growth)	**0.9**	
			72 hour EC_{10} (biomass)	1.0	
			72 hour EC_{10} (yield)	1.1	
			72 hour EC_{10} (growth)	2.3	
			72 hour EC_{20} (biomass)	1.4	
			72 hour EC_{20} (yield)	1.6	
			72 hour EC_{20} (growth)	5.7	
		Anabaena flos-aquae (Blue-green alga)	72 hour NOEC (biomass)	2.7	Soucy, 2013 SYN545974_10091
			72 hour NOEC (yield)	2.7	
			72 hour NOEC (growth)	**2.7**	
			72 hour EC_{10} (biomass)	2.8	
			72 hour EC_{10} (yield)	ND	
			72 hour EC_{10} (growth)	2.8	

Test type	Test item	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
			72 hour EC_{20} (biomass)	3.0	
			72 hour EC_{20} (yield)	2.8	
			72 hour EC_{20} (growth)	3.0	
		Naviculla pelliculosa (Diatom)	72 hour NOEC (biomass)	0.89	Soucy, 2015a SYN545974_10097
			72 hour NOEC (yield)	0.89	
			72 hour NOEC (growth)	**0.89**	
			72 hour EC_{10} (biomass)	0.71	
			72 hour EC_{10} (yield)	0.68	
			72 hour EC_{10} (growth)	0.97	
			72 hour EC_{20} (biomass)	0.98	
			72 hour EC_{20} (yield)	0.97	
			72 hour EC_{20} (growth)	1.1	
		Skeletonema costatum (Diatom)	72 hour NOEC (biomass)	2.4	Soucy, 2014 SYN545974_10105
			72 hour NOEC (yield)	2.4	
			72 hour NOEC (growth)	**2.4**	
			72 hour EC_{10} (biomass)	2.5	
			72 hour EC_{10} (yield)	2.5	
			72 hour EC_{10} (growth)	2.5	
			72 hour EC_{20} (biomass)	2.5	
			72 hour EC_{20} (yield)	2.5	
			72 hour EC_{20} (growth)	2.5	

Test type	Test item	Test species	Endpoint	Value (mg/L)	Reference (Author, date, Syngenta File No.)
Aquatic plant toxicity	PYDIFLUMETOFEN (SYN545974)	*Lemna gibba* (Duckweed)	Frond number 7 day EC_{50} 7 day EC_{20} 7 day EC_{10}	>6.3	Soucy, 2015b SYN545974_10088
			Dry weight 7 day EC_{50} 7 day EC_{20} 7 day EC_{10}	>6.3	

ND = could not be determined

Table 58: Summary of Tier 1 and Tier 2 RAC for aquatic organisms

Endpoints and refinement (Tier 1 and Tier 2 RACs) for toxicity are summarized below:

RACsw (µg a.s./L)	Fish acute	Fish chronic	Aquatic invertebrate acute	Aquatic invertebrate chronic	Algae	Aquatic plant
Tier 1	1.8	2.5	1.2	3.7	160	>630
Tier 2	Geomean = 3.66 SSD = 17.19	EC_{10} = 13	Geomean = 10.37 SSD = 23	Geomean = 5.6	-	-

RACsed (µg a.s./kg)	Sediment dwelling organism acute	Sediment dwelling organism chronic
Tier 1	920	760

The Tier 1 risk assessment for acute and long term presents that:

- FOCUS PEC_{sw} Step 1 and 2 are above Tier 1 RAC and need refinement.
- FOCUS PEC_{sed} Step 2 are below Tier 1 RAC and present acceptable risk for all requested uses.

Refined Tier 2 RACs were presented including geomean and SSD calculation for critical groups of taxa (fish and invertebrates).

The Tier 2 risk assessment for acute and long term presents that:

- FOCUS PEC_{sw} Step 3 are below Tier 2 RAC (based on fish SSD for acute toxicity

No further refinement is needed to present acceptable risk.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

No additional data available.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 59: Acute endpoints relevant to classification

Species group	Species	Lowest representative L/EC50	Reference
Fish	*Oncorhynchus mykiss*	0.18 mg/L	***Anonymous***, 2012
Aquatic invertebrates	*Hyalella azteca*	0.12 mg/L	Brougher *et al*, 2015
Aquatic plants	*Naviculla pelliculosa*	1.6 mg/L	Soucy, 2015b

Based on these results the most sensitive species group are aquatic invertebrates with an EC_{50} = 0.12 mg/L. On this basis, the following classification and labelling of PYDIFLUMETOFEN (SYN545974) is proposed:
Aquatic Acute 1 H400 (Very toxic to aquatic life); as the lowest L(E)C50 is between 0.1 and 1 mg/L the associated M-factor is 1.

Summary of the relevant studies used for acute environmental hazards is presented below:

Brougher D, Gallagher S, Siddiqui A (2015)

According to the OECD 202 (2014), the freshwater amphipod, *Hyalella azteca*, was exposed for 48 hours under static conditions to six mean measured concentrations of SYN545974 ranging from 0.0028 to 0.89 mg a.s./L. The 48-hour LC_{50} value, based on mean measured concentrations, was 0.12 mg a.s./L, with a 95% confidence interval of 0.057 to 0.21 mg a.s./L. The NOEC was 0.009 mg a.s./L.

Test chambers were 250 mL glass beakers filled with approximately 200 mL of test water. The depth of the test water in a representative chamber was 6.8 cm. Two approximately 2x2 cm squares of nylon mesh screen were placed on the bottom of each test compartment prior to test initiation to serve as a substrate for the organisms. The chambers were indiscriminately positioned by treatment group in a temperature-controlled environmental chamber.

A primary stock solution was prepared by mixing a calculated amount of test substance (0.00406 g) in 4000 mL of UV sterilized well water at a nominal concentration of 1.0 mg a.s./L, the highest concentration tested. Aliquots of the primary stock solution were proportionally diluted with UV sterilized well water to prepare five additional test solutions at nominal concentrations of 0.0029, 0.0095, 0.031, 0.10 and 0.31 mg a.s./L. The solutions were stirred for 15 minutes and approximately 250 mL of solution was placed in each of four replicate test chambers per treatment group. The negative control solution was dilution water only.

The test concentrations were verified by analysis of SYN545974. The method used for the analysis of SYN545974 in freshwater consisted of diluting the samples with a ratio of 20 : 80 (v/v) methanol : freshwater. Samples were analyzed by high performance liquid chromatography with tandem mass spectrometric detection (LC/MS/MS).

All organisms were observed periodically to determine the number of mortalities in each treatment group. Mortality was defined as a lack of reaction by the test organism to application of a gentle stimulus. The numbers of individuals exhibiting signs of toxicity or abnormal behaviour also were evaluated. Observations were made approximately 5, 24 and 48 hours after test initiation. Mean measured concentrations of SYN545974 ranged from 84.8 to 99.1% of nominal values According to OECD 202 criteria, the nominal exposure concentration are used to calculate EC_{50}.

Estimates of LC_{50}, slopes of the concentration-response curves, and confidence intervals for both 24 and 48-hour data responses were determined using probit analyis. The protocol stated that the LC_{50} and 95% confidence interval would be calculated by probit analysis, the moving average method, or by binomial probability with nonlinear interpolation using the computer program of C. E. Stephan. However, there was one mortality in the negative control group, and it was noted that algorithm used by Stephan to calculate maximum likelihood estimates of the LD_{50} ignores mortality in the control group. Therefore, the mortality data were analyzed using the CETIS computer program of Tidepool Scientific instead. This program is designed to calculate the LC_{50} value and the 95% confidence interval by probit analysis, and does incorporate control mortality into the maximum likelihood estimate of the LC_{50} and 95% confidence interval. The no-observed-effect concentration (NOEC) was determined using the Jonckheere-Terpstra Step-Down Test. Validity criteria are fulfilled.

To conclude, the freshwater amphipod, *Hyalella azteca*, was exposed for 48 hours under static conditions to six mean measured concentrations of SYN545974 ranging from 0.0028 to 0.89 mg a.s./L. Based on mean measured concentrations, the 48-hour LC_{50} value was 0.12 mg a.s./L, with a 95% confidence interval of 0.057 to 0.21 mg a.s./L. The NOEC was 0.009 mg a.s./L.

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 60: Summary of information on long-term aquatic toxicity relevant for classification

Species group	Species	Lowest representative NOEC	Reference
Fish	*Pimephales promelas*	0.025 mg/L	***Anonymous***, 2015a
Aquatic invertebrates	*Daphnia magna*	0.042 mg/L	Fournier, 2015
Aquatic plants	*Naviculla pelliculosa*	0.89 mg/L	Soucy, 2015a

Based on these results the most sensitive species group to chronic exposure are fish with a NOEC = 0.025 mg/L.

Summary of the relevant studies used for long-term environmental hazards is presented below:

Anonymous, (2015a)

The chronic effects of SYN545974 to fathead minnow (Pimephales promelas) embryos and larvae were determined under flow-through conditions. Fish were exposed to nominal concentrations of 0.010, 0.026, 0.064, 0.16 and 0.40 mg a.s./L alongside a dilution water control and a solvent control. Results were based on the mean measured concentrations of 0.0095, 0.025, 0.064, 0.15 and 0.38 mg a.s./L.

Based on the day 32 (day 28 post-hatch-completion) larval survival, mean length and mean dry weight and mean measured concentrations, the No-Observed-Effect-Concentration (NOEC) was determined to be 0.025 mg a.s./L and the No-Observed-Adverse-Effect Concentration (NOAEC) was determined to be 0.064 mg/L for SYN545974 and fathead minnow.

A flow-through test system was employed. At the start of the test 30 eggs, approximately 22 hours old, were randomly allocated to egg cups and one egg cup suspended in each of four replicate test vessels at each test and control treatment. Hence, 120 eggs were exposed at each treatment. The test was undertaken in a temperature controlled water-bath. A 100 mg a.s./L diluter stock solution was prepared, prior to exposure initiation and as needed throughout the definitive exposure, by adding approximately 1.0 g of SYN545974 to 10 mL of dimethylformamide (DMF), mixed by inversion, and sonicated for less than one minute. A 28 µL/mL solvent stock solution was prepared by diluting 28 mL of DMF to a final volume of 1000 mL with reagent grade water. The control, solvent control and test solutions were delivered to the exposure aquaria (50 L/ aquarium/day) using a Mount and Brungs intermittent-flow proportional diluter at a rate of approximately 7.7 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 7 hours. The concentrations of SYN545974 in test solutions were measured at 0, 4, 11, 17, 27 and 32 days using LC/MS/MS. Observations for time to hatch, hatching success, larval mortality and deformed larvae were made daily during the pre- and post-hatch phases, as appropriate. Day of hatch was considered to be day 4 when no more than 10% unhatched viable embryos remained in any control or solvent control embryo incubation cup. At the end of the test, survival percentage was determined together with lengths and dry weights of the surviving fry.

The mean measured concentrations ranged from 81% to 120% of their nominal concentrations. The limit of quantification (LOQ) for the method validation was 0.151 µg SYN545974/L. It was established that the concentrations of SYN545974 in the exposure solutions were generally consistent and that the delivery apparatus maintained the expected concentration.

Statistical analysis determined a significant difference in percent of live, normal larvae among embryos exposed to the 0.064, 0.15 and 0.38 mg/L treatment levels, compared to the pooled control. The NOEC and LOEC for this endpoint were determined to be 0.025 and 0.064 mg/L, respectively. However, the absolute effect at 0.064 and 0.15 mg/L (i.e. 94 and 93% live and normal larvae post hatch) is minimal compared to the control response (100% pooled control) and within the historical control data. Therefore, the biological significance of this minor statistical difference is questionable. Especially considering larval survival at the end of test was 93% at 0.064 mg/L and well above the performance criterion of 70%. Therefore, the No-Observed-Adverse-Effect Concentration (NOAEC) is considered to be 0.15 mg/L for percent live and normal larvae post hatch.

Mean embryo hatching success and percent live normal larvae at hatch were compared to the mean embryo hatching success and percent live normal larvae at hatch in the pooled control. At exposure termination (28 days post-hatch), larval survival and growth (total length and dry weight) were compared to the mean larval survival and growth in the pooled control.

Based on the day 32 (day 28 post-hatch-completion) larval survival, mean length and mean dry weight and mean measured concentrations, the No-Observed-Effect-Concentration (NOEC) was determined to be 0.025 mg a.s./L and the No-Observed-Adverse-Effect Concentration (NOAEC) was determined to be 0.064 mg/L for SYN545974 and fathead minnow.

