



Draft Assessment Report

Evaluation of Active Substances

Plant Protection Products

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

Pydiflumetofen

Volume 3 – B.7 (AS)

Residue data

Great Britain

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Version History

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B.7. RESIDUE DATA

Preface - metabolite names/codes used the B7:

Code Number (Synonyms)	Description	Commodities in the B7 in which metabolite is found in metabolism studies.
SYN545974 – parent pydiflumetofen	N-methoxy-N-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide 1H-Pyrazole-4-carboxamide,3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-	Crop (wheat, oilseed rape, tomato, rotated crops) Livestock (hen, goat)
SYN545547	3-(difluoromethyl)-1-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]pyrazole-4-carboxamide	Crop (wheat, oilseed rape, tomato, rotated crops) Livestock (hen, goat)
SYN547891	3-(difluoromethyl)-N-methoxy-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1H-pyrazole-4-carboxamide	Crop (wheat, oilseed rape, tomato, wheat, rotated crops) Livestock (hen, goat)
NOA449410	3-(difluoromethyl)-1-methyl-pyrazole-4-carboxylic acid	Livestock (hen, goat)
2,4,6-Trichlorophenol 2,4,6-TCP	2,4,6-trichlorophenol	Livestock (hen, goat); <i>also sought in feeding studies</i>
Sulphate conjugate of 2,4,6-trichlorophenol	Sulphate conjugate of 2,4,6-trichlorophenol	Livestock (hen, goat); <i>also sought in feeding studies</i>
SYN548264 N-desmethoxy SYN548263	2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]propanoic acid	Livestock (goat); <i>also sought in ruminant feeding studies</i>
SYN508272 R423363	3-(difluoromethyl)-1-methyl-pyrazole-4-carboxamide	Livestock (hen, goat); <i>also sought in ruminant feeding studies</i>
SYN547897	3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichloro-3-hydroxy-phenyl)ethyl]pyrazole-4-carboxamide	Livestock (hen, goat); <i>also sought in ruminant feeding studies</i>
SYN548263	2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid	Livestock (goat); <i>also sought in ruminant feeding studies</i>
SYN547948	3-(difluoromethyl)-N-[2-hydroxy-1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-N-methoxy-1-methyl-pyrazole-4-carboxamide	Livestock (hen, goat)

B.7.1. STORAGE STABILITY OF RESIDUES

B.7.1.1. Plant matrices

For the new active substance approval of pydiflumetofen in GB; a storage stability study for residues of pydiflumetofen, in a range of plant matrices, was submitted. This study has been evaluated and is discussed below.

Report: K-CA 6.1/07, [REDACTED], [REDACTED] (2015)
Title: Storage Stability in Crops Stored Frozen for up to 23 months. Final Report
Report No: Report No. S13-02224 (Syngenta Report No. TK0103785).
Document No: VV-414120 (Syngenta File No. SYN545974_10278)
Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.
 EU guidance document 7032/VI/95, rev.5, Appendix H of EC document 1607/VI/97 rev. 2 “Storage Stability of Residue Samples”, 22-Jul-1997.

	OECD guideline 506 for testing of chemicals “Stability of Pesticide Residues in Stored Commodities”, 16-Oct-2007.
Guideline deviations:	None
GLP:	Yes

The stability of pydiflumetofen was investigated for 23 months at -18 °C in the following crops and crop matrices: orange (whole fruit), wheat (grain and straw), Adzuki bean (dry bean w/out pod), rapeseed (seed), potato (tuber) and lettuce (whole plant). These crops cover all 5 major crop categories: high acid (orange), high starch (wheat grain, potato), high protein (Adzuki bean), high oil (rapeseed) and high water (lettuce). Data is also available for wheat straw (no group but representative of dry commodities).

Test System

The test conditions of the storage stability study on pydiflumetofen were as follows:

Test substance: pydiflumetofen

Plant matrices: orange (whole fruit), wheat (grain and straw), Adzuki bean (bean w/out pod), rapeseed (seed), potato (tuber) and lettuce (whole plant)

Storage temperature: approx. -18 °C

Storage intervals (nominal, months): 0, 3, 6, 12, 18, 23

Test commodities

Control materials of orange, Adzuki bean and potato were sourced from a local supermarket. The remaining commodities were control samples from separate residue field trials.

Test methods

Homogenised sub-samples of each test commodity (10 g) were fortified with standard solutions of pydiflumetofen in acetonitrile at a rate of 0.20 mg/kg. The solution was left to soak for a short period of time and samples were then transferred to a deep freezer at -18 °C. Untreated samples of each crop commodity were stored for use as control and procedural recovery samples together with test samples.

Three replicate samples of the zero-time samples were extracted for analysis after fortification. Duplicate stored samples were extracted for analysis at 3, 6 and 12, 18 and 23 months. A control sample and two freshly spiked samples were analysed in parallel with each stored sample (procedural recovery).

Residues were analysed using method ‘GRM061.03A’. Method ‘GRM061.03A’ has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (cereal grain and potato), high protein (dry beans) and dry (cereal straw) commodities, with an LOQ of 0.01 mg/kg. The method validation data covers the crops in the storage stability study except oranges. As the method is validated in a high acid commodity (grape), a reduced validation data set is acceptable for oranges. In the storage stability study three samples were prepared at 0.2 mg/kg for all crops, and two per timepoint were analysed (at day 0 all three samples were analysed). Aside from lettuce head at 370 days, and a procedural recovery for Adzuki bean, the mean recoveries were within the acceptable range of 70-110% and the %RSD was ≤20%. Example chromatograms show the method is specific and no significant interference (>30% of the LOQ) was observed. However, recoveries have only been reported at one fortification level and therefore the data set for oranges does not meet the criteria for a reduced validation data set as outlined in SANCO/3029/99 rev. 4. Nevertheless, there is data at a level appropriate to the storage stability tests and residues were demonstrated to be stable over the course of the stability test. Therefore, the method can be considered fit for regulatory purposes for the determination of pydiflumetofen residues in oranges in this study.

Matrix effects were investigated for each commodity, at each time point. Where matrix effects >20 % were observed, matrix matched standards were used for analysis. Where matrix effects were considered insignificant (i.e., <20 %) solvent or matrix matched standards were used. The matrices for which matrix matched standards were used were for all time points: lettuce, adzuki beans, wheat grain, and oilseed rape. Matrix effects were present

for some, but not all of the time points for orange, potato and wheat straw. For time points where effects were significant, matrix matched standards were used; for the remaining time points, either solvent or matrix matched standards were used.

The majority of samples were analysed within 1 day of extraction. A number of sample extracts, from a range of time points and crop matrices were stored for longer than 1 day. Extracts were stored refrigerated at 5 ± 4 °C. The maximum extract storage period was 15 days for oilseed rape, for the zero-time samples. The stability of pydiflumetofen in oil seed rape extracts has been proven for 12 days (please refer to section B.7.1.3, which also reports that pydiflumetofen is stable in extracts for a range of commodity types).

Results

No residues of pydiflumetofen >30 % of the LOQ were observed in any control sample. The non-corrected results of the freezer storage stability of pydiflumetofen are summarised in Table 7-1. The mean procedural recovery of the freshly spiked sample is also reported to demonstrate the effectiveness of the method at the time of analysis.

Table 7-1 Summary of storage stability data for pydiflumetofen in plant matrices

Commodity	Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
		Individual	Mean		
Lettuce head (high water)	0	0.1944, 0.2115, 0.1929	0.1996	100	100
	92	0.1843, 0.1919	0.1881	94	103
	190	0.2020, 0.1982	0.2001	100	99
	370	0.2217, 0.2302	0.2260	113	97
	552	0.1543, 0.1656	0.1600	80	73
	715	0.1613, 0.1768	0.1691	85	80
Orange fruit (high acid)	0	0.1807, 0.2018, 0.2145	0.1990	100	100
	92	0.1569, 0.1689	0.1629	81	93
	190	0.2054, 0.2016	0.2035	102	88
	370	0.1621, 0.2176	0.1899	95	81
	561	0.1717, 0.1629	0.1673	84	73
	708	0.1776, 0.2351	0.2064	103	110
Wheat grain (high starch)	0	0.2209, 0.2251, 0.1897	0.2120	106	106
	91	0.1657, 0.1381	0.1519	76	84
	186	0.1894, 0.1786	0.1840	92	88
	379	0.1727, 0.1915	0.1821	91	99
	551	0.1747, 0.1900	0.1824	91	92
	697	0.1806, 0.1698	0.1752	88	92
Wheat straw (no group, representative of dry commodities)	0	0.1973, 0.1802, 0.1788	0.1850	93	93
	91	0.1986, 0.2235	0.2111	106	108
	186	0.2109, 0.2049	0.2079	104	92
	379	0.1872, 0.1817	0.1845	92	83
	560	0.1794, 0.1797	0.1796	90	91
	705	0.1896, 0.1869	0.1883	94	79

Commodity	Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
		Individual	Mean		
Potato tuber (high starch)	0	0.1704, 0.1879, 0.1584	0.1720	86	86
	97	0.1751, 0.1838	0.1795	90	97
	186	0.2046, 0.2036	0.2041	102	106
	364	0.1794, 0.1689	0.1742	87	93
	551	0.1787, 0.1850	0.1819	91	78
	697	0.1604, 0.1816	0.1710	86	78
Rapeseed (high oil)	0	0.2195, 0.2041, 0.1972	0.2070	104	104
	92	0.1602, 0.1637	0.1620	81	103
	190	0.1963, 0.1787	0.1875	94	110
	370	0.1373, 0.1467	0.1420	71	77
	552	0.1697, 0.1629	0.1634	82	105
	715	0.1528, 0.1402	0.1465	73	75
Adzuki bean (high protein)	0	0.2158, 0.2102, 0.1989	0.2080	104	104
	116	0.2030, 0.1700	0.1865	93	85
	186	0.2153, 0.2201	0.2177	109	98
	364	0.2229, 0.2145	0.2187	109	109
	551	0.1802, 0.1971	0.1887	94	84
	697	0.1981, 0.2151	0.2066	103	115

Residues of pydiflumetofen are considered stable in lettuce head, orange fruit, wheat grain & straw, potato tuber, rapeseed, and Adzuki bean for **at least** 23 months under frozen conditions ($\leq -18^{\circ}\text{C}$).

As residues of pydiflumetofen have been shown to be stable in all five crop commodity categories (high water, high starch, high oil, high acid and high protein) it can be assumed that pydiflumetofen will remain stable in all other commodities for the same period of time (for at least **up to** 23 months), under the same storage conditions ($<-18^{\circ}\text{C}$).

B.7.1.2. Animal matrices

Animal commodities

For the new active substance approval of pydiflumetofen (SYN545974) in GB; storage stability studies for residues of pydiflumetofen, in animal matrices, were submitted. This study has been evaluated and is discussed below.

Report: K-CA 6.1/01, [REDACTED] (2016, final 2017)
Title: SYN545974 – Storage Stability of SYN545974 in Bovine Muscle, Liver, Milk, Fat and Chicken Eggs
Report No: Report No. 36552
Document No: Document No. VV-414208 (Syngenta File No. SYN545974_10291)
Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Guideline deviations:	OECD guideline 506 for testing of chemicals “Stability of Pesticide Residues in Stored Commodities”, 16-Oct-2007.
GLP:	None
	Yes

The storage stability of pydiflumetofen (SYN545974) was investigated for 24 months at -20°C in the following animal matrices: bovine muscle, liver, fat, milk and chicken eggs.

Test System

The test conditions of the storage stability study on pydiflumetofen were as follows:

Test substance: pydiflumetofen (SYN545974, 98.5% purity)
Animal matrices: bovine muscle, liver, fat, milk and chicken eggs
Storage temperature: *ca* -20°C
Storage intervals (nominal): 1, 3, 6, 9, 12, 18 and 24 months

Test commodities

All untreated samples were sourced from a local retail supplier

Test methods

Untreated subsamples of each matrix (10g) were homogenised with aqueous acetonitrile and fortified at 0.1 mg/kg with a standard solution of pydiflumetofen. The fortified samples were then either analysed immediately or stored at -20°C along with an untreated sample of each animal matrix for use as a control.

Triplicate samples for each matrix were immediately analysed after fortification, at zero time. Duplicate stored samples were extracted for analysis at 1, 3, 6, 9, 12, 18 and 24 months. The untreated control sample and two freshly spiked samples were analysed (procedural recoveries) in parallel with the duplicate stored samples.

Residues were analysed by LC-MS/MS using a procedure validated in Charles River Study No. 224292, based on the draft method ‘GRM061.06A’ (KCA1 4.1.2/29, please refer to Volume 3 CA, B.5.1.2). The LOQ of the method is 0.01 mg/kg for all animal matrices.

Acceptable procedural recoveries in accordance with SANCO/3029/99 rev. 4 were presented for all freshly spiked animal matrices except for one sample of bovine liver, which had a procedural recovery of 113% for the freshly spiked sample prepared to test the storage at 1 month, and one milk sample which had a procedural recovery of 111% for the concurrent 9-month sample. These are still considered acceptable, as they are just outside the acceptable range (for % mean recovery in method validations) in SANCO/3029/99 rev. 4 (70 – 110 %) and within the acceptable range (for % mean recovery in method validations) in OECD guidance ENV/JM/MONO(2007)17 (70 -120%). There are only 2 samples at each fortification level therefore it’s not possible to calculate a reliable %RSD based on this. However, given the recoveries are all within the acceptable range and the replicate results are all within <15% of each other (with the exception of bovine muscle at time 0, 27.5% difference), there are no concerns regarding the lack of %RSD data.

Stability of extracts

Within the storage stability study, the storage period between extraction of the samples and analysis was 10 days for baseline preparation samples and 7 days for storage samples (up to 3 days for egg samples). Pydiflumetofen has been confirmed in a separate study (GRM061.06A) to be stable in sample extracts for 6 days in eggs and 7 days in other matrices, therefore the storage period of extracts is covered.

Results

No residues of pydiflumetofen >30 % of the LOQ were observed in the fresh and stored blank samples. The non-corrected results of the freezer storage stability of pydiflumetofen in animal matrices are summarised in Table 7-2. The mean procedural recovery of the freshly spiked sample is also reported to demonstrate the effectiveness of the method at the time of analysis.

Table 7-2 Summary of storage stability data for pydiflumetofen in Animal Matrices Stored at *ca* -20°C

Commodity	Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
		Individual	Mean		
Bovine muscle	0	0.0936, 0.0919, 0.0969	0.0941	94	77
	28	0.0970, 0.0970	0.0970	97	98
	91	0.0730, 0.0682	0.0706	71	72
	181	0.107, 0.110	0.109	109	107
	273	0.104, 0.101	0.103	103	101
	365	0.101, 0.110	0.106	106	100
	547	0.105, 0.107	0.106	106	99
	730	0.106, 0.108	0.107	107	108
Bovine Liver	0	0.0945, 0.0779, 0.0738	0.0821	82	86
	35	0.118, 0.116	0.117	117	113
	92	0.0823, 0.0627	0.0725	73	70
	180	0.098, 0.102	0.100	100	94
	272	0.111, 0.111	0.111	111	108
	364	0.108, 0.111	0.110	110	103
	547	0.0889, 0.123	0.106	106	102
	729	0.106, 0.109	0.108	108	106
Milk	0	0.0975, 0.0866, 0.0890	0.0910	91	83
	34	0.113, 0.108	0.111	111	107
	98	0.0978, 0.0959	0.0969	97	87
	181	0.0988, 0.0950	0.0969	97	93
	273	0.105, 0.106	0.106	106	111
	365	0.113, 0.101	0.107	107	103
	547	0.0984, 0.0980	0.0982	98	92
	737	0.101, 0.100	0.101	101	109
Bovine fat	0	0.0866, 0.0876, 0.0946	0.0896	90	87
	30	0.111, 0.105	0.108	108	104
	78	0.0823, 0.0854	0.0839	84	77
	182	0.0943, 0.0980	0.0962	96	82
	273	0.0957, 0.0979	0.0968	97	92
	365	0.0976, 0.0927	0.0952	95	81
	546	0.0919, 0.0928	0.0924	92	95
	724	0.0987, 0.105	0.102	102	97
Eggs	0	0.101, 0.0988, 0.102	0.101	101	103
	33	0.108, 0.106	0.107	107	107
	90	0.0474, 0.0792	0.0770	77	75
	180	0.0983, 0.0925	0.0954	95	91
	272	0.103, 0.108	0.106	106	101
	365	0.0995, 0.103	0.101	101	100
	547	0.0883, 0.0922	0.0903	90	86
	736	0.102, 0.103	0.103	103	102

The mean recovery for all stored samples was above 70%, indicating no significant decline.

Any slightly high recoveries (>100%) can mostly be attributed to the procedural recovery on the day of analysis. For samples with recoveries >100%, procedural recoveries recorded for the freshly spiked samples at the same time period are also relatively close to the upper range (110%, usually applied to % mean recovery in method validations). As none of the results show a decline of more than 30% there is no concern of instability in accordance with Commission Regulation (EU) No 283/2013.

Residues of pydiflumetofen were therefore shown to be stable in bovine muscle, liver and fat and in milk and eggs for at least up to 24 months when stored at -20°C. It can be assumed that residues of pydiflumetofen in all other animal commodities will be stable for at least up to 24 months when stored under the same conditions.

Report:	K-CA 6.1/01, (2016)
Title:	SYN545974 – Frozen Storage Stability of Residues of SYN508272, SYN548264, SYN547897 and SYN548263 in Animal Matrices
Report No:	Report No. CEMR-7064
Document No:	Document No. VV-412637 (Syngenta File No. SYN508272_10915)
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OECD guideline 506 for testing of chemicals “Stability of Pesticide Residues in Stored Commodities”, 16-Oct-2007.
Guideline deviations:	None
GLP:	Yes

The storage stability of the pydiflumetofen (SYN545974) metabolites: SYN508272, SYN548264, SYN547897 and SYN548263 was investigated for up to 12 months in animal matrices when stored at -18°C. The following animal matrices were studied: bovine liver, kidney and milk.

Metabolite	Commodity
SYN508272	Milk
SYN548264	
SYN547897	Bovine Liver
SYN548263	Bovine Kidney

Test System

The test conditions of the storage stability study on the pydiflumetofen metabolites SYN508272, SYN548264, SYN547897 and SYN548263 were as follows:

Test substance: SYN508272 (97% purity), SYN548264 (100% purity), SYN547897 (100% purity) and SYN548263 (100% purity)

Animal matrices: bovine liver, kidney and milk

Storage temperature: ca -18°C

Storage intervals (nominal): 1, 2, 3, 6, 9, 11 and 12 months and 5 months for SYN547897 in liver

Test commodities

Untreated samples of bovine liver and kidney were sourced from a local butcher and organic milk from a local supermarket.