PYDIFLUMETOFEN (SYN545974) is not rapidly degradable and has a low potential for bioaccumulation.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

On the basis of the above information on chronic toxicity, the following classification and labelling of PYDIFLUMETOFEN (SYN545974) is proposed;
Aquatic Acute 1 H400 (Very toxic to aquatic life); as the lowest L(E)C50 is between 0.1 and 1 mg/L the associated M-factor is 1.

Aquatic Chronic 1 H410 (Very toxic to aquatic life with long lasting effects); as the lowest NOEC is between 0.01 and 0.1 mg/L the associated M-factor is 1.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS proposed to classify the substance as Aquatic Acute 1 – H400 (M=1) based on a 48 hours mean measured EC_{50} value of 0.12 mg/L for the freshwater amphipod *Hyalella azteca*, and as Aquatic Chronic 1 – H410 (M=1) based on lack of rapid degradation and a 32-d mean measured NOEC value of 0.025 mg/L for fish *Pimephales promelas*.

Degradation

Pydiflumetofen was stable to hydrolysis at acidic, neutral and alkaline pH conditions at 50°C.

Aqueous photolysis of pydiflumetofen was studied in pH 7 buffer (direct photolysis) and in natural water (indirect photolysis). Pydiflumetofen was degraded, primarily by dechlorination and phenyl ring degradation to produce phenyl-hydroxylated metabolites, carboxylic acid metabolites and carbon dioxide. Estimated DT_{50} values were 93 and 35 days (summer sunlight 30-50°N) in pH 7 buffer and natural water, respectively. No photo-degradates reached levels ≥ 5% Applied Radioactivity (AR) via direct photolysis. Photolysis in natural water led to the formation of the degradation products SYN548261 at ≥ 5% AR at two consecutive sampling intervals (maximum 7.3% AR after 21 days) and NOA449410 at a maximum level of 5.8% AR by the end of the experimental period (30 days).

Pydiflumetofen was not considered readily biodegradable under the conditions of the available 28-d ready biodegradability test (OECD TG 301F).

The aerobic mineralisation and degradation of pydiflumetofen in surface water was

determined in the laboratory under dark conditions and light/dark conditions. No significant degradation of the substance was observed throughout the study. Mineralisation was low (< 1%) in all systems tested. DT_{50}s were extrapolated beyond the study period in all incubation groups and ranged from 637 to >1000 days for dark incubation and from 402 to 662 days for light/dark incubation.

The rate and route of degradation of [14C]-pydiflumetofen has been investigated in two water-sediment systems under laboratory aerobic and anaerobic conditions in the dark. In the aerobic systems 70 - 74% of applied pydiflumetofen remained in the total systems after 100 days (end of study). Only one metabolite was observed at levels above 5% AR and this was identified as SYN545547. It increased throughout the duration of the study and accounted for up to 12.3% AR in sediment extracts and 12.8% AR in the total system after 100 days. In the anaerobic systems, 54 - 64% of applied pydiflumetofen remained in the total systems after 100 days. As in the aerobic systems, the only metabolite exceeding 5% of applied radioactivity was SYN545547. It increased throughout the duration of the study and accounted for up to 26.5% AR in sediment extracts, 10.8% in water and 32.4% AR in the total system after 100 days.
The rate of degradation of pydiflumetofen and its metabolite SYN545547 in aquatic systems were assessed from the data from the aerobic water-sediment study according to FOCUS guidance on degradation kinetics (FOCUS 2006, 2011). The persistence endpoints for pydiflumetofen were $DegT_{50}$ of 270 - 299 days ($DegT_{90}$ of 976 - 1100 days) for degradation in the whole system and DT_{50} of 0.74 - 8.03 days ($DegT_{90}$ of 33.1 - 86.9 days) for dissipation in the water column. The modelling endpoints for pydiflumetofen ranged from 244 to 252 days (geometric mean $DegT_{50}$ of 248 days) for degradation in the whole system. For the metabolite SYN545547, persistence endpoints were $DegT_{50}$ of 18.6 - 455 days ($DegT_{90}$ of 61.9 - 1510 days). The modelling whole system degradation endpoints ranged from 18.6 to 455 days (geometric mean $DegT_{50}$ of 92.0 days).

The enantiomeric composition of pydiflumetofen in water was determined at the end of the aerobic mineralisation study, at the end of the aerobic and anaerobic incubations in water/sediment studies and at the end of the irradiation period in the water photolysis study compared to the ratio in the pydiflumetofen application solutions. The pydiflumetofen enantiomer did not change significantly over the course of these degradation studies.

The DS concluded that pydiflumetofen is not considered readily biodegradable. In addition, the results from hydrolysis and water-sediment studies show that pydiflumetofen is not degraded in the aquatic environment to a level > 70 % within a 28 days period. As a consequence, pydiflumetofen is considered not rapidly degradable for the purpose of classification and labelling.

Bioaccumulation

The experimentally derived Log K_{OW} of pydiflumetofen is 3.8 at 25°C (OECD TG 107).

A fish bioconcentration study (OECD TG 305, OPPTS 850.1730, GLP) is available for pydiflumetofen (Anonymous, 2014). Bluegill sunfish (*Lepomis macrochirus)* were exposed to a single concentration (4.9 µg/L) of the mixture of radiolabelled [Phenyl-U-^{14}C]-pydiflumetofen and unlabelled test substance for 19 days in a flow-through system, followed by a 7-d depuration period. The bioconcentration factors $BCF_{SS,\ lipid\text{-}normalised}$ and $BCF_{k,\ lipid\text{-}normalised}$ for whole fish were 31.1 and 189, respectively.

Based on the available data the DS concluded that pydiflumetofen has a low potential for bioconcentration and consequently does not meet the CLP criteria for bioaccumulation.

Aquatic toxicity

Reliable aquatic toxicity data are available for all three trophic levels, and a summary of the relevant information is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). The results of the studies are expressed in terms of mean measured concentrations.

Table: *Summary of relevant information on aquatic toxicity*

Method/Exposure	Test organism	Endpoint	Toxicity values in mg/L	Reference
Short-term toxicity to fish				
OECD TG 203, OPPTS 850.1075, flow-through	*Lepomis macrochirus* (Bluegill sunfish)	96-h LC_{50}	0.48	Anonymous, 2014
OECD TG 203, OPPTS 850.1075, flow-through	*Cyprinus carpio* (Common carp)	96-h LC_{50}	0.33	Anonymous, 2013a
OECD TG 203, EC L142/446 C.1, OPPTS 850.1075, flow-through	*Oncorhynchus mykiss* (Rainbow trout)	96-h LC_{50}	0.18	Anonymous, 2012
OECD TG 203, OPPTS 850.1075, flow-through	*Pimephales promelas* (Fathead minnow)	96-h LC_{50}	0.35	Anonymous, 2013
OECD TG 203, OPPTS 850.1075, flow-through	*Cyprinodon variegatus* (Sheepshead minnow)	96-h LC_{50}	0.66	Anonymous, 2013b
Short-term toxicity to aquatic invertebrates				
OECD TG 202, EC L142/456 C.2, OPPTS 850.1010 Static	*Daphnia magna* (Water flea)	48-h EC_{50}	0.42	Fournier, 2012a SYN545974_10016
No specific guideline but OECD TG 202 was consulted. Static	*Asellus aquaticus* (Water louse)	96-h EC_{50}	4.21	Pickering, 2015 SYN545974_10305
No specific guideline but OECD TG 202 was consulted. Static	*Chaoborus crystallinius* (Phantom midge)	48-h EC_{50}	2.49	Joyce, 2015 SYN545974_10341
No specific guideline but OECD TG 202 was consulted Static	*Chironomus riparius* (Non-biting midge / Harlequin fly)	48-h EC_{50}	0.69	Joyce, 2015a SYN545974_10316 Pickering, 2015a SYN545974_10315
No specific guideline but OECD TG 202 was consulted Static	*Cloeon dipterum* (Mayfly)	48-h NOEC	5.01	Pickering, 2015a SYN545974_10315
No specific guideline but OECD TG 202 was consulted	*Crangonx pseudogracilis* (Freshwater amphipod)	48-h EC_{50}	1.23	Pickering, 2015b SYN545974_10306

Static				
No specific guideline but OECD TG 202 was consulted Static	*Cyclops agilis speratus* (Eastern oyster)	48-h EC_{50}	4.17	Joyce, 2015b SYN545974_10347
No specific guideline but OECD TG 202 was consulted Static	*Lumbriculus variegatus* (Blackworm)	48-h EC_{50}	4.65	Pickering, 2015c SYN545974_10304
No specific guideline but OECD TG 202 was consulted Static	*Lymnaea stagnalis* (Great pond snail)	48-h NOEC	7.3	Pickering, 2015d SYN545974_10303
OECD TG 202, OPPTS 850.1010 Static	*Hyalella azteca* (Freshwater amphipod)	48-h LC_{50}	**0.12**	Brougher *et al*. 2015 SYN545974_10354
OPPTS 850.1035, OPPTS 850.1000 Static	*Americamysis bahia* (Mysid)	96-h LC_{50}	0.16	Fournier, 2012b SYN545974_10015
OPPTS 850.1025 Static	*Crassostrea virginica* (Eastern Oyster)	96-h EC_{50}	0.31	Fournier, 2014a SYN545974_10099
Toxicity to algae and aquatic plants				
OECD TG 201, OPPTS 850.5400, EC 761/2009 C.3 Static	*Pseudokirchneriella subcapitata* (Freshwater Green Alga)	72-h E_rC_{50}	>5.9	Kirkwood, 2013 SYN545974_10013
		72-h E_yC_{50}	3.6	
		72-h E_bC_{50}	4.3	
OECD TG 201, OPPTS 850.5400 Static	*Skeletonema costatum* (Marine diatom)	72-h E_rC_{50}	2.7	Soucy, 2014 SYN545974_10105
		72-h E_yC_{50}	2.7	
		72-h E_bC_{50}	2.7	
OECD TG 201, OCSPP 850.4550 Static	*Anabaena flos-aquae* (Freshwater Blue-Green Alga)	72-h E_rC_{50}	3.6	Soucy, 2013 SYN545974_10091
		72-h E_yC_{50}	3.5	
		72-h E_bC_{50}	3.6	
OECD TG 201, OCSPP 850.4550 Static	*Navicula pelliculosa* (Freshwater Diatom)	72-h E_rC_{50}	1.6	Soucy, 2015 SYN545974_10097
		72-h E_yC_{50}	1.5	
		72-h E_bC_{50}	1.5	
OECD TG 221, OPPTS 850.4400 Semi-static	*Lemna gibba* (Duckweed)	7-d E_rC_{50}	> 6.3	Soucy, 2015a SYN545974_10088
		7-d NOE_rC	> 6.3	
Long-term toxicity to fish				
OECD TG 210, OPPTS 850.1400, EC L.142/603, C.15 Flow-through	*Pimephales promelas* (Fathead Minnow)	32-d NOEC (survival, mean length and mean dry weight)	**0.025**	*Anonymous*, 2015a
		32-d EC_{10} 32-d EC_{20} (Body length)	0.15 0.32	
		32-d EC_{10} (Body weight)	0.13	
OECD TG 210, OPPTS 850.1400 Flow-through	*Cyprinodon variegatus* (Sheepshead Minnow)	32-d NOEC (survival, mean length and mean dry weight)	0.17	*Anonymous*, 2015b
		EC_{10} (embryo	0.34	

		hatching success)		
Long-term toxicity to aquatic invertebrates				
OECD TG 211, OPPTS 850.1300, EC L.142/674, C.20 Static-renewal	*Daphnia magna* (Water flea)	21-d NOEC (survival, reproduction, growth)	0.042	Fournier, 2015 SYN545974_10017
		21-d EC_{10} 21-d EC_{20} (survival)	0.094 > 0.31	
		21-d EC_{10} 21-d EC_{20} (reproduction)	0.085 0.13	
		21-d EC_{10} 21-d EC_{20} (body length)	0.21 > 0.31	
		21-d EC_{10} 21-d EC_{20} (dry weight)	0.16 0.20	
OCSPP 850.1350	*Americamysis bahia* (Mysid shrimp)	28-d NOEC (survival, reproduction, growth)	0.076	Sayers, 2015c SYN545974_10167
Chronic toxicity to algae or aquatic plant				
OECD TG 201, OPPTS 850.5400, OECD TG 201, OPPTS 850.5400, EC 761/2009 C.3 Static	*Pseudokirchneriella subcapitata* (Green alga)	72-h NOE_bC 72-h E_bC_{10} 72-h E_bC_{20}	0.9 1.0 1.4	Kirkwood, 2013 SYN545974_10013
		72-h NOE_yC 72-h E_yC_{10} 72-h E_yC_{20}	0.9 1.1 1.6	
		72-h NOE_rC 72-h E_rC10 72-h E_rC20	0.9 2.3 5.7	
OECD TG 201, OCSPP 850.4550 Static	*Anabaena flos-aquae* (Blue-green alga)	72-h NOE_bC 72-h E_bC_{10} 72-h E_bC_{20}	2.7 2.8 3.0	Soucy, 2013 SYN545974_10091
		72-h NOE_yC 72-h E_yC_{10} 72-h E_yC_{20}	2.7 n.d. 2.8	
		72-h NOE_rC 72-h E_rC_{10} 72-h E_rC_{20}	2.7 2.8 3.0	
OECD TG 201, OCSPP 850.4550 Static	*Navicula pelliculosa* (Diatom)	72-h NOE_bC 72-h E_bC_{10} 72-h E_bC_{20}	0.89 0.71 0.98	Soucy, 2015b SYN545974_10097
		72-h NOE_yC 72-h E_yC_{10} 72-h E_yC_{20}	0.89 0.68 0.97	
		72-h NOE_rC 72-h E_rC_{10} 72-h E_rC_{20}	0.89 0.97 1.1	
OECD TG 201, OPPTS 850.5400 Static	*Skeletonema costatum* (Marine diatom)	72-h NOE_bC 72-h E_bC_{10} 72-h E_bC_{20}	2.4 2.5 2.5	Soucy, 2014 SYN545974_10105

		72-h NOE_yC 72-h E_yC_{10} 72-h E_yC_{20}	2.4 2.5 2.5	
		72-h NOE_rC 72-h E_rC_{10} 72-h E_rC_{20}	2.4 2.5 2.5	
OECD TG 221, OPPTS 850.4400 Semi-static	*Lemna gibba* (Duckweed)	7 day EC_{50} 7 day EC_{20} 7 day EC_{10} (frond number)	>6.3	Soucy, 2015a SYN545974_10088
		7 day EC_{50} 7 day EC_{20} 7 day EC_{10} (dry weight)	>6.3	

Data for sediment-dwelling invertebrates (*Leptocheirus plumulosus*, *Chironomus dilutes* and *Hyalella azteca*) were reported in CLH report but were not used for classification because the endpoint values were presented in relation to sediment concentrations of pydiflumetofen (mg/kg). The only data for sediment-dwelling invertebrates available also in mg/L were the result of the acute toxicity study with freshwater amphipods *Hyalella azteca* which was used for classification of pydiflumetofen.

Acute toxicity

For fish, five studies were available. *Oncorhynchus mykiss* was the most sensitive fish species tested in the acute studies, with a 96-h LC_{50} of 0.18 mg/L.

Twelve studies were available in case of aquatic invertebrates. *Hyalella azteca* was the most sensitive species tested in the acute studies, with a 48-h LC_{50} of 0.12 mg/L.

Five acute toxicity studies were available for algae and aquatic plants. *Navicula pelliculosa* was the most sensitive species with a 72-h E_rC_{50} of 1.6 mg/L.

From the available aquatic toxicity data, invertebrates are the most sensitive trophic level therefore the acute aquatic classification proposed by the DS was based on freshwater amphipod *Hyalella azteca* (48-h LC_{50} = 0.12 mg/L). The DS proposed Aquatic Acute 1, with an M-factor = 1 (0.1 < LC_{50} < 1 mg/L).

Chronic toxicity

For fish, two studies were available. *Pimephales promelas* was the most sensitive fish species tested in the chronic studies, with a 32-d NOEC of 0.025 mg/L.

Long-term toxicity to aquatic invertebrates was assessed based on two available studies. The DS concluded that *Daphnia magna* was the most sensitive species tested in the chronic studies, with a 21-d NOEC of 0.042 mg/L. RAC is of the opinion that *Americamysis bahia* was the most sensitive invertebrate species tested in the chronic studies, with a 28-d NOEC of 0.076 mg/L (explanation provided in RCOM document).

Five chronic toxicity studies were available for algae and aquatic plants. *Navicula pelliculosa* was the most sensitive species tested in the chronic studies, with a 72-h NOEC

of 0.89 mg/L. In the same study, the 72-h E_rC_{10} for *Navicula pelliculosa* (based on the same endpoint growth) was 0.97 mg/L.

The results of long-term aquatic toxicity studies indicate that the fish are the most sensitive taxon with a 32-d NOEC of 0.025 mg/L for *Pimephales promelas*. Therefore, the DS proposed Aquatic Chronic 1, with an M-factor = 1 (0.01 < NOEC ≤ 0.1 mg/L) and the substance is not rapidly degradable.

Comments received during public consultation

Six MSCAs submitted comments on the environmental part of the DS's proposal during the public consultation (PC). Four MSCAs agreed with proposed classification for pydiflumetofen as Aquatic Acute 1, M-factor = 1 and Aquatic Chronic 1, M-factor = 1. One of them considered that the classification should be based on the surrogate approach for the most acutely sensitive endpoints. Two MSCAs indicated minor editorial mistakes in the CLH report.

One MSCA was of the opinion that the results from the study with marine diatom *Skeletonema costatum* (Soucy, 2014) and freshwater blue-green algae *Anabaena flos-aquae* (Soucy, 2013) should be considered as supplementary information because the coefficient of variation of mean daily growth rate in controls is above the validity criteria. In response, the DS stated that all other validity criteria were fulfilled and that the test was considered valid and relevant, and was not challenged during the peer review of the active substance. Moreover those algae studies are not the key studies for acute and chronic environmental hazard classification as algae are not the most sensitive aquatic organisms. Therefore, they do not impact on overall conclusion for classification.

The second commenting MSCA provided general comments regarding missing information in the combined CLH/DAR report (e.g. reliability and validity of the studies, etc.).

The third commenting MSCA pointed out that the bioaccumulation has been addressed for the active substance but no mention is made of the water metabolites SYN548261 (photolysis, max 7.3 % AR), NOAA449410 (photolysis, max 5.8 % AR), SYN545547 (aerobic, max 12.3% AR in total system). The DS responded that pydiflumetofen has a low potential for bioaccumulation and is not rapidly degradable. The latter together with chronic toxicity data drives the proposal for environmental hazard classification. Therefore the comment has no impact on harmonised classification proposal for environmental hazard. RAC agrees with the explanation provided by the DS.

In the view of the fourth commenting MSCA, the EC_{10}/EC_{20} (mean dry weight and mean length) should be used, if available, in preference to the NOEC (survival, mean length and mean dry weight) for *Pimephales promelas*. The same MSCA also asked for the EC_{10}/EC_{20} values for survival, if available. In response, the DS confirmed that the EC_{10} was calculated only for "total length" and "dry weight". The NOEC was based on the "live, normal larvae at hatch" and was considered valid and relevant during the peer review process of the active substance.

The same MSCA also noted that the fish *P. promelas* was not the most acutely sensitive fish species and suggested that the surrogate approach should be considered using the 96-h LC_{50} for *O. mykiss* of 0.18 mg/L. This would result in Aquatic Chronic 1 (M-factor = 1). The DS considered that *P. promelas* and *O. mykiss* have acute sensitivity in the same order of magnitude and therefore chronic data on *P. promelas* were considered relevant

for classification. RAC agrees with DS' explanation.

The MSCA also pointed out that a chronic endpoint is available for *Hyalella azteca* (42-d NOEC 7.6 mg/kg) but the study details are not presented to consider its reliability and the endpoint is based on spiked sediment. On this basis, the MSCA suggested that the surrogate approach should be considered using the 48-h EC_{50} of 0.12 mg/L for *H. azteca* and this would result in Aquatic Chronic 1, with M-factor = 1. The DS considered that *D. magna* and *H. azteca* have acute sensitivity in the same order of magnitude and therefore chronic data on *D. magna* were considered relevant for classification. Classification based on chronic study on *D. magna* would result in classification as Aquatic Chronic 1, with M-factor of 1. RAC is of the opinion that the chronic data for *Americamysis bahia* (NOEC of 0.076 mg/L) should be used for classification instead of chronic data for *Daphnia magna* as selected by the DS (explanation provided below and in RCOM document). *A. bahia* has an acute sensitivity in the same order of magnitude as *H. azteca*, therefore it is appropriate to consider chronic data for *A. bahia* relevant for classification. This does not change the proposed classification.

The fifth commenting MSCA disagreed with selected NOECs for aquatic invertebrates and aquatic plants by the DS. The MSCA pointed out that in line with the current CLP Guidance the preference to the EC_{10} value over the NOEC value is given. This applies in cases where EC_{10}s are available for the same endpoint. In the view of the MSCA the following endpoint should be selected as the most critical one:

- The EC_{10} of 0.085 mg/L for *D. magna* (reproduction) instead of NOEC (survival, reproduction and growth) (0.042 mg/L). The EC_{10} (0.085 mg/L) is however higher than the NOEC for *A. bahia* of 0.076 mg/L (survival, reproduction and growth). For this study, EC_{10} values could not be derived and therefore the NOEC for *A. bahia* is the most critical chronic endpoint for aquatic invertebrates.
- The E_rC_{10} of 0.97 mg/L for *N. pelliculosa* instead of NOE_rC of 0.89 mg/L.

The DS responded that this does not change the initially proposed classification. RAC agrees with the commenting MSCA regarding selection of the most critical chronic endpoint for aquatic invertebrates and algae/aquatic plants.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal that pydiflumetofen does not meet the criteria for rapid degradation following CLP criteria. Based on available hydrolysis, results obtained in a biodegradation study, aerobic mineralisation and degradation in surface water study (no significant degradation, low mineralisation (< 1%)), aerobic water/sediment system study (70-74% remained in the total systems after 100 days) RAC agrees with the DS' conclusion that available degradation information does not indicate that pydiflumetofen is ultimately degraded (> 70%) within 28 days (equivalent to a degradation half-life of < 16 days). Consequently, pydiflumetofen is considered to be not rapidly degradable for the purpose of classification under the CLP Regulation.

Bioaccumulation

RAC agrees with the DS that pydiflumetofen has no potential to bioaccumulate in aquatic organisms. The basis for this is that log K_{OW} value of 3.8 is below the CLP threshold of 4 and both measured fish BCF_{ss} and BCF_k values are below the CLP criterion of ≥ 500.

Aquatic toxicity

Aquatic acute toxicity data are available for all three trophic levels. The lowest reliable short-term aquatic toxicity value is a 48-h LC_{50} of 0.12 mg/L for the invertebrate *Hyalella azteca*. As this is below the threshold value of 1 mg/L, pydiflumetofen meets the criteria for classification as Aquatic Acute 1 (H400). Since this toxicity value is in the range of 0.1 < LC_{50} ≤ 1 mg/L, the acute M-factor is 1.

Chronic toxicity

RAC is of the opinion that in case of pydiflumetofen, adequate chronic toxicity data are available for all three trophic levels, although the data for the most acutely sensitive fish and invertebrate species is not represented. Based on the most sensitive standard test organism data (32-d NOEC of 0.025 mg/L for fish *Pimephales promelas*), and lack of rapid degradability, the substance should be classified as Aquatic Chronic 1 with a chronic M-factor of 1 (as 0.01 < NOEC ≤ 0.1 mg/L). The same conclusion on chronic classification would be reached based on the surrogate approach using *Oncorhynchus mykiss* (96-h LC_{50} of 0.18 mg/L) or *Hyalella azteca* (48-h LC_{50} of 0.12 mg/L) and lack of rapid degradation.
In summary, based on the available data, RAC considers to classify pydiflumetofen as Aquatic Chronic 1 with M-factor of 1.