Test methods

Untreated subsamples of each matrix (10g) were homogenised and fortified at 0.2 mg/kg with a standard solution of the metabolite being studied for that matrix in acetonitrile. Samples of liver were fortified with SYN547897 at 0.2 mg/kg. Samples of kidney were fortified with standard samples of either SYN547897 or SYN548263 at rates of 0.2 mg/kg. Samples of milk were fortified with standard samples of either SYN548264 or SYN508272 at rates

of 0.2 mg/kg. All samples were allowed to equilibrate for 5 minutes after fortification. The fortified samples were then either analysed or stored at -18°C along with untreated samples of each animal matrix for use as controls.

Triplicate samples of SYN548264, SYN508272, SYN547897 and SYN548263 - in each matrix tested for that analyte - were immediately analysed after fortification, at zero time. Duplicate stored samples for each metabolite in each matrix being tested were extracted for analysis at 1, 2, 3, 6, 9, 11 and 12 months. Duplicate stored samples of SYN547897 in liver were also analysed at 5 months. Additionally, a spare set of duplicate samples of SYN547897 in liver and kidney were analysed alongside the standard samples at 9 months. The untreated control sample and two freshly spiked samples (procedural recoveries) were analysed in parallel with the duplicate stored samples for each metabolite in each of their corresponding matrices.

Residues of SYN548264 and SYN508272 in milk were analysed by LC-MS/MS using a modified method based on the method 'GRM061.08A' (KCA1 4.1.2/27, please refer to Volume 3 CA, B.5.1.2). The LOQ of the method is 0.01 mg/kg for both analytes. Residues of SYN547897 in liver and kidney and SYN548263 in kidney were analysed by LC-MS/MS using procedures from method 'GRM061.09A' (KCA1 4.1.2/28, please refer to Volume 3 CA, B.5.1.2). The LOQ of the method is 0.01 mg/kg for both analytes in both matrices.

Acceptable procedural recoveries in accordance with SANCO/3029/99 rev. 4 were presented for all freshly spiked animal matrices, except for the following results: SYN508272 in milk at 0 days (118%), SYN548264 in milk at 367 days (115%), SYN547897 in bovine liver at 27 days (113%) and SYN547897 in bovine kidney at 369 days (112%). However, these are procedural recoveries (means of two values only for each analyte/matrix at the relevant fortification level) and the acceptable range of 70-110% stated in SANCO/3029/99 rev. 4 is for % mean recovery in method validations where there are usually more than two data points contributing to the mean. For a spiking level of 0.2 mg/kg, 70-110% is also the acceptable guideline range for % mean recovery in OECD guidance ENV/JM/MONO(2007)17. All procedural recoveries for SYN548263 in bovine kidney are above 110% (112 – 120%). Method 'GRM061.09A' is validated in section B.5.1.2.5., which is the method used to analyse for residues of SYN548263 in kidney in the storage stability study. All mean recoveries in the method validation were within the acceptable range (70-110%) for both mass transitions for SYN548263, so the over-recovery of results observed in the storage stability study does not appear to be typical for the method.

Stability of extracts

All samples were analysed within 4 days of extraction. Procedural recoveries were stored under the same conditions for the same time period and gave acceptable results, demonstrating stability of the samples in this study. Additionally, as extracts were shown to be stable for at least 7 days for all metabolites in each studied matrix (methods GRM061.08A and GRM061.09A), this is acceptable.

Results

No residues of SYN508272, SYN548264, SYN547897 and SYN548263 >30 % of the LOQ were observed in the fresh and stored blank samples of their respective commodities. The non-corrected results of the freezer storage stability of the pydiflumetofen metabolites SYN508272, SYN548264, SYN547897 and SYN548263 in animal matrices are summarised in Table 7-3, Table 7-4, Table 7-5 and Table 7-6. The mean procedural recovery of the corresponding freshly spiked sample for each metabolite is also reported to demonstrate the effectiveness of the method at the time of analysis

Table 7-3 Stability of SYN508272 residues in milk stored at <-18°C

Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
	Individual	Mean		
0	0.2303, 0.2215, 0.2277	0.2265	113	118
29	0.2167, 0.2159	0.2163	108	110
58	0.2043, 0.2071	0.2057	103	104
101	0.2034, 0.1982	0.2008	100	102

186	0.2063, 0.1982	0.2023	101	103
281	0.1992, 0.2023	0.2008	100	96
338	0.2131, 0.2137	0.2134	107	109
367	0.2005, 0.1947	0.1976	99	104

Table 7-4 Stability of SYN548264 residues in milk stored at <-18°C

Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
	Individual	Mean		
0	0.2154, 0.2169, 0.2184	0.2169	108	107
29	0.1997, 0.2120	0.2059	103	102
58	0.2170, 0.2136	0.2153	108	107
101	0.2122, 0.2065	0.2093	105	108
190	0.2182, 0.2193	0.2187	109	104
281	0.2249, 0.2234	0.2242	112	104
338	0.2133, 0.2127	0.2130	107	101
367	0.2432, 0.2421	0.2426	121	115

Table 7-5 Stability of SYN547897 residues in bovine liver and kidney stored at <-18°C

Commodity	Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%) ¹
		Individual	Mean		
Bovine liver	0	0.1926, 0.1955, 0.1984	0.1955	98	91 (88, 94)
	27	0.2082, 0.2122	0.2102	105	113 (116, 110)
	60	0.1899, 0.1875	0.1887	94	93 (94, 92)
	102	0.1677, 0.1602	0.1640	82	101 (104, 98)
	158	0.1952, 0.1896	0.1924	96	108 (108, 107)
	195	0.1780, 0.1840	0.1810	91	109 (117, 101)
	285	0.1432, 0.1625	0.1529	76	106 (108, 105)
	299	0.1168, 0.1104	0.1136	57	86 (87, 85)
	335	0.1380, 0.1353	0.1366	68	92 (92, 91)
	369	0.1311, 0.1168	0.1240	62	106 (104, 109)
Bovine kidney	0	0.2036, 0.1942, 0.2008	0.1995	100	96 (98, 95)
	27	0.2137, 0.1948	0.2042	102	110 (105, 114)
	60	0.1869, 0.1879	0.1874	94	96 (90, 101)
	102	0.1918, 0.1792	0.1855	93	94 (90, 97)
	195	0.1768, 0.1773	0.1770	89	98 (101, 94)
	285	0.1447, 0.1470	0.1459	73	92 (90, 94)
	299	0.1364, 0.1536	0.1450	73	97 (97, 97)
	335	0.1619, 0.1424	0.1522	76	98 (99, 97)
	369	0.1465, 0.1330	0.1397	70	112 (115, 109)

¹ the individual procedural recoveries are stated in brackets.Table 7-6 Stability of SYN548263 residues in bovine kidney stored at <-18°C

Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
	Individual	Mean		

0	0.2327, 0.2430, 0.2377	0.2378	119	117
27	0.2380, 0.2436	0.2408	120	114
60	0.2362, 0.2383	0.2372	119	119
102	0.2250, 0.2320	0.2285	114	120
195	0.2621, 0.2658	0.2640	132	115
285	0.2267, 0.2302	0.2285	114	112
343	0.2519, 0.2531	0.2525	126	115
369	0.2470, 0.2539	0.2504	125	116

The mean recovery for most of the stored samples was above 70%, indicating no significant decline. However, the following exceptions were noted:

SYN547897 in bovine liver at 9 (one of the two sets of results available), 11 and 12 months is 57%, 68 and 62% respectively. They demonstrate a greater than 30% decline (as outlined in Commission Regulation (EU) No 283/2013) compared to the recovery at time 0. As the procedural recoveries of freshly fortified samples range from 86 – 106%, the low mean recovery is unlikely to result from low procedural recovery on the day of analysis.

SYN547897 in bovine kidney at 12 months was 70%, not indicating an unacceptable decline (>30%). However, there is a general trend of decline with increasing storage time. Additionally, the procedural recovery at the 12-month time point is high (112%), possibly indicating an over-estimation of residue level. If this is taken into account, the decline may exceed 30%. Further discussion of the relevance of this in the context of the feeding studies is given in Volume 1 and in section B.7.4.2.

As discussed above, any high recoveries (SYN548264 in milk at 9 and 12 months, SYN548263 in bovine kidney from 1 – 12 months) can be attributed to relatively high procedural recoveries of freshly spiked samples on the day of analysis. As none of the results show a decline of more than 30% there is no concern of instability in accordance with Commission Regulation (EU) No 283/2013.

SYN508272

Residues of SYN508272 were shown to be stable in milk **up to** at least 12 months when stored at -18°C.

SYN548264

Residues of SYN548264 were shown to be stable in milk **up to** at least 12 months when stored at -18°C.

SYN547897

Residues of SYN547897 were shown to be sufficiently stable in bovine kidneys up to 11 months when stored at -18°C but was only sufficiently stable in bovine liver up to ~9.5 months. After these periods, a start of a decline in residues of SYN547897 was observed towards the end of the study (tested for 12 months).

SYN548263

Residues of SYN548263 were shown to be stable in bovine kidney for **up to** at least 12 months when stored at -18°C.

Report: K-CA 6.1/01, [REDACTED], [REDACTED]. (2016)
Title: SYN545974 – Storage Stability of Residues of Conjugated 2,4,6 Trichlorophenol in Animal Matrices Stored Frozen for up to Twelve Months
Report No: Report No. PTRL Europe ID P 3669 G
Document No: Document No. VV-414155 (Syngenta File No. SYN545974_10280)
Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

Guideline deviations:	OECD guideline 506 for testing of chemicals “Stability of Pesticide Residues in Stored Commodities”, 16-Oct-2007.
GLP:	None
	Yes

The storage stability of conjugated 2,4,6 Trichlorophenol was investigated for up to 12 months at -18°C in the following animal matrices: bovine muscle, liver, kidney, fat, milk and chicken eggs. Samples fortified with conjugated 2,4,6-trichlorophenol were analysed for deconjugated 2,4,6-trichlorophenol.

Test System

The test conditions of the storage stability study on conjugated 2,4,6 Trichlorophenol were as follows:

Test substance: conjugated 2,4,6 Trichlorophenol (ammonium 2,4,6-trichlorophenyl sulfate, SYN548983, 74% purity), and free/deconjugated 2,4,6-Trichlorophenol (12%).

Animal matrices: bovine muscle, liver, kidney, fat, milk and chicken eggs

Storage temperature: *ca* -18°C

Storage intervals (nominal): 1, 2, 3, 6, 11 and 12 months

Test commodities

Untreated samples of bovine muscle, kidney, liver, milk and chicken eggs were sourced from a local supermarket.

Untreated samples of bovine fat were sourced from a butcher.

Test methods

Untreated subsamples of each matrix (10g) were fortified at 0.62 mg/kg with a standard solution of conjugated 2,4,6-trichlorophenol. The reference test item contained mainly conjugated 2,4,6 Trichlorophenol (ammonium 2,4,6-trichlorophenyl sulfate, and also a lower amount of 2,4,6 Trichlorophenol (deconjugated/free); it consisted of 74% conjugated 2,4,6-trichlorophenol (equivalent to approximately 0.5 mg/kg conjugated 2,4,6-trichlorophenol) and 12% of free/deconjugated 2,4,6-trichlorophenol (~ 0.1 mg/kg)) in aqueous acetonitrile. All samples were allowed to equilibrate for 5 minutes after fortification. The fortified samples were then either analysed or stored at -18°C along with untreated samples of each animal matrix for use as controls. Samples were analysed immediately for free/deconjugated 2,4,6-trichlorophenol to establish the stability of conjugated 2,4,6-trichlorophenol. Prior to analysis, all samples were subject to enzyme de-conjugation using β -glucuronidase to give de-conjugated 2,4,6-TCP before the recovery was determined. The enzyme β -glucuronidase also has sulphatase activity so is appropriate to cleave glucuronide and sulphate conjugates.

Triplicate samples for each matrix were immediately analysed for deconjugated 2,4,6-trichlorophenol after fortification and enzyme de-conjugation, at zero time. Duplicate stored samples were extracted for analysis at 1, 2, 3, 6, 9, 11 and 12 months. A control sample and two freshly spiked samples, spiked with 0.5 mg/kg of free/deconjugated 2,4,6-trichlorophenol, were analysed (to assess procedural recoveries) in parallel with the duplicate stored samples for each matrix.

Residues of free/deconjugated 2,4,6-trichlorophenol were analysed by LC-MS/MS using a procedure based on the method ‘GRM061.07A’ validated in PTRL Europe Study P 3613 G. The LOQ of the method is 0.01 mg/kg for all animal matrices. Please refer to Volume 3 CA, B.5.1.2 (KCA1 4.1.2/26) for validation of GRM061.07A.

Stability of extracts

Within the storage stability study, the storage period between extraction of the samples and analysis was 8 days. For this maximum extraction period, samples were kept at room temperature or 37°C for 2 days (18 hours hydrolysis at 37°C required for sample de-conjugation) and 6 days stored frozen (as final extract) prior to analysis. As 2,4,6 – TCP (free and conjugated) has been shown to be stable in fat extracts for up to 10 days and longer for other matrices, the storage period of extracts is covered. Additionally, procedural recovery samples were stored for the same time, under the same conditions, giving acceptable results within the study. This also demonstrates acceptable stability of sample extracts.

Results

No residues of 2,4,6 – TCP >30 % of the LOQ were observed in the fresh and stored blank samples. The non-corrected results of the freezer storage stability of conjugated 2,4,6 – TCP in animal matrices are summarised in Table 7-7. The mean procedural recovery of the freshly spiked sample is also reported to demonstrate the effectiveness of the method at the time of analysis

Table 7-7 Summary of storage stability data of 2,4,6-TCP (free and conjugated) in Animal Matrices Stored at *ca* -18°C

Commodity	Storage period (days)	Residue level in stored sample (mg/kg)		Mean recovery stored sample – uncorrected (% of fortification level)	Mean procedural recovery for freshly spiked sample (%)
		Individual	Mean		
Bovine muscle	0	0.585, 0.585, 0.600	0.590	95	98
	28	0.560, 0.580	0.570	92	91
	56	0.565, 0.525	0.545	88	87
	92	0.515, 0.550	0.533	86	85
	181	0.580, 0.565	0.573	92	83
	330	0.585, 0.590	0.588	95	99
	358	0.550, 0.500	0.525	85	83
Bovine liver	0	0.610, 0.620, 0.620	0.617	100	101
	28	0.451, 0.505	0.478	77	96
	56	0.500, 0.540	0.520	84	89
	92	0.540, 0.585	0.563	91	91
	181	0.590, 0.575	0.583	94	88
	330	0.585, 0.590	0.588	95	98
	358	0.496, 0.483	0.489	79	72
Bovine kidney	0	0.600, 0.575, 0.590	0.588	95	92
	28	0.545, 0.555	0.550	89	86
	56	0.535, 0.540	0.538	87	89
	92	0.459, 0.467	0.463	75	83
	181	0.520, 0.520	0.520	84	84
	330	0.530, 0.535	0.533	86	96
	358	0.488, 0.489	0.488	79	72
Bovine fat	0	0.530, 0.469, 0.465	0.488	79	91
	28	0.520, 0.453	0.487	79	87
	56	0.540, 0.560	0.550	89	91
	92	0.482, 0.464	0.473	76	76
	181	0.480, 0.480	0.480	77	84
	330	0.475, 0.490	0.482	78	95
	358	0.525, 0.515	0.520	84	85
Milk	0	0.590, 0.615, 0.610	0.605	98	93
	28	0.585, 0.570	0.578	93	90
	56	0.625, 0.635	0.630	102	92
	92	0.585, 0.580	0.583	94	91
	181	0.580, 0.545	0.563	91	81
	330	0.540, 0.590	0.565	91	99
	358	0.560, 0.555	0.558	90	86
Eggs	0	0.595, 0.575, 0.570	0.580	94	95
	28	0.515, 0.497	0.506	82	78
	56	0.565, 0.560	0.563	91	86
	92	0.560, 0.515	0.538	87	99

	181	0.555, 0.520	0.538	87	83
	330	0.525, 0.545	0.535	86	98
	358	0.488, 0.494	0.491	79	74

Acceptable procedural recoveries in accordance with SANCO/3029/99 rev. 4 were presented for all freshly spiked animal matrices; the RSD for each commodity was < 20 % (n=14)).

Residues of free and conjugated 2,4,6 – TCP were therefore shown to be stable in bovine muscle, liver, kidney and fat and in milk and eggs for at least up to 12 months when stored at -18°C. It can be assumed that residues of 2,4,6 – TCP in all other animal commodities will be stable for at least up to 12 months when stored under the same conditions.

It is noted that some lower results are observed in bovine liver (77% at 1 month) and bovine fat (76% - 78% at 3 – 11 months), however, there is not an overall trend of decline in either upon storage. Overall, there are no concerns with stability of free and conjugated 2,4,6 – TCP in bovine muscle, liver, kidney and fat and in milk and eggs for at least up to 12 months when stored at -18°C.

B.7.1.3. Stability of residues in sample extracts

No specific information on the stability of residues in sample extracts is provided within the storage stability studies on plant or animal matrices. However, for plant matrices, the stability of sample extracts was considered during the method validation for 'GRM061.03A'. The stability of sample extracts in animal matrices was also considered during the respective method validation report for each metabolite. A summary of the stability for each analytical method is presented in Volume 3 CA B5. For completeness, a summary of the extract stability data is presented below in Table 7-8. The data covering plant extracts is based on storage at 5 ± 4 °C in the dark; the data covering animal matrices is based on storage at 3 – 8 °C in the dark.

Table 7-8 Summary of extract stability data in plant matrices (pydiflumetofen)

Commodity	Storage interval of sample extract (days)	Conclusion
Apple	14	There was no significant difference between the day 0 and day 8-21 results (results were within ±10% of the initial values when re-analysed)
Lettuce	13	
Tomato	14	
Cabbage	19	
Fresh peas	13	
Dry bean	11	
Wheat grain	8	
Wheat straw	11	
Wheat forage	21	
Potato	15	
Rape seed	12	

Table 7-9 Summary of extract stability data in animal matrices

Animal matrix	Storage interval of sample extract (days)					
	Pydiflumetofen ¹	2,4,6 – TCP (free & conjugated) ²	SYN508272 ³	SYN548264 ³	SYN547897 ⁴	SYN548263 ⁴

Bovine meat	7	26	-	-	-	-
Bovine fat	7	10	-	-	-	-
Bovine kidney	7	19	-	-	7	7
Bovine Liver	7	19	-	-	8	-
Milk	7	21	11	11	-	-
Eggs	6	19	-	-	-	-
Conclusion	There was no significant difference between the day 0 and day 6-26 results.					