In conclusion, RAC supports the DS's proposal that pydiflumetofen should be classified according to CLP as:

Aquatic Acute 1 (H400), M-factor = 1

Aquatic Chronic 1 (H410), M-factor = 1

2.9.3 Summary of effects on arthropods

Table 61: Summary of information for bee toxicity

Test type	**Test substance**	**Endpoint**	**Value**	**Reference (Author, date, Syngenta File No.)**
Adult acute	PYDIFLUMETOFEN (SYN545974)	48 h oral LD_{50} 48 h contact LD_{50}	>116 µg a.s./bee >100 µg a.s./bee	Kling, 2012 SYN545974_10010
Adult chronic	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	10 d oral LD_{50} 10 d oral NOED	>138.2 µg a.s./bee/day 138.2 µg a.s./bee/day	Ruhland, 2014 A19649B_10055
		10 d oral LD_{10} LD_{20}	>138.2 µg a.s./bee/day >138.2 µg a.s./bee/day	
Larvae (8 day)	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	8 d oral NOED	55 µg a.s./larva 13.75 µg a.s./larva/day [a]	Kleebaum, 2015 A19649B_10076
Larvae (22 day)	PYDIFLUMETOFEN (SYN545974) [c]	8 d oral NOED	<0.014 µg a.s./larva <0.0035 µg a.s./larva/day	Deslandes, 2015 SYN545974_10279
		22 d oral NOED	<0.014 µg a.s./larva <0.0035 µg a.s./larva/day	
	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	8 d oral NOED	**0.08 µg a.s./larva** **0.02 µg a.s./larva/day**	Deslandes, 2015a A19649B_10184
		8 d oral ED_{10}	0.1 µg a.s./larva 0.026 µg a.s./larva/day	
		8 d oral ED_{20}	0.872 µg a.s./larva 0.218 µg a.s./larva/day	
		22 d oral ED_{10}	0.097 µg a.s./larva 0.024 µg a.s./larva/day	
		22 d oral ED_{20}	0.165 µg a.s./larva 0.041 µg a.s./larva/day	
Semi field studies	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	63 days 7 days exposed in tunnel 56 days post exposure monitoring of hives	NOAER = 200 g a.s./ha	Kleinhenz (2017) A19649B_10314 Gonsior (2017) A19649B_10312

Two semi-fields studies (tunnel) are provided. The NOAEC is determined as 200 g a.s./ha.

Table 62: Summary of information on lnon target arthropods toxicity

Test type	Test species	Exposed life stage	Endpoint	Value	Reference (Author, date, Syngenta File No.)
Tier I Glass plate	*Aphidius rhopalosiphi* (Parasitoid wasp)	Adult	48 h LR_{50}	> 2 000 mL A19649B/ha (equivalent to > 408 g a.s./ha)	Stevens, 2016 A19649B_10049
			48 h NOER mortality	500 mL A19649B/ha (equivalent to 102 g a.s./ha)	
			13 d ER_{50} parasitisation	> 2 000 mL A19649B/ha (equivalent to > 408 g a.s./ha)	
			13 d NOER parasitisation	1 000 mL A19649B/ha (equivalent to 204 g a.s./ha)	
	Typhlodromus pyri (Predatory mite)	Proto-nymphs	7 d LR_{50}	> 2 000 mL A19649B/ha (equivalent to > 408 g a.s./ha)	Fallowfield, 2016 A19649B_10032
			7 d NOER mortality	2 000 mL A19649B/ha (equivalent to > 408 g a.s./ha)	
			14 d ER_{50} reproduction	> 2 000 mL A19649B/ha (equivalent to > 408 g a.s./ha)	
			14 d NOER reproduction	250 mL A19649B/ha (equivalent to 51 g a.s./ha)	
Tier II Barley seedlings	*Aphidius rhopalosiphi* (Parasitoid wasp)	Adult	47 h LR_{50}	> 4 000 mL A19649B/ha (> 800 g a.s./ha)	Stevens, 2015 A19649B_10103
			47 h NOER mortality	4 000 mL A19649B/ha (800 g a.s./ha)	
			14 d ER_{50} parasitisation	> 4 000 mL A19649B/ha (> 800 g a.s./ha)	
			14 d NOER parasitisation	4 000 mL A19649B/ha (800 g a.s./ha)	
Tier II Leaf discs	*Typhlodromus pyri* (Predatory mite)	Proto-nymphs	7 d LR_{50}	> 4 000 mL A19649B/ha (> 800 g a.s./ha)	Fallowfield, 2015 A19649B_10173
			7 d NOER mortality	4 000 mL A19649B/ha (800 g a.s./ha)	
			14 d ER_{50} reproduction	> 4 000 mL A19649B/ha (> 800 g a.s./ha)	
			14 d NOER reproduction	4 000 mL A19649B/ha (800 g a.s./ha)	

The risk assessment for non-target arthropods other than bees was conducted in accordance with ESCORT 2 (Candolfi et al. 2000: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods) and SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology).

Based on the HQ calculations below the trigger of 2, the both in-field and off-field assessment for *A. rhopalosiphi* and *T. pyri* shows acceptable risk, indicating that the risk to in-field non-target arthropods is acceptable following use of A19649B according to the proposed use patterns.

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Table 63: Summary of information on earthworm and non target soil meso- and macro fauna toxicities

Test type	Test substance	Test species	Endpoint	Value	Reference (Author, date, Syngenta File No.)
Acute	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	*Eisenia fetida*	14 day LC_{50}	>1 000 mg a.s./kg soil	Friedrich, 2012 SYN545974_10008
Chronic	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	*Eisenia fetida*	56 day NOEC	171 mg A19649B/kg soil (31.81 mg a.s./kg soil)	Friedrich, 2015 A19649B_10073
			56 day EC_{10}	177 mg A19649B/kg soil (32.92 mg a.s./kg soil)	
			56 day EC_{20}	270 mg A19649B/kg soil (50.22 mg a.s./kg soil)	
Chronic	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	*Folsomia candida*	28 d NOEC	1 000 mg A19649B/kg soil (equivalent to 186.0 mg a.s./kg soil)	Friedrich, 2014 A19649B_10046
			28 d EC_{10}	>1 000 mg A19649B/kg soil (equivalent to >186.0 mg a.s./kg soil)	
			28 d EC_{20}	>1 000 mg A19649B/kg soil (equivalent to >186.0 mg a.s./kg soil)	
	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	*Hypoaspis aculeifer*	14 d NOEC	1 000 mg A19649B/kg soil (equivalent to 186.0 mg a.s./kg soil)	Schulz, 2014 A19649B_10038

The risk assessment for earthworms and other soil macro-organisms was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology).

In accordance with the guidance for substances with a log P_{OW} value of > 2, a correction factor of 2 is typically applied to the NOEC to take into account the relatively high organic matter content (10%) used in the artificial test soil compared to natural soils. As Pydiflumetofen (SYN545974) has a log P_{OW} of greater than 2 (being 3.7), a correction factor would be applicable; however the studies were conducted with a lower peat content of 5% which is more representative of natural soils. Therefore a correction factor is not applied.

The potential long-term risk of A19649B and Pydiflumetofen (SYN545974) to earthworm and other non-target soil meso- and macro-fauna was assessed by calculating long-term TER (TER_{LT}) values by comparing the NOEC values and the maximum $PEC_{S.}$

The long-term TER values exceed the Commission Regulation (EU) No. 546/2011 long-term trigger value of 5, indicating that the long-term risk to both earthworms and soil macro-organisms is acceptable following use of Pydiflumetofen (SYN545974) and A19649B according to the proposed use pattern.

When applied in accordance with the uses supported in this submission Pydiflumetofen (SYN545974) and A19649B pose an acceptable long-term risk to earthworms and soil macro-organisms.

2.9.5 Summary of effects on soil nitrogen transformation

Table 64: Summary of information of effect on soil nitrogen transformation

Test type	Test substance	Endpoint	Value	Reference (Author, date, Syngenta File No.)
Nitrogen transformation	PYDIFLUMETOFEN (SYN545974)	28 d NOEC	2.71 mg a.s./kg soil	Schulz, 2015 SYN545974_10275

The risk assessment for soil micro-organisms was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology).

Pydiflumetofen (SYN545974) had a significant effect on soil micro-organisms at 2.71 mg a.s./kg. This value is approximately 0.5 times below than the maximum accumulated PEC_S of 3.6251 mg a.s./kg following the maximum application to grapes (2 x 200 g a.s./ha). A19649B had no significant effect on soil micro-organisms at 14.61 mg A19649B/kg. This value is approximately 25 times higher than the maximum PEC_{Soil} of 0.585 mg A19649B/kg following the maximum application to grapes. This indicates that the risk to non-target soil micro-organisms is acceptable following use of A19649B according to the proposed use pattern.

When applied in accordance with the uses supported in this submission Pydiflumetofen (SYN545974) and A19649B pose an acceptable long-term risk to soil micro-organisms except for the use on Grapes (2 x 200). Further refinement need to be presented.

2.9.6 Summary of effects on terrestrial non-target higher plants

Table 65: Summary of information on non target tyerrestrial plants toxicity

Test type	Test substance	Test species	Endpoint	Value	Reference (Author, date, Syngenta File No.)
Tier 1 Vegetative vigour	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	Monocotyledonae: *Zea mays, Allium cepa, Lolium perenne, Triticum aestivum* Dicotyledonae: *Brassica oleracea, Beta vulgaris, Brassica napus, Lycopersicon esculentum, Lactuca sativa, Glycine max*	ER_{50}	**>200 g a.s./ha**	Porch *et al*., 2015 A19649B_10077
Tier 1 Seedling emergence	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)		ER_{50} (all species)	**>200 g a.s./ha**	Porch *et al*., 2015a A19649B_10105
Tier 2 Seedling emergence	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)		ER_{50}	>400 g a.s./ha	Porch *et al*., 2015b A19649B_10178

The risk assessment for terrestrial non-target plants was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology).

According to the **Terrestrial Guidance Document**, based on Tier 1 studies, the risk to non-target plants should be considered acceptable if less than 50 % effect on all six species is seen at the maximum application rate. Less than 50 % effect on seedling emergence and vegetative vigour on all ten species was observed at 200 g a.s./ha, the highest proposed label rate for A19649B. It can therefore be concluded that the risk to non-target plants is acceptable following use of A19649B according to the proposed use patterns.

When applied in accordance with the uses supported in this submission A19649B (SYN545974 200 SC) poses an acceptable risk to terrestrial non-target plants.

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

Not required.

2.9.8 Summary of effects on biological methods for sewage treatment

Table 66: Summary of information on effect for seawage treatments

Test substance	Test species	Endpoint	Value	Reference (Author, date, Syngenta File No.)
PYDIFLUMETOFEN (SYN545974)	Activated sludge inoculum	**3h NOEC**	**1000 mg a.s./L**	Eisner G (2013) SYN545974_10061

Pydiflumetofen (SYN545974) had no significant inhibitory effect (< 15%) on respiration rate of activated sludge up to 1000 mg SYN545974/L

2.9.9 Summary of product exposure and risk assessment

Summary of product exposure and risk assessment for terrestrial vertebrates

The risk assessment for birds and mammals is carried out following the latest guidance document by EFSA (Anonymous 2009: Guidance Document on risk assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. European Food Safety Authority).

Based on a screening step and first tier assessment, TER values for PYDIFLUMETOFEN (SYN545974) exceed the Commission Regulation (EU) No. 546/2011 trigger value of 10 for acute and 5 for long term and therefore poses no unacceptable risk for birds and mammals..