¹ Study/report no. 'GRM061.06A'

² Study/report no. 'GRM061.07A'

³ Study/report no. 'GRM061.08A'

⁴ Study/report no. 'GRM061.09A'

B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

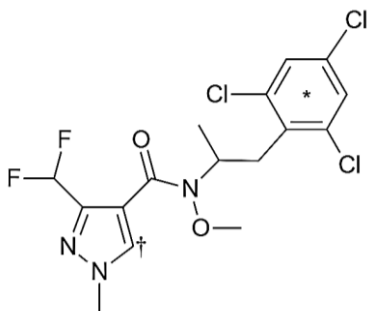
The applicant submitted studies investigating the metabolism of pydiflumetofen in plant (wheat, oilseed rape and tomato) and animal (poultry and goat) matrices which are compliant with current OECD guidelines.

The structure of pydiflumetofen (SYN545974) is shown in Figure 7-1; the label positions are also shown.

The metabolism and distribution of pydiflumetofen in plants and livestock was investigated using the active substance labelled in either the phenyl ring or the pyrazole ring. This is considered appropriate to elucidate sufficient information on the metabolic pathway.

All expressions of the results **in the metabolism studies (section B.7.2)** are given in mg/kg parent equivalents.

Figure 7-1 Structure of pydiflumetofen, with label positions



* [phenyl-¹⁴C]-SYN54974; also referred to as [phenyl-¹⁴C] label

† [pyrazole-5-¹⁴C]-SYN54974; also referred to as [pyrazole-¹⁴C] label

B.7.2.1. Plants

Metabolism in wheat

Report: K-CA 6.2.1/02. [REDACTED], [REDACTED] (2014).
Title: SYN545974 - Metabolism of [¹⁴C]-SYN545974 in Wheat.
Report No: Report Number 33586 (Syngenta Report No. TK0123335)
Document No: VV-411108 (Syngenta File No. SYN545974_10136)
Guidelines: OECD Guideline for the Testing of Chemicals, Metabolism in Crops. Guideline 501 (January 2007)

	OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 64. Series on Pesticides No. 32. Guidance Document on Overview of Residue Chemistry Studies ENV/JM/MONO (2009)31 (July 2009)
	Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market
	Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the 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
	Nature of the Residue - Plants, Livestock; United States Environmental Protection Agency; Residue Chemistry Test Guidelines OPPTS 860.1300; (August 1996)
	Japanese MAFF Guideline on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 2000)
Guideline deviations:	None
GLP:	Yes

Materials and methods

Materials

1. C-label pydiflumetofen (phenyl)

Description: Phenyl-U-¹⁴C – SYN545974 (spec. activity of a.s. 4.11 MBq/mg)

Lot/Batch #: DAD-XIII-38-4

Radiochemical Purity: 98.7 %

2. C-label pydiflumetofen (pyrazole)

Description: Pyrazole-5-¹⁴C – SYN545974 (spec. activity of a.s. 4.01 MBq/mg)

Lot/Batch #: 5222GAR001-1

Radiochemical Purity: 97.7 %

Methods

A study, investigating the metabolism of pydiflumetofen in wheat, was carried out in 2012-2014 at Charles River Laboratories in Edinburgh, Scotland. Spring wheat was treated with two post emergence, foliar spray applications using pydiflumetofen labelled at the [phenyl] or the [pyrazole] position, as shown in Figure 7-1. The experiments with both labels were carried out simultaneously.

Spring wheat seeds (*var. Paragon*) were sown into 8 sample containers, each filled with sandy loam soil. The spring wheat was grown outdoors, the containers were covered with mesh netting to prevent birds and animals from eating the seeds. The containers were treated with [pyrazole-5-¹⁴C]- pydiflumetofen or [phenyl-U-¹⁴C]- pydiflumetofen at a nominal application rate of 250 g a.s./ha (2 applications of 125 g as/ha, per application, 2 x 0.63 N). The radiochemicals were formulated as a suspension concentrate (SC200) and applied to the plants using a hand-held sprayer as spray applications at growth stage BBCH 32-34 for application 1 and BBCH 58 for application 2. The actual application rates achieved were 123.2 g as/ha and 129.1 g as/ha for the [phenyl-¹⁴C]- pydiflumetofen labelled treatments; and 124.2 g as/ha and 128.1 g as/ha for the [pyrazole-¹⁴C]- pydiflumetofen applications.

Daily maximum temperature ranged from 13.3 – 22.3 °C; minimum daily temperature ranged from 3.8 °C – 14.9 °C. There was rainfall (≥8.00 mm) on 8 days out of the 67 days monitored. Wind speed ranged from 6 – 26 mph.

Forage was harvested 10 days after application 1 (BBCH 39) and hay 29 days after application 2 (BBCH 77). Straw and grain were harvested 50 days after application 2, at maturity (BBCH 89). The total radioactive residue (TRR); mg a.s. equivalents per kg of commodity, was measured in each commodity sampled.

Analysis/general procedures

Samples were homogenised and total radioactive residues (TRRs) determined by sample combustion, prior to liquid scintillation counting (LSC). Extraction and further analysis was carried out on samples where the radioactive residue was greater than 0.01 mg/kg. Samples were extracted with aqueous acetonitrile solvent combinations (80 % v/v acetonitrile). Samples were extracted in multiple stages, the solvent: sample ratio remained constant for all samples at each stage of extraction. Where residues exceeded 0.01 mg/kg, the various extracts were combined and concentrated using rotary evaporation, as required. Selected extracts were cleaned up using solid phase extraction (SPE) prior to LC-MS chiral analysis.

Identification of residues in the extracts was conducted using HPLC-UV, with comparison to authentic reference standards of parent pydiflumetofen and its metabolites. Further identification was conducted using 1D-TLC. The following reference standards were used: metabolites which were detected- SYN545547 and SYN547891; metabolites which were not detected-SYN545720, SYN508272, NOA449410, SYN547890, SYN547892, SYN547893, SYN547894, SYN547895, and SYN547897.

The samples were stored (ca.-20 °C) until required for analysis. On the day of harvest, all samples were placed in a freezer set to maintain – 20 °C and stored frozen prior to analysis. Initial analysis of the wheat fractions took place 1 month after harvest although further analysis was conducted on the extracts after this period. The main analysis appeared to have taken place within 10 months of extraction and initial analysis; work on enantiomeric composition of the pydiflumetofen residues took place after this time- see below.

Storage stability investigation -. Storage stability was assessed for the grain and straw by comparing the initial TLC analysis (performed within 1 months of sampling) to the TLC chromatographic profile obtained after 10 months storage (after completion of the main work done on analysis of residues).

Enantiomeric composition: Pydiflumetofen is a racemate. To consider the potential for differential metabolism of the ‘S-’ and ‘R-’ enantiomers of pydiflumetofen, the enantiomeric composition of pydiflumetofen residues in wheat grain and straw was determined by LC-MS (and compared to the composition in spray solution).

Table 7-10 Summary of study design

Label		[phenyl-14C]		[pyrazole-14C]	
Intended use rate (g as/ha)		2 x 125 (total 250)		2 x 125 (total 250)	
Actual application rate (g a.s./ha)		1 st app:	123.2	1 st app:	124.2
		2 nd app:	129.1	2 nd app:	128.1
		Total:	252.3	Total:	252.3
Application timing (BBCH)	1 st app:	32-34			
	2 nd app:	58			
Sampling (DALA)†		Forage: ‡	10	Forage: ‡	10
		Hay:	29	Hay:	29
		Grain:	50	Grain:	50
		Straw:	50	Straw:	50

† Days after last application: growth stages at harvest: forage (BBCH 39); hay (BBCH 77); straw and grain (BBCH 89).

‡ Forage sample taken 10 days after *first* application

Results and discussion

Total radioactive residue (TRR)

The extractable TRR levels were 0.327 mg/kg & 0.445 mg/kg in forage; 0.920 mg/kg & 1.311 mg/kg in hay; 1.232 mg/kg & 1.443 mg/kg in straw, and 0.033 mg/kg & 0.048 mg/kg in grain for the phenyl & pyrazole labels, respectively. Extractability of residues was high for all crop matrices, accounting for $\geq 90\%$ and $\geq 84\%$ TRR for the phenyl and pyrazole labels, respectively.

Table 7-11 Summary of TRR determined by LSC and fractionation – all crop matrices

Label	Crop Commodity	By direct quantification of the sample †	By summation of extracts and debris ‡
[phenyl-14C]	Forage	0.320	0.338
	Hay	1.138	0.977
	Straw	1.250	1.286
	Grain	0.036	0.037
[pyrazole-14C]	Forage	0.456	0.465
	Hay	1.312	1.391
	Straw	1.548	1.527
	Grain	0.055	0.057

† The TRR values of all commodities were initially determined by direct quantification of the radioactivity by combustion/LSC.

‡ The TRR values of all commodities were also determined by the summation of the radioactivity present in the extracts and debris after initial fractionation

The extracted radioactivity was analysed by chromatography. The extracted and identified residues are detailed in Table 7-12 and Table 7-13 for the phenyl & pyrazole label, respectively.

Table 7-12 Summary of identified and characterised residues – all crop matrices – phenyl label

[Phenyl- ¹⁴ C]	forage	hay	straw	grain
DALA	10	29	50	50
BBCH (at sampling)	BBCH 39	BBCH 77	BBCH 89	BBCH 89
extractable TRR % (mg/kg) *	96.5 (0.327)	94.2 (0.92)	95.8 (1.232)	90.4 (0.033)
pydiflumetofen	91.0 (0.307)	84.1 (0.821)	83.6 (1.075)	81.5 (0.030)
SYN545547	1.4 (0.005)	2.4 (0.023)	2.8 (0.036)	2.9 (0.001)
SYN547891	1.2 (0.004)	3.0 (0.029)	2.4 (0.032)	8.3 (0.003)
Unassigned % (mg/kg)	NA	4.2† (0.041)	-	NA
Uncharacterised Extract	-	-	1.9 (0.024)	-
total identified % (mg/kg)	93.6 (0.316)	89.5 (0.873)	88.8 (1.143)	92.7 (0.034)
non extracted TRR % (mg/kg) ‡	3.5 (0.012)	5.8 (0.057)	4.6 (0.059)	9.6 (0.004)
total TRR (by summation) % (mg/kg)	100 (0.338)	100 (0.977)	100 (1.286)	100 (0.037)

† Unassigned extract consisting of at least 3 discrete components, no single one of which $>2.3\%$ TRR, (0.022 mg/kg)

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

Table 7-13 Summary of identified and characterised residues – all crop matrices – pyrazole label

[Pyrazole- ¹⁴ C]	forage	hay	straw	grain
DALA	10	29	50	50
BBCH	BBCH 39	BBCH 77	BBCH 89	BBCH 89
extractable TRR % (mg/kg) *	95.6 (0.445)	94.2 (1.311)	94.5 (1.443)	84.9 (0.048)
pydiflumetofen	84.3 (0.392)	70.5 (0.981)	76.4 (1.167)	81.6 (0.046)
SYN545547	2.7 (0.012)	2.4 (0.034)	3.9 (0.059)	2.6 (0.001)
SYN547891	2.4 (0.011)	3.6 (0.049)	4.3 (0.065)	7.8 (0.004)
Unassigned % (mg/kg)	2.5 (0.012)	13.8† (0.189)	-	3.3‡ (0.002)
Uncharacterised Extract	-	-	1.5 (0.023)	-
total identified % (mg/kg)	89.4 (0.415)	76.5 (1.064)	84.6 (1.291)	92 (0.051)
non extracted TRR % (mg/kg) ¹	4.4 (0.02)	5.7 (0.079)	6.1 (0.093)	15.2 (0.009)
total TRR (by summation) % (mg/kg)	95.6 (0.445)	99.9 (1.391)	101 (1.527)	100 (0.057)

† Unassigned extract comprising at least 12 discrete components, no single one of which >2.6% TRR, (0.036 mg/kg)

‡ Unassigned extract consisting of 1 discrete component, 3.3% TRR, (0.002 mg/kg).

¹ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

Storage stability investigations:

Representative radio chromatograms (TLC) of wheat straw, 10 months after initial analysis were presented in the report (comparisons between TLC before and after the storage period). These representative TLC chromatograms supporting the storage stability work showed the major 'spot' of pydiflumetofen, and then weaker TLC spots for the metabolites SYN545547 and SYN547891. These are sufficient to show that there is no marked qualitative change in the samples over the period of the study, as far as can be seen in the context of the TLC work; these cannot be interpreted quantitatively.

The TLC storage stability results were only able to show the 'weak' TLC spots for the metabolites SYN545547 and SYN547891, and did not cover the low-level peaks (indicated to be more polar in nature)/components that were unassigned (Tables 7-12 and 7-13) during the HPLC analysis and metabolic profiling.

Only limited information is available on stability of the residues from the investigations conducted on stability in this study. Whilst presenting some uncertainty, the data are likely to be sufficient in the context of the conclusions surrounding the proposal for the residue definition. There were unidentified low-level peaks present in the HPLC work. Only pydiflumetofen (SYN545974), and metabolites SYN545547 and SYN547891 were identified in the wheat metabolism study. These three spots (parent and these two identified metabolites) were visible on the TLC radiochromatograms for the 'post storage' wheat straw samples.

Enantiomer composition:

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur. The enantiomeric composition in the spray solution and in grain and straw samples was determined to see whether any change occurred. The enantiomeric fraction was 0.5 in the spray solution. Whilst the LC-MS chromatograms for the grain

and straw samples (enantiomeric analysis) were presented, the peak area estimations were not presented. The R- and S- peaks were similar. Better estimations were possible for the other primary crop metabolism studies on oilseed rape and tomato (see evaluation of these studies below) where the peak area amounts for each of the enantiomers were presented.

HSE is not proposing to consider an assessment factor in the consumer risk assessment to consider the potential changes in isomer ratio/amounts in plants.

See also information derived from published literature reports at the end of section B.7.2.1

The principal component identified in all matrices was pydiflumetofen – accounting for 91.0 % TRR (0.307 mg/kg) and 84.3 % TRR (0.392 mg/kg) in forage, 84.1 % TRR (0.821 mg/kg) and 70.5 % TRR (0.981 mg/kg) in hay, 83.6% TRR (1.075 mg/kg) and 76.4% TRR (1.167 mg/kg) in straw and 81.5 % TRR (0.030 mg/kg) and 81.6 % TRR (0.046 mg/kg) in grain for the phenyl and pyrazole labels, respectively.

The metabolites SYN545547 and SYN547891 were also identified in all commodities. In forage these metabolites accounted individually for <3 % TRR. The remaining extractable residues in the pyrazole label were found to consist of three polar components, none individually exceeding 0.9 % TRR (0.004 mg/kg). In hay they accounted individually for <4 % TRR. The remaining extractable residues for hay were found to consist of 3-12 polar components none of which individually exceeded 2.6 % TRR (0.036 mg/kg), so low %TRR and <0.05 mg/kg (animal feed item).

In straw ≤ 3.9 % TRR (≤ 0.059 mg/kg) was identified as SYN545547 and ≤ 4.3 % TRR (≤ 0.065 mg/kg) as SYN547891. Levels of the metabolite SYN545547 in grain were very low (≤ 2.9 % TRR, ≤ 0.001 mg/kg). SYN547891 was identified at levels ≤ 8.3 % TRR (≤ 0.004 mg/kg). The remaining unassigned extractable residue in grain consisted of a single component of polar radioactivity, accounting for 3.3 % TRR; (0.002 mg/kg) found in the pyrazole label only. No further identification is required for the unassigned extractable residue in grain as the TRR is <10 %, and the concentration is <0.01 mg/kg.

Conclusion

The present study describes the metabolism of pydiflumetofen in wheat, following two foliar spray applications, post emergence. Immature forage was collected 10 days after the first application. Hay was harvested at BBCH stage 77 (29 DALA); straw and grain were harvested at normal commercial harvest, BBCH 89 (50 DALA).

The main observations from the identification work are the following:

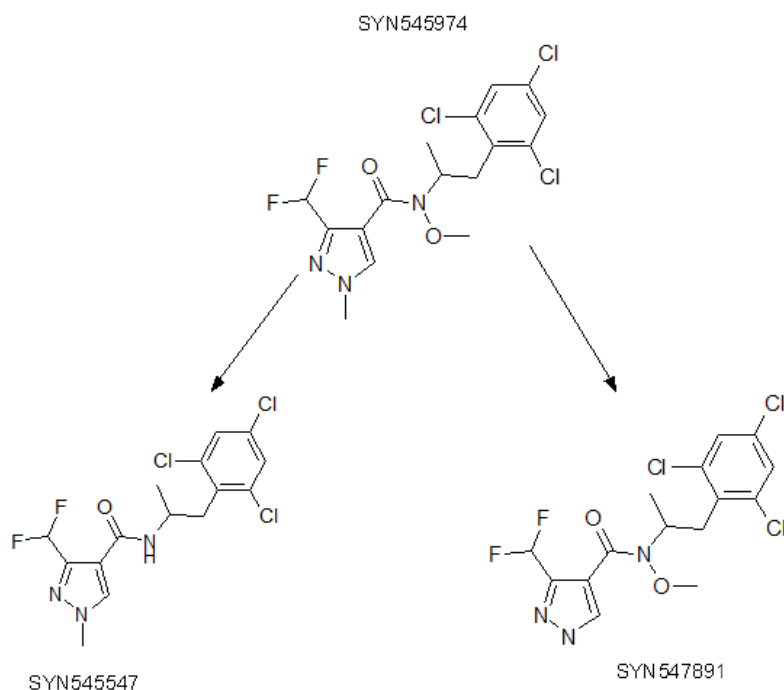
- Parent pydiflumetofen was the principal residue detected in all samples (70.5- 91.0 % TRR, 0.030 – 1.167 mg/kg).
- The highest residue of pydiflumetofen was detected in straw (1.075 – 1.167 mg/kg, ≥ 76.4 % TRR).
- SYN545547 and SYN547891 were detected in each of the samples at lower residues than the parent (max contribution in terms of mg/kg – 0.065 mg/kg for pyrazole labelled SYN547891).
- The highest proportion of the metabolites SYN545547 & SYN547891 was detected in grain: at >11 % TRR in grain samples for each label; next highest contribution of SYN545547 & SYN547891 was 8.2 % in straw, for the pyrazole label.
- Individual levels of each metabolite did not exceed 10 % TRR in any sample.
- Overall, absolute levels of metabolite (in mg/kg) were low in grain: maximum 0.005 mg/kg, for the pyrazole label.
- Levels of SYN545547 and SYN547891 were highest in straw (0.032– 0.065 mg/kg, ≤ 3.9 % TRR).

Unidentified components were present in all samples except straw and in total accounted for 2.5-13.8 % TRR (0.002-0.189 mg/kg); no individual component accounted for >3.3 % (or 0.036 mg/kg in animal feed items (hay) or 0.002 mg/kg (grain)).

The principal biotransformations observed were:

- Demethylation of the pyrazole ring to produce SYN547891.
- Reduction of the parent molecule producing SYN545547.