Toxicity/exposure ratios for terrestrial vertebrates (Regulation (EU) N° 284/2013, Part A, Annex point 10.1)

Grapes at 200 g a.s./ha x 2

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	22.87	170	10
All	Small omnivorous bird	Long-term	5.77	16	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	32.74	150	10
All	Small herbivorous mammal	Long-term	3.83	**9.4**	5
Tier 1 (Mammals)					
Vineyard Application crop directed BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	Long-term	10.73	**3.36**	5
	Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	Long-term	0.34	106	5

Risk from bioaccumulation and food chain behaviour				
Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Long-term	5	18.2	5
Earthworm-eating mammals	Long-term	6.08	5.9	5
Fish-eating birds(PYDIFLUMETOFEN (SYN545974))	Long-term	0.0086[a]	10000	5
Fish-eating birds(SYN545547)	Long-term	0.023[b]	390.0	5
Fish-eating mammals (PYDIFLUMETOFEN (SYN545974))	Long-term	0.0076[a]	4737	5
Fish-eating mammals (SYN545547)	Long-term	0.021[b]	171	5

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
[a] FOCUS Step 3 21 day TWA PECsw [b]Maximum Step 2 PEC_{sw} [c] Defaut correction assuming the metabolite is 10 time more toxic Higher tier : none					

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5
Puddle scenario, Screening step 1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed					

Grapes at 40 g a.s./ha x 2

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	8.26	457	10
All	Small omnivorous bird	Long-term	2.06	43.7	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	7.09	705	10
All	Small herbivorous mammal	Long-term	2.03	15.66	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Higher tier : none				

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5
Puddle scenario, Screening step 1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed					

Pome fruits at 50 g a.s./ha x 3

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
	Small insectivorous bird	Acute	3.74	1 000	10
	Small insectivorous bird	Long-term	0.96	94	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	10.91	460	10

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
All	Small herbivorous mammal	Long-term	3.83	9.4	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			

Higher tier : none

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5

Puddle scenario, Screening step

1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed

Tomatoes at 70 g a.s./ha x 2

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	15.56	240	10
All	Small omnivorous bird	Long-term	3.85	23	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	13.37	370	10
All	Small herbivorous mammal	Long-term	4.29	8.4	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			

Higher tier : none

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5

Puddle scenario, Screening step

1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed

Cucurbits at 50 g a.s./ha x 2

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
	Small omnivorous bird	Acute	11.12	340	10
	Small omnivorous bird	Long-term	2.75	33	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	9.55	520	10
All	Small herbivorous mammal	Long-term	3.07	11.7	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			

Higher tier : none

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5

Puddle scenario, Screening step

1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed

Potatoes at 40 g a.s./ha x 3

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	8.26	460	10
	Small omnivorous bird	Long-term	2.06	44	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	6.16	810	10
All	Small herbivorous mammal	Long-term	1.54	23.4	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			

Higher tier : none

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5

Puddle scenario, Screening step

1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed

Brassicas at 70 g a.s./ha x 2

Growth stage	Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Screening Step (Birds)					
All	Small omnivorous bird	Acute	13.34	280	10
	Small omnivorous bird	Long-term	3.37	27	5
Screening Step (Mammals)					
All	Small herbivorous mammal	Acute	11.46	440	10
All	Small herbivorous mammal	Long-term	3.76	9.57	5

Risk from bioaccumulation and food chain behaviour

Indicator or focal species	Time scale	DDD (mg/kg bw per day)	TER	Trigger
Earthworm-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Earthworm-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating birds	Covered by use on Grapes at 200 g a.s./ha x 2			
Fish-eating mammals	Covered by use on Grapes at 200 g a.s./ha x 2			

Higher tier : none

Risk from consumption of contaminated water

Scenarios	**Indicator or focal species**	**Time scale**	**PEC_{dw}xDWR**	**TER**	**Trigger**
Leaf scenario	Birds	acute	21.62	170	5

Puddle scenario, Screening step

1)Application rate (g a.s./ha)/relevant endpoint <3000 (koc≥500 L/kg), TER calculation not needed

Summary of product exposure and risk assessment for aquatic organisms

The risk assessment for aquatic organisms is carried out following the latest guidance document by EFSA (. EFSA panel on plant protection products and their residues (PPR). European Food Safety Authority (EFSA), Parma, Italy. EFSA Journal 2013;11(7):3290).

Based on a first tier assessment and refined endpoints, TER values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011 for STEP 3 PEC_{sw} calculation indicating an acceptable risk to aquatic organisms for these uses.

Toxicity/exposure ratios for the most sensitive aquatic organisms

Only the South STEP 2 PEC_{sw} are worst-case value and cover the STEP 2 PEC_{sw} North.

$FOCUS_{sw}$ step 1-3 - RACs for Pydiflumetofen – Grapes at 200 g a.s./ha x 2 applications

Scenario	PEC_{sw} (µg L)	fish acute	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates	Aquatic invertebrates prolonged	Aquatic invertebrates prolonged	Algae	Higher plant	PEC sediment (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	51.4	**No**	**No**	**No**	**No**	**No**	**No**	**No**	Yes	Yes	1394	**No**
FOCUS Step 2												
North Europe												
South Europe	8.54	**No**	Yes	Yes	**No**	10.8	**No**	**No**			242	Yes
FOCUS Step 3												
D6	3.83	**No**			**No**		**No**	Yes				
R1 / pond	0.180	Yes			Yes		Yes	Yes				
R1 / stream	2.44	**No**			**No**		Yes	Yes				
R2 / stream	3.37	**No**			**No**		Yes	Yes				
R3 / stream	3.54	**No**			**No**		Yes	Yes				
R4 / stream	2.51	**No**			**No**		Yes	Yes				

$FOCUS_{sw}$ step 1-2 - RACs for Metabolite SYN545547 – Grapes at 200 g a.s./ha x 2 applications as worst case

Scenario	PEC_{sw} (µg L)	fish acute	Aquatic invertebrates	Algae	PEC sediment (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	*Hyalella azteca*	*Navicula pelliculosa*		*Chironomus riparius*
		LC_{50}	EC_{50}	EC_{50}		NOEC
		1400 µg/L	7300 µg/L	4000 µg/L		7200 µg/kg
FOCUS Step 1						
	28	**No**	Yes	Yes	160	Yes
FOCUS Step 2						
North Europe						
South Europe	4.72	Yes				

$FOCUS_{sw}$ step 1 - RACs for Metabolite SYN548261 – Grapes at 200 g a.s./ha x 2 applications as worst case

Scenario	PEC_{sw} (µg L)	fish acute	Aquatic invertebrates	Algae
		Oncorhynchus mykiss	*Daphnia magna*	*Pseudokirchneriella subcapitata*
		LC_{50}	EC_{50}	EC_{50}
		>100 000 µg/L	>100 000 µg/L	>100 000 µg/L
FOCUS Step 1				
	8.45	Yes	Yes	Yes

FOCUS$_{sw}$ step 1 - RACs for Metabolite NOA449410 – Grapes at 200 g a.s./ha x 2 applications as worst case

Scenario	PEC$_{sw}$ (µg L)	fish acute	Aquatic invertebrates	Algae
		Oncorhynchus mykiss	*Daphnia magna*	*Pseudokirchneriella subcapitata*
		LC_{50}	EC_{50}	EC_{50}
		>100 000 µg/L	>100 000µg/L	36 310 µg/L
FOCUS Step 1				
	3.44	Yes	Yes	Yes

$FOCUS_{sw}$ step 1-3 - RACs for Pydiflumetofen – Pome fruits at 50 g a.s./ha x 3 applications

Scenario	PEC_{sw} (µg L)	fish acute	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates	Aquatic invertebrates prolonged	Aquatic invertebrates prolonged	Algae	Higher plant	PEC sediment (µg/kg)	Sed. dweller prolonged
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	23.1	**No**	**No**	**No**	**No**	**No**	**No**	**No**	Yes	Yes	560	13.6
FOCUS Step 2												
North Europe												
South Europe	3.72	**No**	Yes	Yes	**No**	Yes	**No**	Yes				
FOCUS Step 3												
D3	1.84	**No**	Yes		**No**	Yes	Yes					
D4	0.207	Yes	Yes		Yes	Yes	Yes					
D4	1.84	**No**	Yes		**No**	Yes	Yes					
D5	0.211	Yes	Yes		Yes	Yes	Yes					
D5	1.98	**No**	Yes		**No**	Yes	Yes					
R1	0.145	Yes	Yes		Yes	Yes	Yes					
R1	1.41	Yes	Yes		**No**	Yes	Yes					
R2	1.89	**No**	Yes		**No**	Yes	Yes					
R3	1.98	**No**	Yes		**No**	Yes	Yes					
R4	1.41	Yes	Yes		**No**	Yes	Yes					

$FOCUS_{sw}$ step 1-3 - RACs for Pydiflumetofen – Tomatoes at 70 g a.s./ha x 2 applications

Scenario	PEC_{sw} (µg L)	fish acute	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates	Aquatic invertebrates prolonged	Aquatic invertebrates prolonged	Algae	Higher plant	PEC sediment (µg/kg	Sed. dweller prolonged
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	15.5	**No**	Yes	**7.7**	**7.7**	**No**	**No**	**No**	Yes	Yes	464	Yes
FOCUS Step 2												
North Europe												
South Europe	1.72	Yes		Yes	**No**	Yes	Yes	Yes				
FOCUS Step 3												
D6	2.17	**No**			**No**							
R2	0.392	Yes			Yes							
R3	0.468	Yes			Yes							
R4	0.719	Yes			Yes							

$FOCUS_{sw}$ step 1-3 - RACs for Pydiflumetofen – Cucurbits at 50 g a.s./ha x 2 applications

Scenario	PEC_{sw} (µg L)	fish acute	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates	Aquatic invertebrates prolonged	Aquatic invertebrates prolonged	Algae	Higher plant	PEC sediment (µg/kg	Sed. dweller prolonged
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	11.1	**No**	Yes	**No**	**No**	Yes	**No**	**No**	Yes	Yes	332	Yes
FOCUS Step 2												
North Europe												
South Europe	3.36	**No**		Yes	**No**		Yes	Yes				
FOCUS Step 3												
D6	1.51	Yes			**No**							
R2	0.28	Yes			Yes							
R3	0.353	Yes			Yes							
R4	0.481	Yes			Yes							

$FOCUS_{sw}$ step 1-2 - RACs for Pydiflumetofen – Potatoes at 40 g a.s./ha x 3 applications

Scenario	**PEC_{sw} (µg L)**	**fish acute**	**fish acute**	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates**	**Aquatic invertebrates prolonged**	**Aquatic invertebrates prolonged**	**Algae**	**Higher plant**	**PEC sediment (µg/kg**	**Sed. dweller prolonged**
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	13.3	**No**	Yes	**No**	**No**	Yes	**No**	**No**	Yes	Yes	399	Yes
FOCUS Step 2												
North Europe												
South Europe	1.4	Yes		Yes	**No**		Yes	Yes				

$FOCUS_{sw}$ step 1-3 - RACs for Pydiflumetofen – Brassicas at 70 g a.s./ha x 2 applications

Scenario	**PEC_{sw} (µg L)**	**fish acute**	**fish acute**	**fish chronic**	**Aquatic invertebrates**	**Aquatic invertebrates**	**Aquatic invertebrates-prolonged**	**Aquatic invertebrates-prolonged**	**Algae**	**Higher plant**	**PEC sediment (µg/kg**	**Sed. dweller prolonged**
		Oncorhynchus mykiss	SSD	*Pimephales promelas*	*Hyalella azteca*	SSD	*Daphnia magna*	Overall	*Navicula pelliculosa*	*Lemna gibba*		*Hyalella azteca*
		LC_{50}	HC_5	NOEC	EC_{50}	HC_5	NOEC	Geomean	EC_{50}	EC_{50}		NOEC
		180 µg/L	154.74 µg/L	130 µg/L	120 µg/L	92 µg/L	42 µg/L	56 µg/L	1600 µg/L	>6300 µg/L		7600 µg/L
RAC		1.8 µg/L	17.19 µg/L	13 µg/L	1.2 µg/L	23 µg/L	4.2 µg/L	5.6 µg/L	160 µg/L	>630 µg/L		760 µg/L
FOCUS Step 1												
	15.5	**No**	Yes	**No**	**No**	**No**	**No**	**No**	Yes	Yes	464	Yes
FOCUS Step 2												
North Europe												
South Europe	4.7	**No**	Yes	Yes	**No**	Yes	**No**	**No**				
FOCUS Step 3												

D3 (1st crop)	0.443	Yes	Yes	Yes	Yes
D3 (2nd crop)	0.44	Yes	Yes	Yes	Yes
D4 (1st crop)	0.312	Yes	Yes	Yes	Yes
D4 (1st crop)	0.633	Yes	Yes	Yes	Yes
D6 (1st crop)	2.37	**No**	**No**	Yes	Yes
R1 (1st crop)	0.256	Yes	Yes	Yes	Yes
R1 (2nd crop)	0.2	Yes	Yes	Yes	Yes
R1 (1st crop)	0.411	Yes	Yes	Yes	Yes
R1 (2nd crop)	0.378	Yes	Yes	Yes	Yes
R2 (1st crop)	0.392	Yes	Yes	Yes	Yes
R2 (2nd crop)	0.392	Yes	Yes	Yes	Yes
R3 (1st crop)	0.414	Yes	Yes	Yes	Yes
R3 (2nd crop)	0.411	Yes	Yes	Yes	Yes
R4 (1st crop)	0.655	Yes	Yes	Yes	Yes
R4 (2nd crop)	0.642	Yes	Yes	Yes	Yes

*1st and 2nd crop correpond to the both annual period of sewing during a year (see details in Section 8)

Summary of product exposure and risk assessment for bees

The risk assessment for bees is carried out following the latest approved guidance document Guidance Document on Terrestrial Ecotoxicology, Commission document SANCO/10329/2002 rev 2 (2002).

Based on a laboratory and semi-field endpoint assessment, HQ values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011except for larval assessment. However, higher tier assessment using two semi field studies demonstrate no effect of the PYDIFLUMETOFEN (SYN545974) on bee brood and bee colony for the application rate of 200 g a.s./ha. Thus, requested uses present an acceptable risk for bees.