Figure 7-2 Proposed metabolic pathway of pydiflumetofen in wheat



Metabolism in tomatoes

Report:	K-CA 6.2.1/03. [REDACTED], [REDACTED] (2014)
Title:	SYN545974 - Metabolism of [14C]-SYN545974 in Tomatoes.
Report No:	Report Number 34592 (Syngenta Report No. TK0123336)
Document No:	VV-411024 (Syngenta File No. SYN545974_10143)
Guidelines:	OECD Guideline for the Testing of Chemicals, Metabolism in Crops. Guideline 501 (January 2007) OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 64. Series on Pesticides No. 32. Guidance Document on Overview of Residue Chemistry Studies ENV/JM/MONO (2009)31 (July 2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the 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 Nature of the Residue - Plants, Livestock; United States Environmental Protection Agency; Residue Chemistry Test Guidelines OPPTS 860.1300; (August 1996) Japanese MAFF Guideline on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 2000)
Guideline deviations:	None
GLP:	Yes

Materials and methods

Materials

1. C-label pydiflumetofen

Description: Phenyl-U-¹⁴C – SYN545974 (spec. activity of a.s. 5.79 MBq/mg)

Lot/Batch #: RDR-XVI-76

Radiochemical Purity: 99.8 %

2. C-label pydiflumetofen

Description: Pyrazole-5-¹⁴C – SYN545974 (spec. activity of a.s. 5.06 MBq/mg)

Lot/Batch #: 5283RJP001-2

Radiochemical Purity: 98.8 %

Methods

A study, investigating the metabolism of pydiflumetofen in tomato, was carried out in 2012-2014 at Charles River Laboratories in Edinburgh, Scotland. Tomato plants were treated with either 2 post emergence, foliar spray applications or a single soil application at transplanting, using pydiflumetofen labelled at the [phenyl] or the [pyrazole] position, as shown in Figure 7-1. The experiments with both labels were carried out simultaneously.

Tomato seeds (*var. FI Shirley*) were sown into 18 sample containers, each filled with sandy loam soil. The tomatoes were grown in a greenhouse. 10 sample containers were used for the soil treated samples (5 for each label) and 8 containers were used for the foliar spray treated samples (4 for each sample). The radiochemicals were formulated with a blank formulation as an SC200 (A20048A) for all application methods.

Foliar spray

The tomato plants were treated with two foliar spray applications at growth stage BBCH 83 and BBCH 86. Tomato plants were treated with [phenyl-U-¹⁴C]- pydiflumetofen or [pyrazole-5-¹⁴C]- pydiflumetofen, each at a nominal application rate of 200 g a.s./ha per application (total application rate 400 g as/ha). Total achieved application rates were 393.9 g a.s./ha and 400.5 g a.s./ha for the phenyl and pyrazole-labelled experiments, respectively.

Fruit and foliage were harvested from the foliar treated plants 1 DALA and 14 DALA. Foliage was taken as a contingency and was not analysed. Mature foliar treated fruit was surface rinsed by immersion in acetonitrile which was retained for analysis.

Soil treatment

A separate group of tomato plants were treated with [phenyl-U-¹⁴C]- pydiflumetofen or [pyrazole-5-¹⁴C]- pydiflumetofen as a single soil application. The application was made via pipette to soil surrounding 5 plants (per label) at a nominal application rate of 20 mg a.s./plant.

Immature fruit and foliage were harvested from the soil treated experiment at 28 DALA and mature fruit at 103 DALA. Foliage & immature fruit samples were retained deep frozen as a contingency – and were not analysed.

Analysis/general procedures

Samples were homogenised and TRRs determined by sample combustion analysis, followed by liquid scintillation counting (LSC). Extraction and further analysis was carried out on samples where the radioactive residue was >0.01 mg/kg. Samples were extracted with aqueous acetonitrile solvent combinations (80 % v/v acetonitrile). Samples were extracted in multiple stages, the solvent: sample ratio remained constant for trash samples; the ratio gradually decreased for the additional extractions – this was done by adjusting the actual solvent: sample ratio or by adjusting the concentration of aqueous acetonitrile (to 50 % v/v). Fruit samples were surface washed by immersion three times in acetonitrile, rinsings were then combined for direct LSC analysis and chromatography. Where required, the various extracts were combined and concentrated using rotary evaporation. Selected extracts were cleaned up using solid phase extraction (SPE) prior to LC-MS chiral analysis.

Identification of residues present in the principal fractions was conducted using HPLC, with comparison to authentic reference standards of parent pydiflumetofen and its metabolites. Further identification was conducted using 1D-TLC. The following reference standards were used: metabolites which were detected- SYN545547 and SYN547891; metabolites which were not detected- SYN547890.

All samples not analysed immediately were stored in a freezer set to maintain - 20 °C. Initial analysis of the tomato fruit samples took place approximately 1 month after harvest, and further analytical work, including the enantiomeric composition (see below) took place within a further 6 months.

Storage stability investigation -. Storage stability was assessed for the fruit and surface wash by comparing the TLC analysis performed on retained samples to the TLC chromatographic profile obtained after a further period of 4 months (at the end of the study).

Enantiomeric composition: Pydiflumetofen is a racemate. To consider the potential for differential metabolism of the ‘S-’ and ‘R-’ enantiomers of pydiflumetofen, the enantiomeric composition of pydiflumetofen residues in tomato fruits was determined by LC-MS (and compared to the composition in spray solution).

Table 7-14 Summary of study design

Application method	Foliar spray				Soil treatment	
Label	[phenyl-14C]		[pyrazole-14C]		[phenyl-14C]	[pyrazole-14C]
Intended use rate (g as/ha or mg a.s./plant)†	2 x 200 (total 400)		2 x 200 (total 400)		20	20
Actual application rate	1 st app:	198.3	1st app:	226.7	20.0	20.1
	2 nd app:	195.6	2nd app:	173.8		
	Total:	393.9	Total:	400.5		
Application timing (BBCH)	1 st app:	83			At transplanting (~ BBCH 10/11)	
	2 nd app:	86				
Sampling (DALA)	Fruit: 1 & 14				Fruit: 28 & 103	

† Foliar spray – g as/ha; Soil treatment – mg a.s./plant

Results and discussion

Total radioactive residue (TRR)

Extractability was high in all samples, accounting for >97% TRR. For the foliar treated samples surface washing with acetonitrile released 88.9% and 95.0% TRR from the phenyl and pyrazole labelled samples, respectively. Sequential extraction with acetonitrile: water released a further 4.8-10.5% TRR (0.030-0.067 mg/kg). For foliar spray applications, the majority of TRR in mature fruit samples was recovered from surface washes, see Table 7-15 for details. The TRR levels found in mature fruit, following soil treatment, were very low – 0.007 & 0.013 mg/kg for the [phenyl-¹⁴C] and [pyrazole-¹⁴C] labels, respectively. The [phenyl-¹⁴C] sample for soil treated tomato was not analysed further.

Table 7-15 Summary of TRR determined by LSC and fractionation – fruit, tissue samples

Label	Crop Commodity	By direct quantification of the sample †	By summation of extracts and debris ‡
[phenyl-14C]	Fruit (foliar) – 1 DALA	0.013	0.018
	Fruit (foliar) – 14 DALA	0.067	0.071
	Fruit (soil) – 103 DALA ¹	0.007	NS
[pyrazole-14C]	Fruit (foliar) – 1 DALA	0.008	NS
	Fruit (foliar) – 14 DALA	0.032	0.032
	Fruit (soil) – 103 DALA ¹	0.013	0.013

†The TRR values of all commodities were initially determined by direct quantification of the radioactivity by

combustion/LSC.

‡ The TRR values of all commodities were also determined by the summation of the radioactivity present in the extracts and debris after initial fractionation

1. Includes residue from surface wash.

The extracted radioactivity was analysed by chromatography. The extracted and identified residues are detailed in Table 7-16, Table 7-17 and Table 7-18 for the phenyl & pyrazole label, respectively.

Table 7-16 Total radioactive residues in tomato fruit samples treated with [¹⁴C]- pydiflumetofen

Radiolabel	Treatment	Sample†	Radioactive Residue (mg/kg)		TRR‡
			Surface Wash By Direct Quantification of Sample	Tissue By Direct Quantification of Sample	mg/kg
[Phenyl-14C]	Foliar	1 DALA	0.503 (96.8 % TRR)	0.013	0.519
		14 DALA	0.571 (88.9 % TRR)	0.067	0.642
	Soil	103 DALA	NC	0.007	0.007
[Pyrazole-14C]	Foliar	1 DALA	0.473 (98.4 % TRR)	0.008	0.481
		14 DALA	0.601 (95 % TRR)	0.032	0.633
	Soil	103 DALA	NC	0.013	0.013

† Only mature fruit samples were analysed; foliage and immature fruit were collected but not analysed for any label/application type.

‡ mg/kg calculated directly from radioactivity quantified in surface wash (where applicable) and tissue and specific activity

NC – Not conducted

Table 7-17 Summary of identified and characterised residues tomato fruit (combined residue from surface wash and tissue) – phenyl label

Application type	Foliar spray		Soil application
DALA	1	14	103
BBCH	BBCH 86	BBCH 89	BBCH 89
extractable TRR % (mg/kg) † *	100 (0.520)	99.7 (0.640)	-
pydiflumetofen	91.7 (0.477)	92.2 (0.592)	-
SYN545547	3.6 (0.019)	3.3 (0.021)	-
SYN547891	1.4 (0.007)	1.6 (0.011)	-
Unassigned % (mg/kg)	2.1 (0.010) ¹	2.5 (0.015) ²	-
Uncharacterised Extract	-	-	-
total identified % (mg/kg)	96.7 (0.503)	97.1 (0.624)	-
non extracted TRR % (mg/kg) ‡	0.1 (0.001)	0.3 (0.002)	-
total TRR (by summation) % (mg/kg)	100 (0.521)	100 (0.642)	- (0.007)

† Extractable radioactivity inclusive of surface wash and extracted radioactivity quantified from washed fruit.

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

¹ Unassigned extract comprising at least 4 discrete components, no single one of which >0.8 % TRR (>0.004 mg/kg)

² Unassigned extract comprising at least 7 discrete components, no single one of which >0.7 % TRR (>0.004 mg/kg)

Table 7-18 Summary of identified and characterised residues tomato fruit (combined residue from surface wash and tissue) – pyrazole label

Application type	Foliar spray		Soil application
DALA	1	14	103
BBCH	BBCH 86	BBCH 89	BBCH 89
extractable TRR % (mg/kg) †*	98.4 (0.473)	100 (0.632)	97.5 (0.013)
pydiflumetofen	95.9 (0.461)	96.6 (0.611)	4.1 (0.001)
SYN545547	1.8 (0.009)	1.4 (0.009)	0.4 (<0.001)
SYN547891	0.6 (0.003)	1.0 (0.006)	ND
Unassigned % (mg/kg)	ND	1.0 ¹ (0.006)	88.9 ² (0.008)
Uncharacterised Extract	-	-	2.6 (<0.001)
total identified % (mg/kg)	98.3 (0.473)	99 (0.626)	-
non extracted TRR % (mg/kg) ‡	1.6 (0.008)	0.1 (0.001)	2.6 (<0.001)
total TRR (by summation) % (mg/kg)	100 (0.481)	100 (0.633)	100 (0.013)

† Extractable radioactivity inclusive of surface wash and extracted radioactivity quantified from washed fruit.

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

¹ Unassigned extract comprising at least 6 discrete components, no single one of which >0.3 % TRR (>0.002 mg/kg)

² Unassigned extract comprising at least 25 discrete components, no single one of which >11.9 % TRR (>0.002 mg/kg)

High levels of identification were achieved for the foliar treated samples, with ≥ 96.7 % of the TRR identified. The principal component of the residue was, in all cases, parent pydiflumetofen, accounting for 91.7 % and 95.9 % TRR 1 day after application, and 92.2 % and 96.6 % TRR 14 days after application, for the phenyl and pyrazole labels, respectively. Other metabolites identified were SYN547891 accounting for 1.4 % and 0.6 % TRR for the phenyl and pyrazole labels, respectively, and SYN545547 (3.6 % and 1.8 % TRR for the phenyl and pyrazole labels, respectively) for samples taken 1 day after application. These metabolites were also identified in samples taken 14 days after application accounting for 1.0 – 1.6 % TRR for SYN547891 and 1.4 – 3.3 % TRR for SYN545547. All metabolites identified were found in their free non-conjugated form (only solvent extraction performed in the study). No further identification is required for the unassigned extractable residue as the TRR is <10 %, and the concentration is <0.01 mg/kg.

For the tomato samples treated by soil application, only the pyrazole label was extracted with TRR levels greater than 0.01 mg/kg – hence no further analysis was conducted on the phenyl labelled samples. Of the extracted radioactivity (pyrazole label), 4.1 % TRR was identified as parent pydiflumetofen. 88.9 % TRR remained unidentified; however, this radioactivity was associated with up to 25 individual components, none of which accounted for more than 11.9 % TRR or 0.002 mg/kg.

Storage stability investigations:

Representative radio chromatograms (TLC) of fruit and surface wash were presented in the report (comparisons between TLC before and after the frozen storage period of 4 months). These representative TLC chromatograms supporting the storage stability work showed the major ‘spot’ of pydiflumetofen, and then weaker TLC spots for the metabolites SYN545547 and SYN547891. These are sufficient to show that there is no marked qualitative

change in the samples over the period of the study, as far as can be seen in the context of the TLC work; these cannot be interpreted quantitatively.

The TLC storage stability results were only able to show the ‘weak’ TLC spots for the metabolites SYN545547 and SYN547891, and did not cover the low-level peaks (indicated to be more polar in nature)/components that were unassigned (Tables 7-17 and 7-18) during the HPLC analysis and metabolic profiling. However, these unassigned peaks were individually present at very low levels (<1% TRR or <0.004 mg/kg in the fruits following foliar application and <0.002 mg/kg for the soil application).

Overall, the experimental work in this metabolism study was completed within about 7 months after sampling (with extraction taking place one month after sampling).

Enantiomer composition:

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur. The enantiomeric composition in the spray solution and in tomato fruit samples was determined to see whether any change occurred. The enantiomeric fraction was 0.5 in the spray solution and in the fruits. Based on these determinations, the % change in enantiomeric excess¹ was estimated to be <1% in the tomato fruits (and under 2% in the surface wash). The ‘R’ enantiomer of pydiflumetofen was slightly more prevalent in fruit samples.

HSE is not proposing to consider an assessment factor in the consumer risk assessment to consider the potential changes in isomer ratio/amounts in plants.

See also information derived from published literature reports at the end of section B.7.2.1

Conclusion

The present study describes the metabolism of pydiflumetofen in tomato plants, following two post emergence foliar spray applications, and, separately, a single soil application at transplanting. For the foliar treated plants: fruit were harvested 1 DALA and 14 DALA and analysed. For the soil treated plants: mature fruit sampled at 103 DALA was analysed.

The main observations from the identification work are as follows:

Foliar:

- Parent pydiflumetofen was the principal residue detected in all samples (95.9 – 96.6 % TRR, 0.461 – 0.611 mg/kg).
- The highest residue of pydiflumetofen was detected in mature fruit, 14 DALA – this is true for both labels (0.640 and 0.632 mg/kg, for the phenyl and pyrazole labels, respectively).
- The majority of the TRR was recovered from the surface wash extracts – the proportion of residue incorporated into the fruit increased slightly from the 1 DALA sample to the 14 DALA.
- SYN545547 and SYN547891 were detected in each of the samples at lower levels than the parent (≤ 3.6 % TRR, ≤ 0.019 mg/kg).
- The proportion of the metabolites SYN545547 & SYN547891 was broadly similar across all samples (2.4 – 5.1 % TRR). The highest % TRR was present in the phenyl labelled sample at 14 DALA.

¹ Enantiomeric excess is explained in the EFSA guidance on stereoisomers (2019, “Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers”)

- Overall, absolute levels of metabolite (in mg/kg) were low in mature fruit: maximum 0.032 mg/kg, for the pyrazole label.
- Levels of SYN545547 and SYN547891 did not vary significantly between 1 DALA and 14 DALA for either label.

Soil:

- Uptake into the fruit from plants grown in treated soil was low and showed extensive metabolism into multiple low-level metabolites
- Maximum observed TRR was low – 0.013 mg/kg for the pyrazole label
- Parent pydiflumetofen was detected at a low level – 4.1 % TRR (0.001)
- Unassigned metabolites represented 88.9 % of the total TRR (0.008 mg/kg) – no single metabolite was present at greater than 11.9 % TRR.

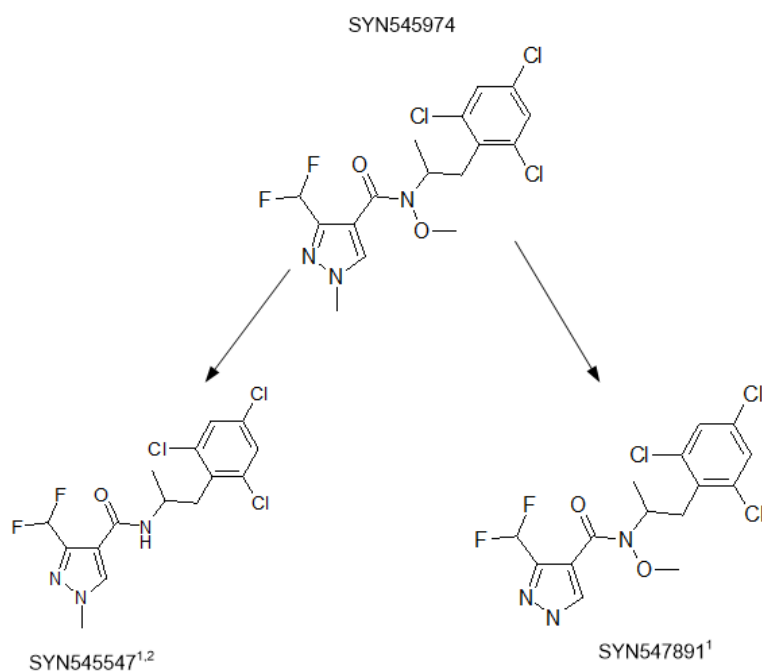
Unidentified components were present in all foliar (treatment) fruit samples (except the pyrazole labelled sample at 1 DALA) at low overall levels - accounting for between 1.0 – 2.5 % of the total TRR (0.006 – 0.015 mg/kg). Individual unassigned levels were low (<1%TRR and max 0.004 mg/kg) for the foliar treatment fruits. The soil treated samples contained at least 25 discrete components, no single one of which >11.9 % TRR (>0.002 mg/kg).

As tissue samples were surface washed with acetonitrile, the residue levels identified in the fruit may not be representative of the expected levels found under normal field conditions. No impact is expected for the conclusion on the metabolic pathway in tomato plants.

The principal biotransformations observed were:

- Demethylation of the pyrazole ring to produce SYN547891 (not observed in soil treated sample).
- Reduction of the parent molecule producing SYN545547.

Figure 7-3

Proposed metabolic pathway of pydiflumetofen in tomato

¹ Identified in foliar spray treated tomatoes

² Identified in soil treated tomatoes

Metabolism in oilseed rape

Report:	K-CA 6.2.1/01. [REDACTED], [REDACTED], [REDACTED] (2015).
Title:	SYN545974 - Metabolism of [14C]-SYN545974 in Oilseed Rape
Report No:	Report Number 33587 (Syngenta Report No. TK0123337)
Document No:	VV-412847 (Syngenta File No. SYN545974_10225)
Guidelines:	OECD Guideline for the Testing of Chemicals, Metabolism in Crops. Guideline 501 (January 2007) OECD Environment, Health and Safety Publications. Series on Testing and Assessment No. 64. Series on Pesticides No. 32. Guidance Document on Overview of Residue Chemistry Studies ENV/JM/MONO (2009)31 (July 2009) Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the 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 Nature of the Residue - Plants, Livestock; United States Environmental Protection Agency; Residue Chemistry Test Guidelines OPPTS 860.1300; (August 1996) Japanese MAFF Guideline on the Application for Agricultural Chemicals Registration (12 Nohsan No. 8147, November 2000)
Guideline deviations:	None
GLP:	Yes

Materials and methods

Materials

1. C-label pydiflumetofen

Description: Phenyl-U-14C – SYN545974 (spec. activity of a.s. 4.11 MBq/mg)

Lot/Batch #: DAD-XIII-38-4

Radiochemical Purity: 98.7 %

2. C-label pydiflumetofen

Description: Pyrazole-5-14C – SYN545974 (spec. activity of a.s. 4.01 MBq/mg)

Lot/Batch #: 5222GAR001-1

Radiochemical Purity: 97.7 %

Methods

A study, investigating the metabolism of pydiflumetofen in oilseed rape, was carried out in 2012-2015 at Charles River Laboratories in Edinburgh, Scotland. Oilseed rape plants were treated with 1 post emergence, foliar spray application, using pydiflumetofen labelled at the [phenyl] or the [pyrazole] position, as shown in Figure 7-1. The experiments with both labels were carried out simultaneously.

Oilseed rape (*Brassica napus*) were sown into 8 sample containers, each filled with sandy loam soil. The oilseed rape was grown outdoors, the containers were covered with mesh netting to prevent birds and animals from eating the seeds. The containers were treated with [pyrazole-5-14C]- pydiflumetofen or [phenyl-U-14C]- pydiflumetofen at an intended nominal application rate of 150 g a.s./ha (0.75N). The radiochemicals were formulated as a suspension concentrate (SC200), using the blank SC formulation 'A20048A'; and applied to the plants as spray applications at growth stage BBCH 65. The actual application rates achieved were 134.2 g a.s./ha (0.67N) and 146.6 g a.s./ha (0.73N) for the phenyl and pyrazole-labelled experiments, respectively.

Foliage was harvested 21 DALA; this was taken as a contingency and was not analysed. Seeds and trash* were harvested at crop maturity (BBCH 89; 62 DALA).

*Oilseed rape trash refers to straw – in this study report, seeds were separated from the pods, and the pods were then incorporated into the trash.

Analysis/general procedures

Samples were homogenised and TRRs determined by sample combustion analysis, followed by liquid scintillation counting (LSC). Extraction and further analysis was carried out on samples where the radioactive residue was >0.01 mg/kg. Samples were extracted with aqueous acetonitrile solvent combinations (80 % v/v). Seed samples were extracted using a combination of aqueous acetonitrile (80 % v/v) and hexane – the organic and aqueous layers were analysed separately. Samples were extracted in multiple stages, the solvent: sample ratio remained constant for trash samples; the ratio decreased for subsequent extractions of seeds. Where residues exceeded 0.01 mg/kg, the various extracts were combined and concentrated using rotary evaporation, as required. Liquid-liquid partition, using diethyl ether, was carried out on the concentrated extracts from trash samples. Partitioned extracts were analysed using LSC. Selected extracts were cleaned up using solid phase extraction (SPE) prior to LC-MS chiral analysis. Further extractions (acid/alkali, followed by aqueous rinse) were performed on the post extraction solids of the trash samples.

Identification of residues present in the principal fractions was conducted using HPLC, with comparison to authentic reference standards of parent pydiflumetofen and its metabolites. Further identification was conducted using 1D-TLC. The following reference standards were used: metabolites which were detected- SYN545547 and SYN547891; metabolites which were not detected- SYN547890, SYN545720, SYN 508272, SYN547890, SYN SYN547892, SYN547893, SYN547894, SYN547895, SYN547897.

All samples not analysed immediately were stored in a freezer set to maintain - 20 °C. Initial analysis of the oilseed rape fractions samples took place approximately within 2 months after harvest. The analytical phase of the work study was completed within around 2 years and 4 months (a further 28 months after extraction and initial analysis).

Storage stability investigation -. Storage stability was assessed for oilseed rape trash samples by comparing the TLC analysis performed on the initially analysed samples to the TLC chromatographic profile obtained after a further period of 28 months (at the end of the study).

Enantiomeric composition: Pydiflumetofen is a racemate. To consider the potential for differential metabolism of the ‘S-’ and ‘R-’ enantiomers of pydiflumetofen, the enantiomeric composition of pydiflumetofen residues in oilseed rape seed and trash was determined by LC-MS (and compared to the composition in spray solution).

Table 7-19 Summary of study design

Label	[phenyl-14C]		[pyrazole-14C]	
Intended use rate (g as/ha)	150		150	
Actual application rate (g as/ha)	134.2		146.6	
Application timing (BBCH)	65			
Sampling (DALA)†	Foliage:	21	Foliage:	21
	Trash:	62	Trash:	62
	Seeds:	62	Seeds:	62

† Days after last application

Results and discussion

Total radioactive residue (TRR)

The extractable TRR levels were 0.051 mg/kg & 0.046 mg/kg in trash, and 0.015 mg/kg & 0.014 mg/kg in seeds, for the phenyl & pyrazole labels, respectively. Extractability was reasonably high in all samples, accounting for ≥71.8 % TRR. Extractability was marginally lower in seed, compared to trash.

Table 7-20 Summary of TRR determined by LSC and fractionation

Label	Crop Commodity	By direct quantification of the sample †	By summation of extracts and debris ‡
[phenyl-14C]	Seeds	0.018	0.020
	Trash	0.059	0.062
[pyrazole-14C]	Seeds	0.014	0.019
	Trash	0.062	0.061

† The TRR values of all commodities were initially determined by direct quantification of the radioactivity by combustion/LSC.

‡ The TRR values of all commodities were also determined by the summation of the radioactivity present in the extracts and debris after initial fractionation

The extracted radioactivity was analysed by chromatography. The extracted and identified residues are detailed in Table 7-22 and Table 7-23 for the phenyl & pyrazole label, respectively.

Table 7-21 Total radioactive residues in oilseed rape samples treated with [¹⁴C]- pydiflumetofen

Radiolabel	Sample†	Radioactive Residue (mg/kg)		TRR (by summation)
		Extractable Radioactivity	Unextracted Radioactivity	mg/kg
[Phenyl-14C]	Seed	0.015 (74.5 % TRR)	0.005 (25.5 % TRR)	0.020
	Trash	0.051 (81.3 % TRR)	0.012 ‡ (18.7 % TRR)	0.062
[Pyrazole-14C]	Seed	0.014 (71.8 % TRR)	0.005 (28.2 % TRR)	0.019
	Trash	0.046 (75.6 % TRR)	0.015 ‡ (24.4 % TRR)	0.061

† Foliage samples were not analysed.

‡ Additional analysis (acid/alkali) was conducted on the post extraction solids of trash samples. Further low-level residues were extracted during this stage (all < 10 % TRR), no further identification was conducted on any extracts – hence they have not been included in the tables below.

Table 7-22 Summary of identified and characterised residues – oilseed rape – phenyl label

[Phenyl- ¹⁴ C]	Seeds	Trash
DALA	62	62
BBCH	89 (maturity)	89 (maturity)
extractable TRR % (mg/kg) *	74.5 (0.015)	81.3 (0.051)
pydiflumetofen	62.6 (0.012)	50.9 (0.032)
SYN545547	ND	3.7 (0.002)
SYN547891	2.7 (0.001)	5.1 (0.003)
Unassigned % (mg/kg) †	6.0 (0.001)	6.2 (0.004)
Uncharacterised Extract ¹	-	23.7 (0.015)
total identified % (mg/kg)	65.3 (0.013)	59.7 (0.037)
non extracted TRR % (mg/kg) ‡	25.5 (0.005)	6.5 (0.004)
total TRR (by summation) % (mg/kg)	100 (0.02)	100 (0.062)

† Seeds: Unassigned extract consisting of 1 discrete component, no single one of which >6.0 % TRR, (0.001 mg/kg). Trash: Unassigned extract consisting of at least 3 discrete components, no single one of which >2.9 % TRR, (0.002 mg/kg)

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

¹ Extractable residues produced during processing that were too low for analysis: 6 individual fractions, no single one of which >9.2%TRR, (0.006 mg/kg)

Table 7-23 Summary of identified and characterised residues – all crop matrices – pyrazole label

[Pyrazole- ¹⁴ C]	Seeds	Trash
DALA	62	62
BBCH	89 (maturity)	89 (maturity)
extractable TRR % (mg/kg) *	71.8 (0.014)	75.6 (0.046)
pydiflumetofen	39.2 (0.007)	30.0 (0.018)
SYN545547	6.1 (0.001)	2.8 (0.002)
SYN547891	ND	3.3 (0.002)
Unassigned % (mg/kg) †	8.6 (0.002)	34.9 (0.022)
Uncharacterised Extract ¹	-	13.6 (0.008)
total identified %	45.3 (0.008)	36.1 (0.022)
non extracted TRR % (mg/kg) ‡	28.2 (0.005)	7.1 (0.004)
total TRR (by summation) % (mg/kg)	100 (0.019)	100 (0.061)

† Seeds: Unassigned extract consisting of 1 discrete component, no single one of which >4.3 % TRR, (0.001 mg/kg). Trash: Unassigned extract consisting of at least 3 discrete components, no single one of which >8.4 % TRR, (0.005 mg/kg)

* The extractable TRR was calculated from the residues determined in the extracts and debris, by summation.

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

¹ Extractable residues produced during processing that were too low for analysis – 5 individual fractions, no single one of which >6.8%TRR, (0.004 mg/kg).

Reasonable levels of identification were achieved for the samples, with $\geq 59.7\%$ and $\geq 36.1\%$ of the TRR identified, for the phenyl and pyrazole labels, respectively. Lower levels of identification were achieved for the pyrazole label, compared to the phenyl labelled samples. The principal component of the residue was, in all cases, parent pydiflumetofen, accounting for 65.3 % and 45.3 % TRR for seed samples; and 59.7 % and 36.1 % TRR for trash samples, for the phenyl and pyrazole labels, respectively. Additional acid/alkali extraction was performed on debris samples of trash, in an attempt to extract further radioactivity. A total of 7.8 % TRR and 12.4 % TRR, from the phenyl and pyrazole labels, respectively, was extracted from the 4 additional washes (1 x acid, 1 x alkali and 2 x water). No further identification was performed on these samples as the % TRR and absolute amount (mg/kg) were low.

Other metabolites identified were SYN547891 and SYN545547. For seeds: SYN547891 was only observed in the phenyl label where it accounted for 2.7 % of the TRR; SYN545547 was only observed in the pyrazole label, where it accounted for 6.1 % of the TRR. Similarly, for trash: SYN545547 accounted for 3.7 % and 2.8 % of the TRR, for the phenyl and pyrazole labels respectively; SYN547891 accounted for 5.1 % and 3.3 % of the TRR, for the phenyl and pyrazole labels, respectively.

Storage stability investigations:

Representative radio chromatograms (TLC) of oilseed rape trash, were presented in the report (comparisons between TLC before and after the frozen storage period of 28 months). These representative TLC chromatograms supporting the storage stability work showed the major 'spot' of pydiflumetofen, and then weaker TLC spots for the metabolites SYN545547 and SYN547891. These are sufficient to show that there is no marked qualitative change in the samples over the period of the study, as far as can be seen in the context of the TLC work; these cannot be interpreted quantitatively.

The TLC storage stability results were only able to show the 'weak' TLC spots for the metabolites SYN545547 and SYN547891, and did not cover the low-level peaks (indicated to be more polar in nature)/components that were unassigned (Tables 7-22 and 7-23) during the HPLC analysis and metabolic profiling.

Only limited information is available on stability of the residues from the investigations conducted on stability in this study. Whilst presenting some uncertainty, the data are likely to be sufficient in the context of the conclusions surrounding the proposal for the residue definition. There were unidentified peaks present in the HPLC work. Only pydiflumetofen (SYN545974), and metabolites SYN545547 and SYN547891 were identified in the oilseed metabolism study. However, the unassigned and 'uncharacterised extract' peaks were individually present at low levels (up to 0.001 mg/kg in oilseed and up to 0.006 mg/kg in trash). These three TLC spots (parent and the two identified metabolites) were visible on the TLC radiochromatograms for the 'post storage' wheat straw samples.

Enantiomer composition:

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur. The enantiomeric composition in the spray solution and in oilseed seed and trash samples was determined to see whether any change occurred. The enantiomeric fraction was 0.5 in the spray solution and 0.56 in the seeds and 0.52 in the trash. Based

on these determinations, the % change in enantiomeric excess² was estimated to be up to 12.4% in the seed and up to 4.8% in the trash. The 'R' enantiomer of pydiflumetofen was more prevalent in these crop samples.

HSE is not proposing to consider an assessment factor in the consumer risk assessment to consider the potential changes in isomer ratio/amounts in plants.

See also information derived from published literature reports at the end of section B.7.2.1

Conclusion

The present study describes the metabolism of pydiflumetofen in oilseed rape plants, following one post emergence foliar spray application at BBCH 65. Seeds and trash were harvested at NCH (BBCH 89; 62 DALA). Foliage was also taken as a contingency but was not analysed.

The main observations from the identification work are the following:

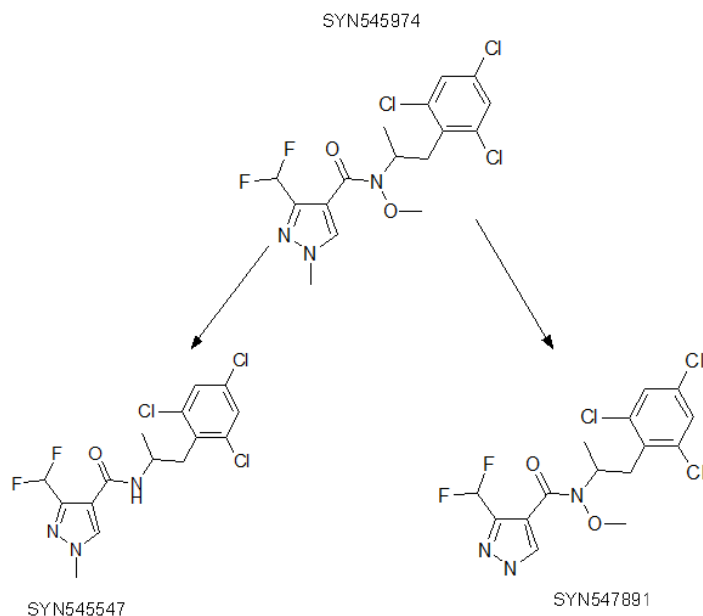
- Parent pydiflumetofen was the principal residue detected in all samples (30.0 – 62.6 % TRR, 0.007 – 0.032 mg/kg).
- The highest residue of pydiflumetofen was detected in rapeseed trash – this is true for both labels (0.032 and 0.018 mg/kg, for the phenyl and pyrazole labels, respectively).
- SYN545547 and SYN547891 were detected in each of the samples at lower levels than the parent (≤ 6.1 % TRR, ≤ 0.003 mg/kg).
- The proportion of the metabolites SYN545547 & SYN547891 was broadly similar across all samples (2.7 – 8.8 % TRR). The highest % TRR was present in the phenyl labelled trash sample.
- Overall, absolute levels of metabolite (in mg/kg) were low in seeds: maximum 0.001 mg/kg, for each label. This was the case for identified and unidentified (unassigned) peaks in seeds.

Unidentified components were present in all samples and in total accounted for 6.0-34.9 % TRR (0.001-0.022 mg/kg); no individual component accounted for >8.4 % (0.005 mg/kg).

The principal biotransformations observed were:

- Demethylation of the pyrazole ring to produce SYN547891.
- Reduction of the parent molecule producing SYN545547.

² Enantiomeric excess is explained in the EFSA guidance on stereoisomers (2019, "Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers")

Figure 7-4 Proposed metabolic pathway of pydiflumetofen in oilseed rapeEnantiomer composition: Updated literature review – summary of relevant published papers:

In the updated literature review (see section B.7.7.2) that the applicant submitted, there were three published papers that provided information on enantiomeric composition in plants and animals. These are summarised below (with HSE evaluator comments).

Pydiflumetofen has a single chiral centre, with two enantiomeric forms (R- and S-).

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

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

The various published papers used the different terms (R- S-) and ('+' and '-'). The applicant has used the terms (R- S-). It should be noted that it is not possible to transpose directly between the two different naming conventions. HSE have not determined the attribution of 'R-' and 'S-' for the (+) and (-) enantiomers used in the ████ *et al.*, 2020, study outlined below.

Enantioselective Metabolic Mechanism and Metabolism Pathway of Pydiflumetofen in Rat Liver Microsomes: In Vitro and In Silico Study

Zhen Wang, Rui Li, Qiqi Wu, Jinsheng Duan, Yuting Tan, Xiaofang Sun, Rou Chen, Haiyan Shi, and Minghua Wang

J. Agric. Food Chem. 2022, 70, 2520–2528

The enantioselective metabolism of pydiflumetofen (PYD) was explored via *in vitro* and *in silico* methods. Consistent results were observed between metabolism and enzyme kinetics experiments, with the S-isomer of pydiflumetofen (S-PYD) metabolising faster than R-isomer of pydiflumetofen (R-PYD) in rat liver microsomes.

The rat liver microsomes were evaluated in incubation experiments, where the reaction with pydiflumetofen was quenched after 0, 2, 5, 10, 15, 20, 30, and 40 minutes. In this study, the enantiomeric composition of PYD was evaluated based on the enantiomeric fraction (EF).