Effects on bees (Regulation (EU) N° 283/2013, Annex Part A, point 8.3.1 and Regulation (EU) N° 284/2013 Annex Part A, point 10.3.1)*

* This section does reflect the new EFSA Guidance Document on bees which has not yet been noted by the Standing Committee on Plants, Animals, Food and Feed.

Species	Test substance	Risk quotient	HQ/ETR	Trigger
Apis mellifera	PYDIFLUMETOFEN (SYN545974)	HQcontact	<1.7	50
Apis mellifera	PYDIFLUMETOFEN (SYN545974)	HQoral	<2	50
Apis mellifera	A19649B	HQcontact	<0.97	50
Apis mellifera	A19649B	HQoral	<1.1	50
Apis mellifera	A19649B	ETRchronic adult oral	430	1
Apis mellifera	A19649B	ETRlarvae	0.53	1

In addition, semi-field studies have been conducted to support the larval component of the bee risk assessment for the registration of PYDIFLUMETOFEN (SYN545974) globally (***Gonsior, 2017; Kleinhenz, 2017***). The objective of these studies was to determine potential effects on honeybees from exposure to flowering *Phacelia tanacetifolia* treated once at the start of flowering during daily bee flight with A19649B under semi-field conditions. Test item treatment groups included 75, 125 and 200 g a.s./ha.

Honeybee colonies were placed in the tunnels at the start of flowering. The mortality, foraging activity, behaviour of the bees, development of the bee brood assessed in individually marked cells and condition of the colonies were examined prior to and post application. The colonies were monitored at a remote location for two further brood cycles following the initial detailed brood assessments (first brood cycle). The influence of PYDIFLUMETOFEN (SYN545974) was evaluated by comparing the assessment data of the three test item groups (75, 125 and 200 g a.s./ha) to the reference item group and the control group, and by comparing the pre-application data to the post-application data.

Samples of forager bees (for preparation of pollen and nectar), leaves, flowers and samples of soil were collected during the exposure phase. Samples of pollen and nectar (in-hive products), pollen (from pollen trap) and dead bees (from dead bee traps and from the hive bottoms) were collected during the monitoring phase of the study. Samples of pollen and nectar (prepared from forager bees), leaves, flowers, samples of in-hive products and pollen from pollen trap were analysed for residues of PYDIFLUMETOFEN (SYN545974).

There were no detectable residues of PYDIFLUMETOFEN (SYN545974) in any of the samples taken in the control group throughout the study period or in the samples from the test item treatment groups (75, 125 and 200 g a.s./ha) taken prior to application. During the exposure phase in the tunnels, residues of PYDIFLUMETOFEN (SYN545974) were found in leaves, flowers and in pollen and nectar samples from forager bees after application at 0DAA in all treatment groups and decreased within 6 days after application.

In both trials, during the post-application period, no effect on honeybee mortality was observed in the test item treatment groups compared to the control. No test item related effects were observed regarding foraging activity. Slight, but not test item related behavioural changes were observed during the post-application period. The brood and compensation indices and termination rates for eggs, young larvae and old larvae were not statistically

different from the control on any assessment date. The overall honeybee colony development in the test item treatment groups, measured as mean number of cells covered with the different types of brood (eggs, larvae and pupae) or food (nectar, pollen) per colony were not significantly different when compared to the control (except ***Gonsior, 2017*** mean amount of nectar, 75 g a.s./ha, DAA9).

Overall, there was no test item related effect on honeybee mortality, foraging activity, behaviour and brood development in both studies. The results support the conclusion of the initial risk assessment that there is an acceptable risk to larval honeybees from application of PYDIFLUMETOFEN (SYN545974) according to the proposed use pattern (maximum application of 200 g a.s./ha).

Summary of product exposure and risk assessment for non target arthropods

The risk assessment for arthropods other than bees is carried out according to the Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods, ESCORT 2.

Based on a laboratory endpoint assessment, HQ values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011 and pose an acceptable risk for non-target arthropods other than bees.

First tier risk assessment for – Grapes at 200 g a.s./ha x 2 applications (cover other representative uses)

Test substance	Species	Effect (LR_{50} g/ha)	HQ in-field	HQ off-field[1]	Trigger
A19649B	*Typhlodromus pyri*	>408	<0.83	<0.33	2
A19649B	*Aphidius rhopalosiphi*	>408	<0.83	<0.33	2

Summary of product exposure and risk assessment for non-target soil meso- and macro fauna

The risk assessment for earthworms and other soil non-target macro-organisms was carried out according to the Guidance Document on Terrestrial Ecotoxicology, Commission document SANCO/10329/2002 rev 2 (2002).

Based on a laboratory endpoint assessment, TER values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011 and pose an acceptable risk for non-target soil meso- and macro fauna.

Toxicity/exposure ratios for soil organisms

Grapes at 200 g a.s./ha x 2 applications

Test organism	Test substance	Time scale	Soil PEC[1]	TER	Trigger
Earthworms					
Eisenia fetida	A19649B	Chronic	3.6251	8.8	5
Other soil macroorganisms					
Folsomia candida	A19649B	Chronic	3.6251	674	5
Hypoaspis aculeifer	A19649B	Chronic	3.6251	674	5

[1]$PEC_{soil\ accu}$ from the active substance

Summary of product exposure and risk assessment for soil nitrogen transformation

The risk assessment for soil non-target micro-organisms was carried out according to the Guidance Document on Terrestrial Ecotoxicology, Commission document SANCO/10329/2002 rev 2 (2002).

Based on a laboratory endpoint assessment, TER values for PYDIFLUMETOFEN (SYN545974) exceed the $PEC_{s\ accu}$ and poses an acceptable risk for non-target soil micro-fauna except for use on Grapes (2 x 200 g a.s./ha).

Risk assessment on soil micro-organisms exposed to PYDIFLUMETOFEN (SYN545974)

Test substance	NOEC (mg/kg)	Crop and GAP (g a.s./ha)	Maximum PEC_S (mg/kg)	Acceptable risk (Y/N)
PYDIFLUMETOFEN (SYN545974)	2.71	Grapes (2 x 200)	3.6251	**N**
		Grapes (2 x 40)	0.906	Y
		Pome fruits (3 x 50)	1.3589	Y
		Cucurbits (2 x 50)	0.1998	Y
		Tomatoes (2 x 70)	0.1865	Y
		Potatoes (3 x 40)	0.3201	Y
		Brassicas (2 x 70)	0.5597	Y

Summary of product exposure and risk assessment for non target higher plants

The risk assessment for non-target plants was carried out according to the Guidance Document on Terrestrial Ecotoxicology, Commission document SANCO/10329/2002 rev 2 (2002).

Based on a laboratory endpoint assessment, TER values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011 and pose an acceptable risk for non target higher plants.

Risk assessment on non-target plants exposed to PYDIFLUMETOFEN (SYN545974)

Species	Test substance	ER_{50} (g a.s./ha) vegetative vigour	ER_{50} (g a.s./ha) emergence	Exposure (g a.s./ha)[2]	TER	Trigger
Monocotyledonae: *Zea mays*, *Allium cepa*, *Lolium perenne*, *Triticum aestivum* Dicotyledonae: *Brassica oleracea*, *Beta vulgaris*, *Brassica napus*, *Lycopersicon esculentum*, *Lactuca sativa*, *Glycine max*	A19649B (PYDIFLUMETOFEN (SYN545974) 200 SC)	>200	>200	200 (maximum requested application rate)	>12.47	5

2.10 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA *[SECTIONS 1-6 OF THE CLH REPORT]*

2.10.1 Identity of the substance [section 1 of the CLH report]

2.10.1.1 Name and other identifiers of the substance

All of the information in this section is also available under section 1.3.

2.10.1.2 Composition of the substance

Table 67: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
N-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide; pydiflumetofen	≥ 98% (w/w)		

Table 68: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
No relevant impurity There are confidential impurities. Further information can be found in IUCLID.				

Table 69: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
No relevant additive					

Table 70: Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Pydiflumetofen; PYDIFLUMETOFEN (SYN545974) pure substance 99.5%	99.5% w/w			See table 1 of physico-chemical properties
Pydiflumetofen; PYDIFLUMETOFEN (SYN545974) Technical substance 98.5%	98.5% w/w			See table 1 of physico-chemical properties

2.10.2 Proposed harmonized classification and labelling

2.10.2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 71: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	Not applicable										
Dossier submitters proposal	616-RST-VW-Y	*N*-methoxy-*N*-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide; pydiflumetofen	Not allocated	1228284-64-7	Aquatic acute 1 Aquatic chronic 1	H400 H410	GHS09 Wng	H410		Acute M-factor = 1 Chronic M-factor = 1	
Resulting Annex VI entry if agreed by RAC and COM											

2.10.2.2 Additional hazard statements / labelling

Table 72: Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	Conclusive but no sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	hazard class not applicable	No
Flammable solids	Conclusive but no sufficient for classification	Yes
Self-reactive substances	Hazard class not assessed in the dossier	Yes
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	Hazard class not assessed in the dossier	Yes
Self-heating substances	Conclusive but no sufficient for classification	Yes
Substances which in contact with water emit flammable gases	Hazard class not assessed in the dossier	Yes
Oxidising liquids	hazard class not applicable	No
Oxidising solids	Conclusive but no sufficient for classification	Yes
Organic peroxides	hazard class not applicable	No
Corrosive to metals	Hazard class not assessed in the dossier	Yes
Acute toxicity via oral route	Conclusive but no sufficient for classification	Yes
Acute toxicity via dermal route	Conclusive but no sufficient for classification	Yes
Acute toxicity via inhalation route	Conclusive but no sufficient for classification	Yes
Skin corrosion/irritation	Conclusive but no sufficient for classification	Yes
Serious eye damage/eye irritation	Conclusive but no sufficient for classification	Yes
Respiratory sensitisation	Conclusive but no sufficient for classification	Yes
Skin sensitisation	Conclusive but no sufficient for classification	Yes
Germ cell mutagenicity	Conclusive but no sufficient for classification	Yes
Carcinogenicity	Conclusive but no sufficient for classification	Yes
Reproductive toxicity	Conclusive but no sufficient for classification	Yes
Specific target organ toxicity-single exposure	Conclusive but no sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Conclusive but no sufficient for classification	Yes
Aspiration hazard	hazard class not applicable	No
Hazardous to the aquatic environment	Aquatic acute 1; H400 Aquatic chronic 1;	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
	H410	
Hazardous to the ozone layer	Conclusive but no sufficient for classification	Yes

2.10.3 History of the previous classification and labelling

Not applicable. Pydiflumetofen has no previous classification and labelling.

2.10.4 Identified uses

Pydiflumetofen is a new broad-spectrum foliar fungicide used on various crops. For more details, please refer to the GAP table on 1.5.

2.10.5 Data sources

Please refer as well to DAR Volumes 3 CA, B1, B2, B6, B8 and B9.

2.11 RELEVANCE OF METABOLITES IN GROUNDWATER

There are no metabolites formed in amounts triggering a groundwater risk assessment.

2.11.1 STEP 1: Exclusion of degradation products of no concern

Not applicable.

2.11.2 STEP 2: Quantification of potential groundwater contamination

Not applicable.

2.11.3 STEP 3: Hazard assessment – identification of relevant metabolites

2.11.3.1 STEP 3, Stage 1: screening for biological activity

Not applicable.

2.11.3.2 STEP 3, Stage 2: screening for genotoxicity

Not applicable.

2.11.3.3 STEP 3, Stage 3: screening for toxicity

Not applicable.

2.11.4 STEP 4: Exposure assessment – threshold of concern approach

Not applicable.

2.11.5 STEP 5: Refined risk assessment

Not applicable.

2.11.6 Overall conclusion

Not applicable.

2.12 CONSIDERATION OF ISOMERIC COMPOSITION IN THE RISK ASSESSMENT

2.12.1 Identity and physical chemical properties

See section 2.2.1.

2.12.2 Methods of analysis

Analytical method SA-97/1 (Mink C. 2015a&b) for the determination of pydiflumetofen (enantiomeric ratio) in technical active substance has been provided and validated according to guidance SANCO3030/99/rev.4.

2.12.3 Mammalian toxicity

The ratio of the PYDIFLUMETOFEN (SYN545974) enantiomers has been examined in samples from crop metabolism studies (see Section 1.5). The data from all these studies show consistently that the ratio of the PYDIFLUMETOFEN (SYN545974) did not change significantly over the course of these studies. Given the lack of potential for interconversion of the PYDIFLUMETOFEN (SYN545974) enantiomers and stability in the enantiomer ratios in all of the samples examined it is concluded that the enantiomers of PYDIFLUMETOFEN (SYN545974) degrade at similar rates and that there is no preferential metabolism of either enantiomer in plant matrices. Therefore the substance tested in all toxicological studies is a true reflection of the exposure to PYDIFLUMETOFEN (SYN545974).

2.12.4 Operator, Worker, Bystander and Resident exposure

The ratio of the PYDIFLUMETOFEN (SYN545974) enantiomers has been examined in environmental fate compartments and in samples from crop metabolism studies. The data from all these studies show consistently that the ratio of the PYDIFLUMETOFEN (SYN545974) did not change significantly over the course of these studies. Given the lack of potential for interconversion of the PYDIFLUMETOFEN (SYN545974) enantiomers and stability in the enantiomer ratios in all of the samples examined it is concluded that the enantiomers of PYDIFLUMETOFEN (SYN545974) degrade in the environment at similar rates and that there is no preferential metabolism of either enantiomer in plant matrices. These findings confirm that consideration of an enantiomer ratio of 1.0 (i.e. an enantiomer fraction ratio of 50:50) is appropriate for the operator risk assessment of PYDIFLUMETOFEN (SYN545974). Therefore the substance tested in all operator, worker, bystander and resident exposure studies is a true reflection of the exposure in the environment and the enantiomer ratio has no impact on the risk assessment

2.12.5 Residues and Consumer risk assessment

The ratio of the PYDIFLUMETOFEN (SYN545974) enantiomers has been examined in selected samples from the 3 primary crop metabolism studies and from the confined rotational crop study. The data from these studies show consistently that the ratio of the PYDIFLUMETOFEN (SYN545974) enantiomers did not change significantly over the course of the study in any of the commodities analysed. Given the lack of potential for interconversion of the PYDIFLUMETOFEN (SYN545974) enantiomers and stability in the enantiomer ratios in all the commodities tested it is concluded that there is no preferential metabolism of either enantiomer in plant matrices. These findings confirm that consideration of an enantiomer ratio of 1.0 (i.e. an enantiomer fraction ratio of 50:50) is appropriate for the dietary risk assessment of PYDIFLUMETOFEN (SYN545974).

Due to the generally very low levels of parent PYDIFLUMETOFEN (SYN545974) remaining in the animal tissues from the ^{14}C goat and hen metabolism studies, compared to that present in crop metabolism studies, no attempt was made to measure the enantiomer ratio in animal commodities.

2.12.6 Environmental fate

The enantiomeric composition of PYDIFLUMETOFEN (SYN545974) in soils was determined at the end of the aerobic and anaerobic incubations in soil, at the end of the irradiation period in the soil photolysis study, at the end of the aerobic mineralisation study, at the end of the aerobic and anaerobic incubations in water/sediment studies,

and at the end of the irradiation period in the water photolysis study, compared to the ratio in the PYDIFLUMETOFEN (SYN545974) application solutions. The PYDIFLUMETOFEN (SYN545974) enantiomer did not change significantly over the course of these degradation studies. Given the lack of potential for interconversion of the PYDIFLUMETOFEN (SYN545974) enantiomers, these findings confirm that consideration of an enantiomer ratio of 1 (*i.e.* an enantiomer fraction ratio of 50:50) is appropriate for the assessment of the environmental risk from PYDIFLUMETOFEN (SYN545974).

2.12.7 Ecotoxicology

The ratio of the PYDIFLUMETOFEN (SYN545974) enantiomers has been examined in environmental fate comparents and in samples from crop metabolism studies (sees **Document N5**). The data from all of these studies show consistently that the ratio of PYDIFLUMETOFEN (SYN545974) enantiomers did not change significantly over the course of these studies. Given the lack of potential for interconversion of the PYDIFLUMETOFEN (SYN545974) enantiomers and stability in the enantiomer ratio in all of the samples examined, it is concluded that the enantiomers of PYDIFLUMETOFEN (SYN545974) degrade in the environment at similar rates and that there is no preferential metabolism of either enantiomer in plant matrices. Therefore the substance tested in all ecotoxicological studies is a true reflection of the exposure in the environment and the enantiomer ratio has no impact on the ecological risk assessment.

2.13 RESIDUE DEFINITIONS

2.13.1 Definition of residues for exposure/risk assessment

Food of plant origin: PYDIFLUMETOFEN (SYN545974)
Food of animal origin:

- **All matrices:** Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974)

- **Ruminant liver:** Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897

- **ruminant kidney:** Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-trichlorophenol (free and conjugated) and SYN548263 expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897

Soil: PYDIFLUMETOFEN (SYN545974)

Groundwater: PYDIFLUMETOFEN (SYN545974)

Surface water: PYDIFLUMETOFEN (SYN545974), SYN548261 and NOA449410

Sediment: PYDIFLUMETOFEN (SYN545974), SYN545547

Air: PYDIFLUMETOFEN (SYN545974)

2.13.2 Definition of residues for monitoring

Food of plant origin: Pydiflumetofen (SYN545974, parent)

Food of animal origin: Pydiflumetofen (SYN545974, parent) and its metabolite 2,4,6-TCP (free and conjugates) expressed as PYDIFLUMETOFEN (SYN545974)

Soil: Pydiflumetofen (SYN545974 parent)

Groundwater: Pydiflumetofen (SYN545974 parent)

Surface water: Pydiflumetofen (SYN545974 parent)

Sediment: Pydiflumetofen (SYN545974 parent)

Air: Pydiflumetofen (SYN545974 parent)

Level 3

PYDIFLUMETOFEN

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 BACKGROUND TO THE PROPOSED DECISION

3.1.1 Proposal on acceptability against the decision making criteria – Article 4 and annex II of regulation (EC) No 1107/2009

3.1.1.1 Article 4				
		Yes	No	
i)	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.	X		RMS considers that Pydiflumetofen can be approved under Regulation (EC) 1107/2009 and that authorizations of PPP can be granted in at least one member States.
3.1.1.2 Submission of further information				
		Yes	No	
i)	It is considered that a complete dossier has been submitted	X		RMS considers that a complete dossier was submitted. However, please refer to Table 3.1.4
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.			
3.1.1.3 Restrictions on approval				
		Yes	No	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance				
Dossier				
		Yes	No	
	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		Please refer to Level 2.6
	It is considered that the dossier contains the information necessary to	X		Please refer to Level 2.7

	carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		The information provided is sufficient to describe the fate and behaviour of PYDIFLUMETOFEN (SYN545974) in soil, water and air, and to estimate the exposure in soil, groundwater, surface water, sediment and air for all intended uses. The information provided is sufficient to evaluate the impact on non-target species
Efficacy				
		Yes	No	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		The field trials data supporting effectiveness of A19649B against these targets comprise 173 trials conducted over 2 years. These trials have shown the interest of A19649B against a broad range of diseases in grapes, pome fruits, potato, Brassicae, Cucurbits and tomato.
Relevance of metabolites				
		Yes	No	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		There are no metabolites formed in amount triggering a groundwater risk assessment. However, additional information is requested to determine the toxicological profile on metabolite SYN547897 in order refine the consumer risk assessment (see below 3.1.4)

Composition				
		Yes	No	
	It is considered that the specification defines the minimum degree of purity, the identity and maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.	X		Pydiflumetofen is manufactured with a minimum purity of 980 g/kg
	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.	NA	NA	Pydiflumetofen is a new active substance, therefore, no FAO specification exist.
	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted	NA	NA	
Methods of analysis				
		Yes	No	
	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		Analytical methods for the determination of Pydiflumetofen and its manufacturing impurities, in technical material, were evaluated and considered acceptable and relevant in terms of current standards and test guidelines. Nevertheless, a reagent blank solvent should be provided to validate the specificity of the analytical method. For the significant impurities see Volume 4 of the RAR. See level 2, part 2.5.2.
	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.	X		See level 2, part 2.5.2.
	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		
Impact on human health				
Impact on human health - ADI, AOEL, ARfD				
		Yes	No	
	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be	X		The **ADI** is set at **0.092 mg/kg bw per day**, based on the 80-week mouse

	established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.			carcinogenicity study and by using a safety factor of 100 (see Level 2.6.10.1). The **AOEL** is set at **0.1 mg/kg bw per day**, based on the rabbit developmental toxicity study and by using a safety factor of 100. No correction for the extent of oral absorption is necessary (see Level 2.6.10.3). The **ARfD** is set at **0.1 mg/kg bw**, based on the rabbit developmental toxicity study and by using a safety factor of 100 (see Level 2.6.10.2). The **AAOEL** is set at **0.1 mg/kg bw per day**, based on the rabbit developmental toxicity study and by using a safety factor of 100. No correction for the extent of oral absorption is necessary (see Level 2.6.10.4).
Impact on human health – proposed genotoxicity classification				
		Yes	No	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B.**		X	Based on the results of *in vitro* and *in vivo* genotoxicity studies, pydiflumetofen is considered to be not genotoxic (a confirmatory *in vivo* genotoxicity assay is required) (see Level 2.6.4).
Impact on human health – proposed carcinogenicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B.**		X	Pydiflumetofen did not show carcinogenic effect in the rat or female mouse in a 2-year rat study and a 18-month mouse study respectively. In male mice, increased incidences of liver adenomas and carcinomas were observed in a 18-month mouse study. However, due to the demonstrated human non-relevance of these liver tumors, pydifllumetofen did not meet the criteria for classification (see level 2.6.5).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans			

	and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed reproductive toxicity classification				
		Yes	No	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B.**		X	Pydiflumetofen did not show effects on fertility in a 2-generation rat toxicity study. No severe developmental effects were observed after exposure of pregnant rats or rabbits to pydiflumetofen (see Level 2.6.6).
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Impact on human health – proposed endocrine disrupting properties classification				
		Yes	No	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **carcinogenic category 2 and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties**		X	Pydiflumetofen is not classified or proposed to be classified as carcinogenic category 2 and toxic for reproduction category 2.
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, as **toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**		X	Pydiflumetofen is not classified or proposed to be classified as toxic for reproduction category 2. Moreover, pydiflumetofen did not show effects on endocrine organs (see Level 2.6.8).
iii)	Linked to either i) or ii) immediately above.			

	It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.		X	The criterion for persistence is fulfilled. The criterion for bioaccumulation is not fulfilled. The criterion for long range transport is not fulfilled.
Persistent, bioaccumulative and toxic substance (PBT)				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.		X	The criterion for persistence is fulfilled. The criterion for bioaccumulation is not fulfilled. The criterion for toxicity is not fulfilled.
Very persistent and very bioaccumulative substance (vPvB).				
		Yes	No	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.		X	The criterion for persistence is fulfilled. The criterion for bioaccumulation is not fulfilled.
Ecotoxicology				
		Yes	No	
	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.	X		Please refer to Level 2 section 2.9

	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	No relevant effect of endocrine disruption properties of the active substance has been observed.
	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			No relevant effect of endocrine disruption properties of the active substance has been observed.
	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behaviour.	X		Based on a laboratory and semi-field endpoint assessment, HQ values for PYDIFLUMETOFEN (SYN545974) exceed the trigger set by Commission regulation (EU) 546/2011except for larval assessment. However, hier tier assessment using two semi field studies demonstrate no effect of the PYDIFLUMETOFEN (SYN545974) on bee brood and bee colony for the application rate of 200 g a.s./ha. Thus, representative uses present an acceptable risk for bees.
Residue definition				
		Yes	No	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		Residue definition for monitoring and risk assessment in plant commodities : PYDIFLUMETOFEN (SYN545974) Residue definition for monitoring in livestock: Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) Residue definition for risk assessment in livestock: - All matrices: Sum of PYDIFLUMETOFEN (SYN545974) and 2,4,6-trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) - Ruminant liver: Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-

				trichlorophenol (free and conjugated) expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897 - Ruminant kidney: Sum of PYDIFLUMETOFEN (SYN545974), 2,4,6-trichlorophenol (free and conjugated) and SYN548263 expressed as PYDIFLUMETOFEN (SYN545974) and separately SYN547897
Fate and behaviour concerning groundwater				
		Yes	No	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		PECgw for PYDIFLUMETOFEN (SYN545974) are < 0.1 µg/L in all scenarios for all intended uses. Therefore the potential for groundwater exposure by PYDIFLUMETOFEN (SYN545974) above the parametric drinking water limit of 0.1 µg/L from the representative uses is expected to be low in geoclimatic situations that are represented by the relevant FOCUS groundwater scenarios. There are no metabolites formed in amounts triggering a groundwater risk assessment.