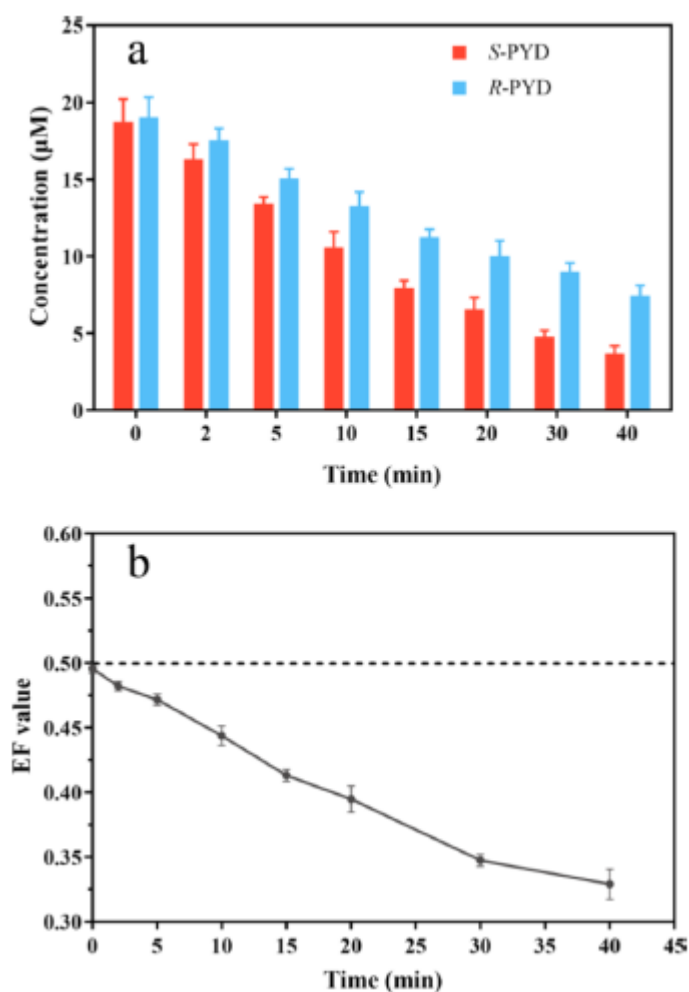
$$EF = CS/(CS + CR)$$

(where CS and CR are the concentrations of S and R-enantiomers).

The results showed that, *in vitro* (rat liver microsomes), S-PYD was preferentially degraded/metabolised over R-PYD. Therefore there was an enrichment of R-PYD.

(a) Degradation of PYD enantiomers

(b) EF values of PYD.



The mechanism for this enantioselective metabolism was also investigated. CYP3A1 and carboxylesterase 1 were found to be major enzymes participating in the metabolism of PYD. Based on the computational results, S-PYD bound with CYP3A1 and carboxylesterase 1 more tightly with lower binding free energy than R-PYD.

HSE evaluator note: This is a report of an *in vitro* study, which has focussed on rat liver (microsomes). This has not been underpinned in the overall pydiflumetofen dossier submission by an analysis of the extent of enantiomeric selective metabolism occurring in live animals by the determination of residues in animal product samples. In this published study, it was shown that the R-isomer predominates over time. The extent of change was more marked than in the crop (published) studies below (which considered a shorter timeperiod of investigation), although it is noted that this conclusion is limited to liver *in vitro* (microsomes) assessment.

Enantioselective separation and dissipation of pydiflumetofen enantiomers in grape and soil by supercritical fluid chromatography–tandem mass spectrometry

Xiuming Wu, Fengshou Dong, Jun Xu, Xingang Liu, Xiaohu Wu, and Yongquan Zheng

J Sep Sci 2020;43:2217–2227

The enantiomeric composition of grapes and soil samples was analysed using chiral analysis. The study reported the method of analysis, the optimization of analytical approaches for successful chiral separation of the enantiomers and supporting method validation data.

This published paper uses the (+)/(-) nomenclature which refers to the direction of optical rotation of plane-polarised light by an enantiomer. The applicant has used the standard ‘R-’ and ‘S-’ nomenclature in the naming of the enantiomers of pydiflumetofen. It should be noted that it is not possible to transpose directly between the two different naming conventions. HSE have not determined the attribution of ‘R-’ and ‘S-’ for the (+) and (-) enantiomers described in this published study. The study included structural diagrams showing the chemical structure of the two enantiomers of pydiflumetofen as mirror images, but did not label these ((+)/(-)).

Evaluation of the results was by considering degradation rates and enantiomeric fraction given by the following equations:

The degradation kinetics of the two enantiomers in grape and soil samples were estimated using first-order kinetics. The half-life ($T_{1/2}$) of each enantiomer was also calculated using the following equations:

$$C = C_0 e^{-kt}$$

$$T_{1/2} = \frac{\ln 2}{k}$$

where C_0 and C indicate the concentrations of the enantiomers at time 0 and time t , respectively. k is the degradation rate constant and $\ln 2 = 0.693$.

The enantiomeric fraction (EF), which was used to investigate the enantioselective dissipation of pydiflumetofen enantiomers in grape and soil samples, was described using the following equation:

$$EF = \frac{(+)\text{A}}{(+)\text{A} + (-)\text{A}}$$

where (+)A and (-)A represent the concentrations of the two enantiomers. Thus EF = 0.5 represents a racemic mixture, and values for EF range from 0 to 1.

The residue concentrations of both pydiflumetofen enantiomers in the grape and soil samples decreased gradually with time after foliar application. The half-lives were estimated as follows.

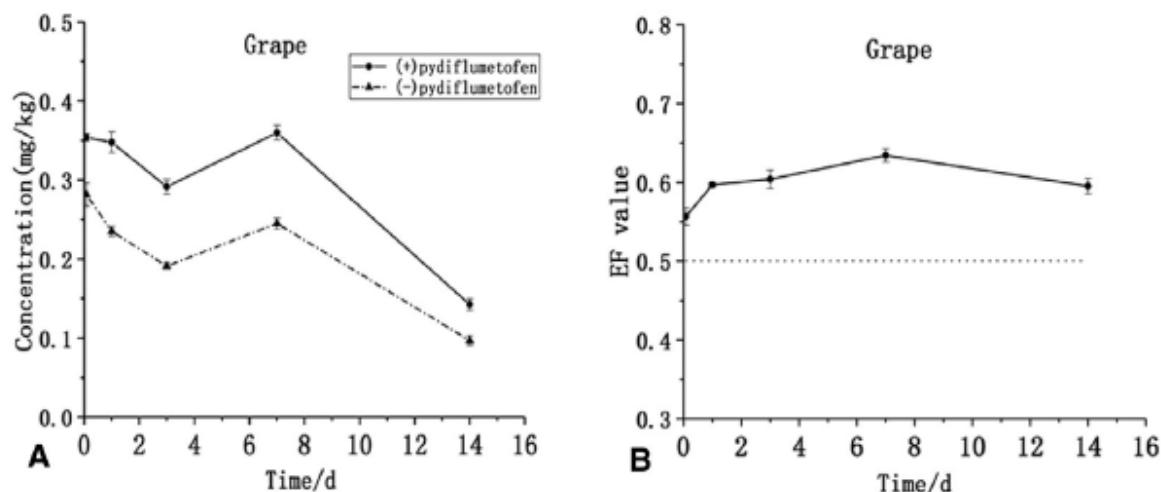
Grape: (+)-pydiflumetofen: estimated half-life of 11.75 days

Grape: (-)-pydiflumetofen: estimated half-life of 10.66 days

Soil: (+)-pydiflumetofen: estimated half-life of 13.33 days

Soil: (-)-pydiflumetofen: estimated half-life of 14.75 days

The concentrations determined over time and the associated EF values are in the following graphs:



In grapes, (-)-pydiflumetofen was degraded more rapidly than (+)-pydiflumetofen, leading to an enrichment of (+)-pydiflumetofen residues.

In soil, (+)-pydiflumetofen was preferentially degraded, leading to an enrichment of (-)-pydiflumetofen residues.

HSE evaluator notes:

The crop treatment aspect (the trial conditions leading to the grape samples being taken following foliar application) was not detailed in the study. The paper describes the time profile as an assessment of dissipation in grape, having determined the residues ‘in grapes’ (so not as a surface wash). However the report does not say about treatment and harvesting of the grape samples. It is assumed that time 0 represents analysis immediately after the foliar application to the grapes.

It is noted that at the start of the study (time 0), the EF value was around 0.55, and not 0.50. Whilst the EF raised to slightly greater than 0.6 after 7 days, by 14 days, the EF had declined to below 0.6. The most marked change in the EF was within the first day. This could be when most of the residue is on the surface of the fruits.

Taking account of the extent of change in EF (without considering the direction of the change) over the course of the study, the values are not too dissimilar from the results observed in the primary crop metabolism studies. In section B.7.2.1, it is reported how the enantiomeric peak areas were not estimated for the wheat metabolism study, the enantiomeric change in tomato fruits was slight, and that the enantiomeric fraction in treated oilseed rape samples was 0.56 in the oilseed rape seed and 0.52 in the oilseed rape trash (the EF in the spray solution was determined as 0.5). In the primary crop metabolism studies, the change was that the R-enantiomer (+ enantiomer) predominated in the primary crop samples.

Since HSE has not determined the attribution of ‘R-’ and ‘S-’ for the (+) and (-) enantiomers described in this published study, it is not possible to compare the direction of change (representing differential metabolism of the two pydiflumetofen enantiomers) in this study with the direction of change in the other non-GLP published studies and the GLP regulatory residues studies.

Evaluation of exploitive potential for higher bioactivity and lower residue risk enantiomer of chiral fungicide pydiflumetofen

Zhen Wang, Rui Li, Jing Zhang, Shiling Liu, Zongzhe He and Minghua Wang

Pest Manag Sci 2021; 77: 3419–3426

In this study, the biological activity of the isomers of pydiflumetofen was investigated. The residues in crops of the isomers were also investigated.

The degradation half-lives of S- and R-pydiflumetofen in three vegetables (cucumber, eggplant (aubergine), and cowpea (black-eyed pea)) under field conditions were 2.56–3.12 days and 2.48–2.76 days, respectively, which reveals that R-pydiflumetofen degrades faster than S-pydiflumetofen.

The field part of the study involved treatment of the vegetables. Whilst the study said that these were treatments in a greenhouse in Nanjing, China, the methods also reports use of experimental plots that were fields that had not used pydiflumetofen before. The trial fields were divided into three plots and isolated by buffer zones. When the vegetables were grown in appropriate stages (growth stages were not stated), a 200 g/litre suspension concentrate (SC) of pydiflumetofen was sprayed onto the treatment plots at 292.4 g as/ha for cucumber, eggplant, and cowpea plants. Representative vegetable fruit samples were collected from each plot at 2 hours and 1, 3, 5, 7, 10, and 14 days after spraying.

The method of analysis and validation data were presented in the publication for the chiral analysis of the two isomeric forms.

The half-lives were estimated according to the equation:

$$C = C_0 e^{-kt}$$

$$T_{1/2} = \frac{\ln 2}{k}$$

The enantiomeric fraction in this published paper was given as the equation given below. In the current study (2021) a value >0.5 means that the S-isomer predominates:

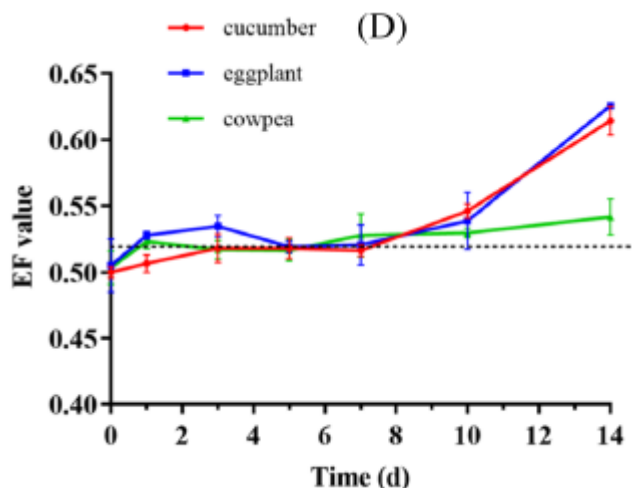
$$EF = C_S / (C_S + C_R)$$

The half-lives were estimated as follows:

cucumber: (R)-pydiflumetofen: estimated half-life of 2.76 days
 cucumber: (S)-pydiflumetofen: estimated half-life of 3.12 days
 eggplant: (R)-pydiflumetofen: estimated half-life of 2.56 days
 eggplant: (S)-pydiflumetofen: estimated half-life of 2.84 days
 cowpea: (R)-pydiflumetofen: estimated half-life of 2.48 days
 cowpea: (S)-pydiflumetofen: estimated half-life of 2.56 days

The statistical evaluation observed that, there were significantly different half-lives of stereoisomers in cucumber and eggplant by Student's paired t-test ($P < 0.05$), but there was no significant difference in cowpeas ($P > 0.05$).

Considering the change in the ratio of the enantiomers, the EF values in cucumber, eggplant, and cowpeas were 0.50, 0.51, and 0.50 at 2 h after foliar spray treatment. The EF value increased when the time increased (0.61 for cucumber, 0.63 for eggplant, 0.54 for cowpea at 14 days).



HSE evaluator notes:

In this published paper, following the investigations of residues in cucumber, aubergine and cowpea, the S-enantiomer of pydiflumetofen predominated in the residues samples. The change in this study indicated a tendency for the R-isomer to be more rapidly degraded than the S-isomer. This is a different observation to that observed in the primary crop metabolism samples in the applicant's data submission (GLP regulatory residues studies).

Overall conclusions from published papers: Information on enantiomeric composition of crop (or animal) residues

Primary crops:

The published papers reported a level of change that was not too dissimilar from the degree of change oilseed rape primary crop metabolism study (GLP study in the main regulatory dossier submitted). [oilseed rape metabolism study, maximum EF observed was 0.56 in oilseed rape seed, with R-isomer 'enrichment']

The published papers considered the enantiomeric composition in samples that had been directly treated. These are primary treated crops (rather than the assessment of rotational crop residues).

In a study on grape (2020 published paper), where residues were assessed over a period of 0 to 14 days, in terms of change, the residues were found to have predominantly (+)-isomer of pydiflumetofen. In a study on cucumber, aubergine, and cowpea (2021 published paper), where residues were assessed over a period of 0 to 14 days, in terms of change, the residues were found to have predominantly S-isomer.

Rotational crop consideration:

In the grape (2020 published paper), soil residues were also analysed. (-)-pydiflumetofen residues were found to predominate in the soil samples ((+)-isomer degraded more rapidly in soil). In the regulatory residues (GLP) studies, in the rotational crop metabolism study (please see section B.7.6.1), wheat straw samples were analysed in terms of enantiomeric composition. In this study S-isomer predominated at the 270 DAT (270 days after treatment plant back interval) wheat straw, whereas R-isomer predominated at the 120 DAT (120 days after treatment plant back interval) wheat straw.

Animal studies:

There is an *in vitro* published study using rat liver microsomes. This showed that over the time course of study the S-isomer is preferentially metabolised, leaving the R-isomer predominating.

Overall (published papers on enantiomeric composition):

The assessment of these published papers does not impact the proposals made based only on the assessment of the GLP regulatory residues studies submitted. HSE is proposing not to apply a form of correction in the consumer risk assessment for residues in plants. However, on a precautionary basis, HSE is suggesting to use a 'x 2 form of adjustment' for the consumer risk assessment relating to residues in livestock products (to account for possible differential metabolism of the isomers of pydiflumetofen). Residues in products of animal origin that were predicted from the feeding studies were doubled prior to inclusion in the consumer risk assessment (see sections, B.7.2.2 (poultry metabolism) , B.7.2.3 (ruminant metabolism) and B.7.4 (feeding studies) below and see Volume 1 section 2.7.5 (livestock residues) and section 2.7.9 (consumer risk assessment)).

B.7.2.2. Poultry

In this section the relationship between the actual doses used in the study, and the anticipated use rates (the N rate) following the assessment of the uses considered in this evaluation, are expressed considering the following various assessment scenario following presentation to the Expert Committee on Pesticides (ECP) in the process of seeking Independent Scientific Advice (ISA) and taking account of both rotational crop and primary crop residues. The fate parameters (on estimation of residues in soil), affect the rotational crop residues which in turn feed into the animal dietary burden. Two different scenarios have been assessed in this residues evaluation—'Tier 1 10 year use' and 'Tier 2 long term use'. These are The approach is further explained in Vol 1 section 2.7.7 (rotational crops) and Vol 1 section 2.7.5 (animal dietary burden).

Report:	K-CA 6.2.2/01, [REDACTED], [REDACTED] (2015)
Title:	SYN545974 – Metabolism of [¹⁴ C]-SYN545974 in the Laying Hen
Report No:	Report No. 33964
Document No:	Document No. VV-414163 (Syngenta File No. SYN545974_10282)
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OECD guideline 503 for testing of chemicals "Metabolism in Livestock", 08-Jan-2007.
Guideline deviations:	In accordance with OECD guideline 503, 10 hens are recommended per dose group. In the study below, 6 hens we used per label (12 hens in total). The dose rate is the same across both label groups, however there are label specific metabolites identified for both poultry and ruminant. This means there might be some uncertainty in the assessment relating to the use of only 6 hens per label group. Despite having a more complex metabolic profile, the ruminant profile does not include any metabolites different to poultry that are formed directly from parent; all additional metabolites (that were found in ruminants compared to poultry) are further breakdown products of other metabolites common to both animal groups. This suggests that there are unlikely to be label specific metabolites that were not identified as a result of the use of fewer hens and, as the dose rate of parent is the same for both labels, this study is still considered acceptable.
GLP:	Yes

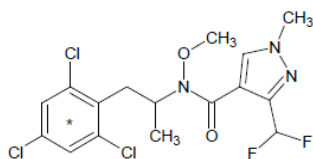
Materials and methods*Materials*1. C-label pydiflumetofen

Description: [Phenyl-U-¹⁴C]-SYN545974 (Spec. Activity of a.s. 5.7905 MBq/mg)

Lot/Batch #: RDR-XV-94

Radiochemical Purity: 99.1%

Structures:
(* marks position of
radiolabel)



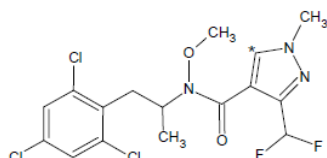
2. C-label pydiflumetofen

Description: [Pyrazole-5-¹⁴C]-SYN545974 (Spec. Activity of a.s. 5.0616 MBq/mg)

Lot/Batch #: 5271GAR001-4

Radiochemical Purity: 99.2%

Structures:
(* marks position of
radiolabel)



Methods

The metabolism of pydiflumetofen (SYN545974) was investigated in laying hens (breed *Hy-line brown*) following repeated oral administration of pydiflumetofen labelled either at the [phenyl] or the [pyrazole] position, as shown in the structural diagrams above. The experiments with both labels were carried out simultaneously.

A total of 12 hens were dosed with pydiflumetofen radiolabelled at either the [phenyl] or the [pyrazole] position (6 hens per label) at an intended/nominal rate of 30 mg as equivalents/kg, dry matter intake. The actual mean dose rates (over the duration of the study) were approximately 56.3 and 56.9 mg as equivalents/kg dry matter in the diet respectively (3.3 and 3.6 mg as equivalents/kg bodyweight) for 14 consecutive days. The dosing rates of 3.3 and 3.6 mg as equivalents/kg bodyweight represent 73N and 80 N 43N (Tier 1–10 year use) and 51N (Tier 2 term use) and 47N (Tier 1–10 year use) and 55N (Tier 2 term use) respectively. Details of the study outline are summarized in Table 7-24. The specific activity of the [phenyl] and [pyrazole] radiolabelled pydiflumetofen were adjusted to 1.85 (MBq/mg) and 1.93 MBq/mg respectively using non-radiolabelled pydiflumetofen.

The test items were prepared in gelatine capsules, orally administered once daily. The actual dose was calculated based on the food consumption (dry weight). Dose capsules were prepared on the day before the first dose, and all capsules were sealed and stored at *ca* -20 °C. Additional capsules were prepared so that purity, stability and concentration checks could be done. Prior to the preparation of the actual dose formulations of phenyl and pyrazole labelled [¹⁴C]- pydiflumetofen in solvent, stability tests were carried out on the dose formulations over a 15-day period to ensure they remained stable over the daily dosing period when stored at *ca* -20 °C. For each radiolabel, a capsule was analysed prior to the first dose and following the final dose to determine the radiochemical purity by high performance liquid chromatography (HPLC). The capsule that was analysed after the final dosing was stored at *ca* -20 °C until analysis.

Table 7-24 Dosing of laying hens with pydiflumetofen

Radiolabel	Animal No.	Treatment period (days)	Nominal daily dose	Actual daily dose ^{1, 2}		N rate (Tier 1–10 year use)	N rate (Tier 2 long term use)	Time of sacrifice (hours) ³
			[mg/kg feed]	[mg/kg dry matter feed]	[mg/kg bw/d]			
Phenyl	6	14	30	56.3	3.3	73N 43N	51N	11
Pyrazole	6	14	30	56.9	3.6	80N 47N	55N	11

¹ Mean value of day 1-13

² Using the mean animal weight at application day 1

³ Hours after last dose

The hens were all acclimatised for at least 14 days prior to the start of dosing. Just prior to the start of dosing they were examined by a veterinarian and deemed acceptable for the study. The appearance and behaviour of the hens continued to be assessed twice daily for the duration of the study.

Excreta and cage wash samples (using a solution of methanol: water, 1:1 v/v) were collected prior to dose administration and at each 24 h period until sacrifice. The weights of the individual samples were recorded. After the addition of acetone: methanol: water (4:1:1, v/v) and homogenisation, the weight of the subsequent homogenates were recorded. Aliquots of each sample were taken for radioassay measurements by combustion / LSC. The remaining excreta samples were stored in a freezer at *ca* -20 °C following collection, whilst cagewash samples were stored at ambient temperature.

Eggs were collected twice daily (am and pm) during the acclimatisation and 14-day dosing period. After removal of the shells (retained but not analysed), the eggs were separated into yolks and whites and the weight of each recorded. After weighing, a composite egg yolk sample was prepared for each radiolabel dose group by combining the yolk samples from each hen. This process was repeated for each dose group for the egg whites. Aliquots of each homogenate were mixed with scintillation fluid for radioassay measurements by LSC.

The hens were sacrificed approximately 11 hours after the administration of the final dose. Edible tissues (liver, kidney, fat (peritoneal and subcutaneous) and muscle (leg/thigh and breast), GI tract (plus contents), blood, carcass, bile and any partially formed eggs were removed postmortem and weighed. Partially formed eggs were stored at *ca* 4°C and the remaining samples stored at *ca* -20°C.

Analysis/general procedures

Samples of liver, peritoneal fat, subcutaneous fat (skin attached), breast muscle, leg and thigh muscle were homogenized prior to analysis. The tissues were homogenised frozen with the aid of dry ice to form a fine powder. The remaining samples of blood and composite egg (yolk and white samples) were also homogenised. Following processing and analysis, each tissue sample was stored at *ca*. -20 °C.

For most of the samples, composite samples for each label were prepared by combining the tissues and eggs of all hens from the respective label groups (phenyl and pyrazole). Liver from each label group was combined at *ca* 75% of the total tissue weight. Where radioactive residues in muscle (breast and leg/thigh) and fat (peritoneal and skin with subcutaneous fat) were ≥ 0.01 mg eq/kg and of similar magnitude, composite samples for each label (phenyl and pyrazole) were prepared by combining the respective tissues. For muscle, the breast and leg/thigh muscle were combined in a ratio of 1:1. Samples of peritoneal fat and skin/subcutaneous fat were also combined in a 1:1 ratio. For the hens dosed with pydiflumetofen labelled at the phenyl position, egg yolk samples from the 240-312 h (10 to 13 day) timepoints (when the TRR in egg yolk had reached a plateau) were combined as a fixed percentage (*ca* 50%) of the total weight. For the same label the egg whites were combined as a fixed percentage (*ca* 20%) of the total weight from the 144-312 h (6-13 day) timepoints (TRR in egg white had reached a plateau). For hens dosed with pydiflumetofen labelled at the pyrazole position, yolk samples were combined as a fixed percentage (*ca* 20%) of the total weight from the 168-312 h (7-13 day) timepoints (TRR in egg yolk had reached a plateau) whilst egg white samples were combined as a fixed percentage (*ca* 20%) of the total weight from the 168-312 h (7-13 day) timepoints (TRR in egg white had reached a plateau).

Samples of liver, egg yolk, egg white and muscle were extracted two times with acetonitrile: water (4:1, v/v) and once with acetonitrile: water (1:1, v/v). Samples of fat were extracted two times with acetonitrile: water (4:1, v/v) + hexane and once with acetonitrile: water (1:1, v/v). For the first extraction, the nominal expected moisture content of the liver, muscle and eggs was accounted for when calculating the volume of water required. After centrifugation to separate the aqueous/organic layers from the solid debris, the respective acetonitrile and water extracts (liver, egg yolk, egg white and muscle) or acetonitrile, water and hexane (fat) were adjusted to a defined volume with appropriate solvents and the aliquots underwent LSC analysis. The solid residue after solvent extraction was subjected to combustion analysis followed by LSC.

To quantify and identify radioactive components, thin layer chromatography (TLC, 1D and 2D) were performed on samples and the plates were subject to bioimage analysis to detect the radioactive areas. Reverse phase HPLC was also performed on all types of sample (tissue and egg extracts) to quantify and identify the residues. Where required, organic solvent/water partition and enzyme hydrolysis were performed prior to chromatographic analysis.

If required, enzyme hydrolysis was performed using β -glucuronidase, which also had sulphatase activity and was therefore able to cleave glucuronide and sulphate conjugates. Aliquots of the aqueous partition fraction from organic solvent/water partitioning were adjusted in pH prior to the addition of β -glucuronidase and then samples were incubated overnight in a shaking water bath at 37°C. After this, the enzyme was denatured, and a liquid-liquid partition was performed via shaking.

Chromatographic comparison of the residues was done with synthetic reference standards of pydiflumetofen and its metabolites.

Characterisation of unextracted residues

Significant levels of radioactivity in liver (phenyl: 48.0% TRR; 0.195 mg eq/kg, pyrazole: 47.5% TRR, 0.100 mg eq/kg) and egg yolk (phenyl: 13.0% TRR; 0.047 mg eq/kg, pyrazole: 18.7% TRR, 0.020 mg eq/kg) remained unextracted in the debris following solvent extraction. For both commodities, the unextracted residues were investigated further by solubilisation either with the surfactant sodium dodecyl sulphate (SDS) or proteolytic enzyme hydrolysis.

Subsamples of egg yolk (both labels) and liver (both labels) were treated with SDS solution to create a micellar suspension of proteins which were then precipitated out of solution, and the aliquot was centrifuged. For both egg yolk and liver, the resultant protein pellet accounted for approximately half of the radioactivity from the original debris. Chromatographic analysis of the radioactivity remaining in the supernatant fraction was not possible due to the high levels of surfactant.

To a separate set of subsamples of liver and egg yolk, protease was added to the aliquots and incubated. The residues released by hydrolysis were separated into soluble and insoluble material and the soluble material was radioassayed. Using 1D-TLC it was determined that this soluble fraction of liver extract was comprised of a complex mixture of polar un-assigned species. For the pyrazole label, there were a minimum of 14 unassigned regions on the chromatogram, no region accounted for more than 7.6% TRR (0.016 mg eq./kg). The phenyl label showed a similar profile. The extracts of egg yolk also underwent 1D-TLC, but a more chromatographically forcing solvent system still gave little separation of species from the origin.

Storage stability investigation:

The poultry study was conducted over a period of around 2.5 years (time between necropsy and final analytical work). Samples were kept frozen over the period of storage. The samples were stored for 4 months frozen prior to extracting the residues. The initial analyses of the samples (metabolic profiling) were done within the 4-to-6-month period.

OECD Guidelines (503, livestock metabolism) indicate that metabolism studies should be completed within an analysis period of six months, or otherwise be appropriately supported by storage stability investigations performed in the context of the metabolism studies.

The applicant included some storage stability analyses (chromatographic comparisons obtained in the metabolism study context) to compare HPLC analysis generated at the initial analysis (within 6 months) time period and to compare to HPLC determinations done in the later period of the study. This was done for representative samples of fat and egg yolk.

Results and discussion

All mg/kg levels reported are in mg/kg parent equivalents.

Total radioactive residue (TRR)

The overall recovery of radioactive residues is provided in Table 7-25 as % administered dose. The recoveries were 103.4% for the phenyl labelled pydiflumetofen and 88.0% for the pyrazole label.

The main fraction was excreted via excreta, accounting for approximately 99.1 % (phenyl label) and 84.3 % (pyrazole label). Radioactive residues recovered in the cage wash and rinse accounted for up to 3.6 % (phenyl

label) and 3.2 % (pyrazole label). Radioactive residues associated with edible portions (egg and tissues) accounted for up to <0.1 % of the administered dose for both labels.

Table 7-25 Recovery of radioactive material (results expressed as % dose administered)

Matrix	% of dosed radioactivity recovered	
	[phenyl- ¹⁴ C]-pydiflumetofen	[pyrazole- ¹⁴ C]-pydiflumetofen
Excreta	99.1	84.3
Egg White	<0.1	<0.1
Egg Yolk	<0.1	<0.1
Part Formed Eggs	<0.1	<0.1
Breast Muscle	<0.1	<0.1
Leg and Thigh Muscle	<0.1	<0.1
Skin & Fat	<0.1	<0.1
Peritoneal Fat	<0.1	<0.1
Liver	<0.1	<0.1
GI Contents	0.5	0.3
GI Tract	0.2	0.2
Cage Wash	3.6	3.2
Blood	<0.1	<0.1
Total	103.4	88.0

GI – Gastrointestinal tract

Unless otherwise stated, % TRR values are calculated using the TRR calculated as the sum of ERR (extractable radioactive residue) and RRR (residual radioactive residue) after extraction of the residues (TRR calculated).

The extractable TRR levels were generally high, ranging from 87.0 % TRR to 98.8 % TRR, except for egg yolk (pyrazole label; 81.2% TRR) and liver (51.7% TRR and 52.5% TRR for the phenyl and pyrazole labels, respectively). Muscle was also relatively low for the phenyl label at 84.2% but this was not investigated further. Radioactive residues in the RRR obtained after the initial extraction of egg yolk and liver was 18.7 – 48.3 % TRR (pyrazole label for both), which were further investigated. The RRR of all other relevant matrices were below or equal to 9.9 % TRR (0.002 mg eq/kg) or 8.4% (0.003 mg eq/kg) for the pyrazole label. For the phenyl label, muscle RRR was 15.8 % TRR (0.004 mg eq/kg) and egg yolk RRR was 13.0% (0.047 mg eq/kg). All other matrices had RRR well below this level for this label.

In the edible matrices, the highest TRR concentrations were found in liver (0.210 – 0.404 mg eq/kg) and egg yolk (0.106 – 0.358 mg eq/kg). For all other matrices, the TRR was in a range from 0.027 – 0.101 mg eq/kg (phenyl label) and from 0.021 – 0.052 mg eq/kg (pyrazole).

Table 7-26 Summary of total radioactive residues and extractability in tissue and egg samples from laying hens treated with [¹⁴C]-pydiflumetofen

Radiolabel	Sample	Extractable radioactivity		Non-extractable radioactivity		TRR ¹	TRR ²
		%TRR	mg eq/kg	%TRR	mg eq/kg	mg eq/kg	mg eq/kg
[Phenyl-U- ¹⁴ C]-pydiflumetofen	Liver	51.7	0.209	48.3	0.195	0.374	0.404
	Egg yolk	87.0	0.311	13.0	0.047	0.353	0.358
	Egg white	97.7	0.052	2.3	0.001	0.055	0.053
	Muscle ³	84.2	0.023	15.8	0.004	0.028	0.027
	Skin and fat ⁴	95.8	0.096	4.3	0.004	0.090	0.101
[Pyrazole-5- ¹⁴ C]-pydiflumetofen	Liver	52.5	0.111	47.5	0.100	0.203	0.210
	Egg yolk	81.2	0.087	18.7	0.020	0.103	0.106
	Egg white	98.8	0.051	1.2	0.001	0.051	0.052
	Muscle ³	90.1	0.018	9.9	0.002	0.022	0.021

	Skin and fat ⁴	91.5	0.029	8.4	0.003	0.028	0.032
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1 – mg eq/kg calculated from combustion/LSC analysis of composite samples

2 – mg eq/kg calculated directly from radioactivity extracted, radioactivity in the debris and specific activity.

3 – Composite muscle samples (includes breast and combined leg/thigh muscle).

4 – Composite fat samples (includes peritoneal fat and subcutaneous fat with skin attached).

The concentration of radioactive residues in eggs are provided in Table 7-27. Daily egg samples were obtained on the fourteen consecutive days of the study. For the phenyl label, the level of radioactive residues increased to a plateau concentration of 0.064 mg eq/kg (egg white) and 0.344 mg eq/kg (egg yolk), at 6 and 10 days respectively. There was no sample available for day 14 for the phenyl label. For the pyrazole label, a plateau of 0.062 mg eq/kg (egg white) and 0.116 mg eq/kg (egg yolk) was reached after 7 days. The plateau of residues in eggs has been further considered in the feeding study for poultry, [REDACTED] (2015).

Table 7-27 Concentration of Radioactive Residues in Eggs from Laying Hens Dosed with [Phenyl-¹⁴C] and [Pyrazole-¹⁴C]- pydiflumetofen

Timepoint (h)	Study Day	TRR Phenyl- ¹⁴ C (mg eq/kg)			TRR Pyrazole- ¹⁴ C (mg eq/kg)		
		Mean Egg White	Mean Egg Yolk	Mean Whole Egg (a)	Mean Egg White	Mean Egg Yolk	Mean Whole Egg (a)
24	1	0.015	0.035	0.021	0.014	0.005	0.011
48	2	0.058	0.034	0.051	0.055	0.015	0.043
72	3	0.088	0.100	0.092	0.061	0.033	0.052
96	4	0.072	0.161	0.098	0.055	0.051	0.054
120	5	0.087	0.218	0.127	0.071	0.073	0.072
144	6	0.064	0.269	0.126	0.092	0.094	0.093
168	7	0.060	0.284	0.123	0.062	0.116	0.078
192	8	0.052	0.311	0.128	0.053	0.085	0.063
216	9	0.063	0.322	0.139	0.062	0.114	0.077
240	10	0.060	0.344	0.140	0.064	0.105	0.077
264	11	0.061	0.355	0.147	0.058	0.119	0.077
288	12	0.061	0.359	0.150	0.055	0.108	0.071
312	13	0.049	0.331	0.130	0.073	0.106	0.084
324	14	N/S	N/S	N/S	0.061	0.093	0.070

(a) Whole egg data calculated from yolks and whites

N/S No Sample

The extracted radioactivity was analysed by chromatography. The identified residues are given in Table 7-28 & Table 7-29 for the phenyl & pyrazole label, respectively. The full distribution of residues, including identified, characterised and non-extracted residues are summarised in the tables following (Table 7-30 to Table 7-34).

Table 7-28 Summary of the identified components in samples of laying hens treated with [phenyl-¹⁴C]- pydiflumetofen

Component	Egg Yolk		Egg White		Liver		Muscle		Fat	
	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)
pydiflumetofen	3.0	0.011	46.5	0.025	5.3	0.021	8.7	0.002	16.6	0.017
2,4,6 TCP (as conjugates*)	67.8 (67.8)	0.242 (0.242)	14.5 (14.5)	0.008 (0.008)	-	-	48.4 (48.4)	0.013 (0.013)	29.3 (26.5)	0.030 (0.027)
SYN547948 (as conjugates*)	N/D	N/D	7.1	0.004	0.7 (N/D)	0.003 (N/D)	3.4	0.001	3.0	0.003
SYN547897 (as conjugates*)	2.3	0.008	-	-	2.4 (0.4)	0.009 (0.001)	N/D	N/D	1.7	0.002

SYN545547 (as conjugates*)	N/D	N/D	-	-	1.2 (0.6)	0.005 (0.003)	-	-	-	-
SYN547891 (as conjugates*)	N/D	N/D	-	-	0.2 (0.2)	0.001 (0.001)	-	-	-	-
SYN508272 (as conjugates*)	-	-	-	-	-	-	-	-	-	-
NOA449410 (as conjugates*)	-	-	-	-	-	-	-	-	-	-

* If applicable

Table 7-29 Summary of the identified components in samples of laying hens treated with [pyrazole-¹⁴C]-pydiflumetofen

Component	Egg Yolk		Egg White		Liver		Muscle		Fat	
	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)
pydiflumetofen	11.0	0.012	26.6	0.014	0.5	0.001	4.7	0.001	30.6	0.010
2,4,6 TCP (as conjugates)	-	-	-	-	-	-	-	-	-	-
SYN547948 (as conjugates)	1.3	0.001	5.5	0.003	3.2 (N/D)	0.007 (N/D)	1.6	<0.001	4.1	0.001
SYN547897 (as conjugates)	6.7	0.007	-	-	0.9 (0.9)	0.002 (0.002)	1.1	<0.001	2.6	0.001
SYN545547 (as conjugates)	3.9	0.004	-	-	3.3 (3.3)	0.007 (0.007)	-	-	-	-
SYN547891 (as conjugates)	2.5	0.003	-	-	N/D (N/D)	N/D (N/D)	-	-	-	-
SYN508272 (as conjugates)	7.2 (2.6)	0.008 (0.003)	34.3	0.018	2.4 (2.4)	0.005 (0.005)	46.3	0.010	9.6	0.003
NOA449410 (as conjugates)	6.6 (0.8)	0.007 (0.001)	15.4	0.008	-	-	-	-	3.1	0.001

* If applicable

Characterisation and identification

Pydiflumetofen and metabolites were identified and quantified using reversed phase HPLC and co-chromatography experiments with radiolabelled and non-radiolabelled reference samples.