3.1.2 Proposal – Candidate for substitution

Candidate for substitution				
		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution		X	Toxicology: No (It is to be noted that proposed reference values for pydiflumetofen are not significantly lower than those of the majority of active substances taking into account the threshold mentioned in the Commission document *Questions and Answers on Candidates for Substitution* Rev. 1, January 2015 in which threshold for ADI is 0.001 mg/kg bw/d, threshold for ARfD is 0.004 mg/kg bw and threshold for AOEL is 0.001 mg/kg bw/d). Fate and behaviour in the environment: No Ecotoxicology: No

3.1.3 Proposal – Low risk active substance

Low-risk active substances				
		Yes	No	
	It is considered that the active substance **shall be considered of low risk.** In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	The active substance is persistent in soil.

3.1.4 List of studies to be generated, still ongoing or available but not peer reviewed

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going.	**Study on-going and anticipated date of completion**	**Study available but not peer-reviewed**
3.1.4.1 Identity of the active substance or formulation				
Not necessary				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation				
Shelf life following storage at ambient temperature *Fumeaux J., 2017a (A19649B_10311)*			X Finished around 07/2017	
3.1.4.3 Data on uses and efficacy				
Not necessary				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
Not necessary				
3.1.4.5 Methods of analysis				
Reagent blank solvent chromatograms for the determination of impurities in technical active substance		X		
A cross validation with acetonitrile:water 50:50 should be provided on all plant matrices group to validated the extraction efficiency		X		

The study Richter, S. 2015 (Syngenta File No. SYN545974_10169) should be updated with the determination of pydiflumetofen (SYN545974) in muscle.		X		
3.1.4.6 Toxicology and metabolism				
Comparative *in vitro* metabolism study with the active substance	Relevant for all representative uses.		X (no anticipated date of completion but expected for the EFSA evaluation)	
Safety data sheets of starting materials according to EU requirements	Relevant for all representative uses.	X		
A confirmatory genotoxicity test like a COMET assay on male mouse (gastrointestinal tract and liver)	Relevant for all representative uses.	X		
3.1.4.7 Residue data				
One additional residue trial on potato conducted in the South of Europe to confirm the non-residue situation	Relevant for all representative uses.		X December 2017	
Additional field rotational crop studies on an allium (onion genus), a legume and a leguminous crop	Relevant for all representative uses.	X		
Additional data for intermediate plant back intervals in field rotational crops	Relevant for all representative uses.	X		
3.1.4.8 Environmental fate and behaviour				
Information to address the effect of water treatment processes on the nature of the residues that might be present in water when it is	Relevant for all representative uses	X		

abstracted for drinking water.				
PECsw calculations for PYDIFLUMETOFEN (SYN545974) (Step 1 to 3) and its metabolites (Step 1-2) for the use on pome fruits to cover applications between BBCH 56-69	Relevant for pome fruits	X		
PECsw calculations for PYDIFLUMETOFEN (SYN545974) in Step 3 for the use on potatoes with correct interval of 14 days (for multiple applications, late application window)	Relevant for potatoes	X		
3.1.4.9 Ecotoxicology				
A new study with higher tested dose for soil-microorganisms	Relevant for use on Grapes (2 x 200 g a.s./ha)			X

3.1.5 Issues that could not be finalised

An issue is listed as an issue that could not be finalised where there is not enough information available to perform an assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

Area of the risk assessment that could not be finalised on the basis of the available data	**Relevance in relation to representative use(s)**
Information to address the effect of water treatment processes on the nature of the residues that might be present in water when it is abstracted for drinking water.	Relevant for all representative uses
Based on available data, the risk assessment cannot always be finalised for soil micro-organisms	Grape (2 x 200 g a.s./ha)

3.1.6 Critical areas of concern

An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

Critical area of concern identified	**Relevance in relation to representative use(s)**
None	N.A.

3.1.7 Overview table of the concerns identified for each representative use considered

(If a particular condition proposed to be taken into account to manage an identified risk, as listed in 3.3.1, has been evaluated as being effective, then 'risk identified' is not indicated in this table.)
All columns are grey as the material tested in the toxicological studies has not been demonstrated to be representative of the technical specification.

Representative use		Pome fruit	Grape	Potato	Tomato	Cucurbits (Cucumber, Zucchini, Melon, Water Melon	Cabbage (Cabbage, Broccoli, Cauliflower, Kale, Brussels sprouts, Kohlrabi)
Operator risk	Risk identified						
	Assessment not finalised						
Worker risk	Risk identified						
	Assessment not finalised						
Bystander risk	Risk identified						
	Assessment not finalised						
Consumer risk	Risk identified						X (acute risk for kale)
	Assessment not finalised						
Risk to wild non target terrestrial vertebrates	Risk identified						
	Assessment not finalised						
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified						
	Assessment not finalised		X (Soil micro-organisms)				
Risk to aquatic organisms	Risk identified						
	Assessment not finalised						
Groundwater exposure active substance	Legal parametric value breached						
	Assessment not finalised						
Groundwater exposure metabolites	Legal parametric value breached						

	Parametric value of 10µg/L[(a)] breached						
	Assessment not finalised						
Comments/Remarks							

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a): Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	**Justification**
Fate and behaviour	Discussion on the appropriate solvent extraction systems for determining route and rate of degradation in soil. This discussion would be relevant for this active substance but also on a more general perspective.

3.1.9 Critical issues on which the Co RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process should be listed.

Issue on which Co-RMS disagrees with RMS	**Opinion of Co-RMS**	**Opinion of RMS**
None		

3.2 PROPOSED DECISION

It is proposed that:

Pydiflumetofen can be approved under Regulation (EC) No 1107/2009

It is considered that the following is specified in Part A of the Commission Implementing Regulation for the approval of the active substance:

Not applicable.

It is considered that the following be specified in Part B of the Commission Implementing Regulation as areas requiring particular attention from Member States when evaluating applications for product authorisation(s):

- Rotation with legume, leguminous crops or allium crops are not recommended
- A plant back interval of 365 days should be respected for roots and leafy crops

It is considered that it should be specified that conditions of use shall include risk mitigation measures, where appropriate.

Not applicable

It is proposed that the Member States concerned shall request the submission of confirmatory information:

Not applicable

3.3 RATIONAL FOR THE CONDITIONS AND RESTRICTIONS TO BE ASSOCIATED WITH THE APPROVAL OR AUTHORISATION(S), AS APPROPRIATE

3.3.1 Particular conditions proposed to be taken into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
- Rotation with fruiting and legume vegetables, pulses or bulb vegetables are not recommended - A plant back interval of 365 days should be respected for roots and leafy crops	*All uses which can be rotated*

3.4 APPENDICES

GUIDANCE DOCUMENTS USED IN THIS ASSESSEMENT

General

Common template to be used for Assessment Reports and proposals for harmonised Classification and Labelling (CLH report). SANCO/12592/2012 –rev. 1

EFSA Guidance on Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011;9(2):2092.

Section identity, physical chemical and analytical methods

Section physico chemical properties
Manual on development and use of FAO and WHO specifications for pesticides, November 2010 - second revision of the First Edition, WHO, Rome 2010

Chemicals Regulation Directorate, DATA REQUIREMENTS HANDBOOK, (Version 2.2, June 2012)
Technical monograph N°17, 2nd edition, Guidelines for Specifying the Shelf Life of Plant Protection Products, June 2009

Evaluation Manual for the Authorisation of plant protection products and biocides according to Regulation (EC) No 1107/2009, EU part, Plant Protection Products, Chapter 2 Physical and chemical properties, version 2.0; January 2014, Board
Guidance ST/SG/AC 10/11/Rev.5 for the safety properties

CLP regulation 1272/2008

Regulation (UE) N°283/2013 (1st March 2013) setting out data requirements for active substances, in accordance with regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

Regulation (UE) N°284/2013 (1st March 2013) setting out data requirements for plant protection products, in accordance with regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market

Section analytical methods
SANCO/3030/99 rev.4: Technical Material and preparations: guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414

SANCO/3029/99 rev .4: Residues: guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, section 4) and Annex III (part A, Section 5) of directive 91/414

SANCO/825/00 rev.8.1: Guidance document on pesticide residues analytical methods

Section Data on application and efficacy
SANCO/10054/2013 – rev. 3: Guidance document on data requirements on efficacy for the dossier to be submitted for the approval of new active substances as defined under Regulation (EC) No 1107/2009 contained in plant protection products

Section Toxicology
EFSA Panel on Plant Protection Products and their Residues (PPR); Guidance on Dermal Absorption. EFSA Journal 2012;10(4):2665. [30 pp.] doi:10.2903/j.efsa.2012.2665

Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. OJ L 353, 31.12.2008, 1-1355.

ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, version 4.1 June 2015.

Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014;12(10):3874

OECD 2012: guidance document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452 and 453. 2nd edition. ENV/JM/MONO(2011)47.

Section Residue and consumer risk assessment
FAO (Food and Agriculture Organization of the United Nations), 2009. Submission and evaluation of pesticide residues data for the estimation of Maximum Residue Levels in food and feed**.** Pesticide Residues. 2nd Ed. FAO Plant Production and Protection Paper 197, 264 pp.

OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in crops. No. 501, OECD, Paris 2007.

OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in rotational crops. No 502, Paris 2007.

OECD, 2007, OECD Guidelines for the testing of chemicals – Metabolism in livestock, No. 503, OECD, Paris 2007.

OECD, 2007, OECD Guidelines for the testing of chemicals – Residues in rotational crops (limited field studies). No 504, Paris 2007.

OECD, 2007. OECD Guidelines for the testing of chemicals – Stability of pesticide residues in stored commodities. No 506, OECD, Paris 2007.

OECD, 2007. OECD Guidelines for the testing of chemicals – Nature of the pesticide residues in processed commodities, high temperature hydrolysis. No 507, Paris 2007.

OECD, 2008. OECD Guidelines for the testing of chemicals – Magnitude of pesticide residues in processed commodities. No 508, Paris 2008.

OECD, 2009. OECD Guidelines for the testing of chemicals – Crop field trial. No 509, Paris 2009

Section fate and behavior in environment

European Commission (2014) "Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU" Report of the FOCUS Ground Water Work Group, EC Document Reference Sanco/13144/2010 version 3, 613 pp.

European Food Safety Authority, 2014. EFSA Guidance Document for evaluating laboratory and field dissipation studies to obtain DegT50 values of active substances of plant protection products and transformation products of these active substances in soil. EFSA Journal 2014;12(5):3662, 37 pp., doi:10.2903/j.efsa.2014.3662

FOCUS (1997) Soil persistence models and EU Registration - The Final Report of the Soil Modelling Workgroup of FOCUS (Forum for the Co-ordination of Pesticide Fate Models and their Use) – 29 February 1997.

FOCUS (2000) FOCUS Groundwater Scenarios in the EU Review of Active Substances. Report of the FOCUS Groundwater Scenarios Workgroup. EC Document Reference Sanco/321/2000 rev.2, 202 pp.

FOCUS (2001). "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.1. 221 pp.

FOCUS (2006). Guidance document on estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005, version 2.0, 434 pp.

FOCUS (2008) "Pesticides in Air: Considerations for Exposure Assessment". Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.

FOCUS (2011). Generic guidance for estimating persistence and degradation kinetics from environmental fate studies on pesticides in EU registration. Version 1.0, 436 pp.

FOCUS (2014) "Generic guidance for Tier 1 FOCUS groundwater assessments". Version 2.2, May 2014.

FOCUS (2015) Generic guidance for FOCUS surface water Scenarios, Version: 1.4, Date: May 2015

Section ecotoxicology

EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals on request of EFSA. EFSA Journal 2009; 7(12):1438.

EPPO/OEPP (2001) EPPO Standards PP1/170(3) Test methods for evaluating the side –effects of plant protection products on honey bees. Bulletin OEPP/EPPO Bulletin 31, 323-330

European Commission, 2002. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2 final, 17 October 2002.

European Commission, 2002. Guidance Document on Aquatic Ecotoxicology Under Council Directive 91/414/EEC. SANCO/3268/2001 rev 4 (final), 17 October 2002.

Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Panel on Plant Protection Products and their Residues, 2013, EFSA Journal 2013;11(7):3290

SETAC (Society of Environmental Toxicology and Chemistry), 2001. Guidance Document on Regulatory Testing and Risk Assessment procedures for Plant Protection Products with Non-Target Arthropods. ESCORT 2.

3.5 REFERENCE LIST

Section identity, physical chemical and analytical methods

None

Section Data on application and efficacy

None

Section Toxicology

None

Section Residue and consumer risk assessment

None

Section fate and behavior in environment

None

Section ecotoxicology

None