Distribution of pydiflumetofen and metabolites in eggs and tissues

Liver

Four (phenyl label) and five (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in liver. For the phenyl label, parent compound pydiflumetofen and the metabolites SYN547897 and SYN545547 accounted for the main portions at 0.021 mg eq/kg (5.3% TRR), 0.009 mg eq/kg (2.4% TRR) and 0.005 mg eq/kg (1.2% TRR), respectively. Metabolites SYN547948 and SYN547891 accounted for 0.003 mg eq/kg (0.7% TRR) and 0.001 (0.2% TRR) respectively. For the pyrazole label, the metabolites SYN547948 and SYN545547 accounted for the main portion with 0.007 mg eq/kg (3.2% TRR) and 0.007 mg eq/kg (3.3% TRR), respectively. Parent compound pydiflumetofen and metabolites SYN547897, SYN547891 and SYN508272 ranged from 0.001 to 0.005 mg eq/kg (0.5 – 2.4% TRR).

After extraction with aqueous acetonitrile there was a significant amount of unextracted radioactivity in debris; 0.195 mg eq/kg (48.3%) for the phenyl label and 0.1 mg eq/kg (47.5%) pyrazole label. The RRR of the phenyl and pyrazole label was further examined by protease solubilization. Organosoluble unassigned residues accounted for 0.078 mg eq/kg (19.4%) for the phenyl label and 0.023 mg eq/kg (11.3%) for the pyrazole, which reduced to 0.023 mg eq/kg (6.1%) and 0.012 mg eq/kg (6.0%) post-enzyme hydrolysis for phenyl and pyrazole respectively.

Other fractions not chromatographed accounted for 0.03 mg eq/kg (7.4% TRR) for the phenyl label and 0.017 mg eq/kg (8.6% TRR) for the pyrazole label. For the phenyl label this consisted of two fractions, neither of which accounted for more than 0.027 mg eq/kg (6.7% TRR). For the Pyrazole label, 4 fractions were present, none of which accounted for more than 0.009 mg eq/kg (4.3% TRR).

Egg Yolk

Five (phenyl label) and six (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in egg yolk. For the phenyl label, the metabolite 2,4,6 TCP accounted for the main portion, 0.242 mg eq/kg (67.8% TRR). Parent compound pydiflumetofen and metabolites SYN547948, SYN547897, SYN545547 and SYN547891 ranged from 0.008 to 0.011 mg eq/kg (2.3 – 3.0% TRR). For the pyrazole label, parent compound pydiflumetofen accounted for the main portion at 0.012 mg eq/kg (11% TRR). The metabolites SYN547897, SYN508272 and NOA449410 accounted for 0.007 mg eq/kg (6.6% TRR), 0.008 mg eq/kg (7.2% TRR) and 0.007 mg eq/kg (6.6% TRR) respectively. The metabolites SYN547948, SYN545547 and SYN547891 ranged from 0.001 to 0.004 mg eq/kg (1.3 – 3.9% TRR).

After the initial extraction, 0.047 mg eq/kg (13.0% TRR) of the phenyl label remained unextracted and 0.02 mg eq/kg (18.7% TRR) pyrazole. The RRR of the pyrazole label was further examined by protease solubilization. Organosoluble unassigned residues accounted for 0.020 mg eq/kg (18.7% TRR) for the pyrazole label, a reduction to 0.008 mg eq/kg (6.8% TRR) was observed post-enzyme hydrolysis.

Other fractions not chromatographed that were obtained from the initial extraction with acetonitrile: water accounted for 0.009 mg eq/kg or 2.6% TRR for the phenyl label. This consisted of at least 3 fractions, none of which accounted for more than 0.006 mg eq/kg (1.7% TRR).

Egg White

Two (phenyl label) and three (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in egg white. For the phenyl label, parent compound pydiflumetofen and the metabolites 2,4,6 TCP and SYN547948 accounted for 0.025 mg eq/kg (46.5% TRR), 0.008 mg eq/kg (14.5% TRR) and 0.004 mg eq/kg (7.1% TRR) respectively. For the pyrazole label, the parent compound and metabolite SYN508272 accounted for the main portion at 0.014 mg eq/kg or 26.6% TRR and 0.018 mg eq/kg or 34.3% TRR, respectively. SYN547948 and NOA449410 accounted for 0.003 mg eq/kg or 5.5% TRR and 0.008 mg eq/kg or 15.4% TRR, respectively.

After extraction with aqueous acetonitrile 0.001 mg eq/kg (2.3% TRR) of the phenyl label and 0.001 mg eq/kg (1.2% TRR) of the pyrazole label remained unextracted. No further extraction procedures were undertaken.

Unassigned radiocomponents chromatographed by HPLC comprised of at least 7 discrete components for the phenyl label, no single one of which accounted for >12.3% TRR (>0.006 mg eq/kg). For the pyrazole label there were at least 3 discrete components, no single one of which accounted for >5.0% TRR (>0.003 mg eq/kg).

Muscle

Three (phenyl label) and three (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in muscle. For the phenyl label, the metabolite 2,4,6 TCP accounted for the main portion at 0.013 mg eq/kg or 48.4% TRR. Parent compound pydiflumetofen and metabolites SYN547948 and SYN547897 ranged from 0.001 to 0.002 mg eq/kg (3.4 – 8.7% TRR). For the pyrazole label, the metabolite SYN508272 accounted for the main portion, 0.010 mg eq/kg or 46.3%. Parent compound pydiflumetofen and metabolites SYN547948 and SYN547897 ranged from <0.001 to 0.001 mg eq/kg (1.1 – 4.7% TRR).

Other fractions not chromatographed accounted for 0.001 mg eq/kg (2.4% TRR) for the phenyl label and 0.002 mg eq/kg (7.0% TRR) for the pyrazole label. No fraction accounted for more than 0.001 mg eq/kg (4.2% TRR).

After extraction with aqueous acetonitrile 0.004 mg eq/kg (15.8% TRR) of the phenyl label and 0.002 mg eq/kg (9.9% TRR) of the pyrazole label remained unextracted. No further extraction procedures were undertaken.

Skin and fat

Three (phenyl label) and four (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in skin and fat. For the phenyl label, the metabolite 2,4,6 TCP accounted for the main portion, 0.030 mg eq/kg or 29.3% TRR. Parent compound pydiflumetofen and the metabolites SYN547948 and SYN547897 accounted for 0.017 mg eq/kg (16.6% TRR), 0.003 mg eq/kg (3.0% TRR) and 0.002 mg eq/kg (1.7% TRR) respectively. For the pyrazole label, parent compound pydiflumetofen accounted for the main portion at 0.01 mg eq/kg (30.6% TRR). The metabolites SYN547948, SYN547897, SYN508272 and NOA449410 ranged from 0.001 to 0.003 mg eq/kg (2.6 – 9.6% TRR).

Chromatographed but unassigned fractions accounted for 0.015 mg eq/kg or 13.8% TRR for the phenyl label and 0.012 mg eq/kg or 32.3% TRR for the pyrazole label. For the phenyl label this consisted of 5 fractions, none of which accounted for more than 0.004 mg eq/kg (3.7% TRR). For the pyrazole label, 9 fractions were present, none of which accounted for more than 0.002 mg eq/kg (6.4% TRR).

Other fractions not chromatographed accounted for 0.021 mg eq/kg or 20.5% TRR for the phenyl label and 0.001 mg eq/kg or 2.7% TRR for the pyrazole label. No single fraction for either label comprised more than 0.006 mg eq/kg (6.0% TRR).

Table 7-30 Summary of the characterisation and identification of components in liver from laying hens treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation, mg eq/kg		0.404 ¹		0.210 ¹	
TRR by direct quantification, mg eq/kg		0.374 ²		0.203 ²	
Percentage of TRR for chromatography, %		39.2		27.9	
Origin of component	Component	%TRR	Residue mg eq/kg	%TRR	Residue mg eq/kg
Chromatographed ³	pydiflumetofen	5.3	0.021	0.5	0.001
	SYN547948	0.7	0.003	3.2 ⁴	0.007 ⁴
	(as conjugates)	(N/D)	(N/D)	(N/D)	(N/D)
	SYN547897	2.4	0.009	0.9 ⁴	0.002 ⁴
	(as conjugates)	(0.4)	(0.001)	(0.9)	(0.002)
	SYN545547	1.2	0.005	3.3 ⁴	0.007 ⁴
	(as conjugates)	(0.6)	(0.003)	(3.3)	(0.007)
	SYN547891	0.2	0.001	N/D	N/D
	(as conjugates) ⁵	(0.2)	(0.001)	(N/D)	(N/D)
	SYN508272	N/A	N/A	2.4	0.005
	(as conjugates)	(N/A)	(N/A)	(2.4)	(0.005)
	Unassigned in pre-enzyme hydrolysis organosoluble ⁶	19.4	0.078	11.3	0.023
	Unassigned in post enzyme hydrolysis organosoluble ⁷	6.1	0.023	6.0	0.012
	Post enzyme hydrolysis water soluble ⁸	4.1	0.017	4.7	0.010
	Other fractions ⁹	7.4	0.030	8.6	0.017
	Unextracted ¹⁰	48.3	0.195	47.5	0.100
	Losses/gains on fractionation ¹¹	5.1	0.022	11.3	0.026
		(Loss)	(Loss)	(Loss)	(Loss)
Total		100.0	0.404	100.0	0.210

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - The components of the TRR that were derived from chromatographic analysis.

- 4 - For pyrazole label the reference standards: SYN547897, SYN547948 and SYN545547 are not resolved by 2D-TLC analysis. HPLC analysis was used to determine which components were present and, in the case of SYN547897 and SYN545547, derive the ratio of these components and determine the approximate levels of the two metabolites by 2D-TLC analysis
- 5 - Due to low residue levels, SYN547891 could not be detected by confirmatory chromatographic system
- 6 - Unassigned radiocomponents in pre-enzyme hydrolysis organo-soluble fraction chromatographed by HPLC for phenyl label and by 2D-TLC pyrazole label.
Phenyl label: comprising at least 27 discrete components, no single one of which >2.3% TRR (>0.009 mg eq/kg).
Pyrazole label: comprising at least 9 discrete components, no single one of which >2.3% TRR (>0.005 mg eq/kg).
- 7 - Unassigned radiocomponents in post enzyme hydrolysis organo-soluble fraction chromatographed by HPLC phenyl label and by 2D-TLC for pyrazole label.
Phenyl label: comprising at least 16 discrete components, no single one of which >1.6% TRR (>0.006 mg eq/kg).
Pyrazole label: comprising at least 8 discrete components, no single one of which >1.4% TRR (>0.003 mg eq/kg).
- 8 - Post enzyme hydrolysis water soluble fraction chromatographed by TLC.
Phenyl label: comprising at least 8 discrete components, no single one of which >1.0% TRR (>0.004 mg eq/kg).
Pyrazole label demonstrated to consist of radioactivity located on the origin, characterised to be similar to that present in the liver debris.
- 9 - Phenyl label: consists of two fractions no individual one of which accounts for >6.7% TRR (>0.027 mg eq/kg). Although this particular fraction was not chromatographed, a corresponding fraction from the initial phenyl liver analysis was analysed and shown to consist of radioactivity located on the origin, characterised to be similar to that present in the liver debris.
Pyrazole label: consists of 4 fractions none of which accounts for >4.3% TRR (0.009 mg eq/kg).
- 10 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. The nature of this residue was characterised further by SDS extraction and a protease digestion experiment and shown to be similar in nature in both radiolabelled experiments and comprise a complex mixture of polar unassigned radiocomponents.
- 11 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-31 Summary of the characterisation and identification of components in egg yolk from laying hens treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation, mg eq/kg		0.358 ¹		0.106 ¹	
TRR by direct quantification, mg eq/kg		0.353 ²		0.103 ²	
Percentage of TRR for chromatography, %		82.3		64.8	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed ³	pydiflumetofen	3.0	0.011	11.0	0.012
	2,4,6 TCP ⁴ (as conjugates)	67.8 (67.8)	0.242 (0.242)	N/A (N/A)	N/A (N/A)
	SYN547948	N/D	N/D	1.3	0.001
	SYN547897	2.3	0.008	6.7	0.007
	SYN545547	N/D	N/D	3.9	0.004
	SYN547891	N/D	N/D	2.5	0.003
	SYN508272 (as conjugates)	N/A (N/A)	N/A (N/A)	7.2 (2.6)	0.008 (0.003)
	NOA449410 (as conjugates)	N/A (N/A)	N/A (N/A)	6.6 (0.8)	0.007 (0.001)

	Unassigned ⁵	6.6	0.024	N/A	N/A
	Unassigned in pre-enzyme hydrolysis organosoluble ⁶	N/A	N/A	18.7	0.020
	Unassigned in post enzyme hydrolysis organosoluble ⁷	N/A	N/A	6.8	0.008
	Other fractions ⁸	2.6	0.009	N/A	N/A
	Other fractions ⁹	2.2	0.008	5.5	0.006
	Unextracted ¹⁰	13.0	0.047	18.7	0.020
	Losses/gains on fractionation ¹¹	2.5 (Loss)	0.009 (Loss)	11.0 (Loss)	0.010 (Loss)
	Total	100.0	0.358	100.0	0.106

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - The components of the TRR that were derived from chromatographic analysis.
- 4 - Found exclusively as the 2,4,6 TCP sulphate conjugate.
- 5 - Unassigned radiocomponents in pre-enzyme hydrolysis aqueous soluble fraction, chromatographed by HPLC. Phenyl label: comprising at least 3 discrete components, no single one of which >3.5% TRR (>0.013 mg eq/kg)
- 6 - Unassigned radiocomponents in pre-enzyme hydrolysis organo-soluble fraction, chromatographed by HPLC. Pyrazole label: comprising at least 9 discrete components, no single one of which >5.9% TRR (>0.006 mg eq/kg).
- 7 - Unassigned radiocomponents in post enzyme hydrolysis organo-soluble fraction, chromatographed by HPLC. Pyrazole label: comprising at least 5 discrete components, no single one of which >4.3% TRR (>0.005 mg eq/kg).
- 8 - Initial concentrated acetonitrile: water (1:1, v/v) fraction analysed by 2D-TLC, comprising at least 3 discrete components, no single one of which >1.7% TRR (>0.006 mg eq/kg).
- 9 - Extractable residue fractions which were not analysed. No single fraction comprised $\geq 4.3\%$ TRR (≥ 0.005 mg eq/kg) in either radiolabelled experiment.
- 10 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. The nature of this residue was characterised further by protease digestion and SDS extraction and characterised to be similar in nature in both radiolabelled experiments.
- 11 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-32 Summary of the characterisation and identification of components in egg white from laying hens treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.053 ¹		0.052 ¹	
TRR by direct quantification mg eq/kg		0.055 ²		0.051 ²	
Percentage of TRR for chromatography, %		92.7		91.8	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed ³	pydiflumetofen	46.5	0.025	26.6	0.014
	2,4,6 TCP ⁴ (as conjugates)	14.5 (14.5)	0.008 (0.008)	N/A (N/A)	N/A (N/A)
	SYN547948	7.1	0.004	5.5	0.003
	SYN508272	N/A	N/A	34.3	0.018
	NOA449410	N/A	N/A	15.4	0.008
	Unassigned ⁵	24.6	0.013	10.0	0.006
	Unextracted ⁶	2.3	0.001	1.2	0.001
	Losses/gains on	5.0	0.002	7.0	0.002

fractionation ⁷	(Loss)	(Loss)	(Loss)	(Loss)
Total	100.0	0.053	100.0	0.052

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - The components of the TRR that were derived from chromatographic analysis.
- 4 - Found exclusively as the 2,4,6 TCP sulphate conjugate.
- 5 - Unassigned radiocomponents chromatographed by HPLC
Phenyl label: comprising at least 7 discrete components, no single one of which >12.3% TRR (>0.006 mg eq/kg)
Pyrazole label: comprising at least 3 discrete components, no single one of which >5.0% TRR (>0.003 mg eq/kg)
- 6 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. No further work was carried out on the debris.
- 7 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-33 Summary of the characterisation and identification of components in muscle from laying hens treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.027 ¹		0.021 ¹	
TRR by direct quantification mg eq/kg		0.028 ²		0.022 ²	
Percentage of TRR for chromatography, %		74.8		75.2	
Origin of component	Component	%TRR	Residue mg eq/kg	%TRR	Residue mg eq/kg
Chromatographed ³	pydiflumetofen	8.7	0.002	4.7	0.001
	2,4,6 TCP ⁴ (as conjugates)	48.4 (48.4)	0.013 (0.013)	N/A (N/A)	N/A (N/A)
	SYN547948	3.4	0.001	1.6	<0.001
	SYN547897	N/D	N/D	1.1	<0.001
	SYN508272	N/A	N/A	46.3	0.010
	Unassigned ⁵	14.3	0.004	21.3	0.004
	Other fractions ⁶	2.4	0.001	7.0	0.002
	Unextracted ⁷	15.8	0.004	9.9	0.002
	Losses/gains on fractionation ⁸	7.0 (Loss)	0.003 (Loss)	7.9 (Loss)	0.002 (Loss)
	Total	100.0	0.027	100.0	0.021

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - The components of the TRR that were derived from chromatographic analysis.
- 4 - Found exclusively as the 2,4,6 TCP sulphate conjugate.
- 5 - Unassigned radiocomponents chromatographed by HPLC
Phenyl label: comprising at least 4 discrete components, no single one of which >5.0% TRR (>0.001 mg eq/kg)

Pyrazole label: comprising at least 6 discrete components, no single one of which >6.0% TRR (>0.001 mg eq/kg)

- 6 - Extractable residue fractions which were not analysed. No single fraction comprised > 4.2% TRR (>0.001 mg eq/kg) in either radiolabelled experiment.
- 7 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. No further work was carried out on the debris.
- 8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-34 Summary of the characterisation and identification of components in skin and fat from laying hens treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.101 ¹		0.032 ¹	
TRR by direct quantification mg eq/kg		0.090 ²		0.028 ²	
Percentage of TRR for chromatography, %		64.6		82.3	
Origin of component	Component	%TRR	Residue mg eq/kg	%TRR	Residue mg eq/kg
Chromatographed ³	pydiflumetofen	16.6	0.017	30.6	0.010
	2,4,6 TCP (as conjugates) ⁴	29.3 (26.5)	0.030 (0.027)	N/A (N/A)	N/A (N/A)
	SYN547948	3.0	0.003	4.1	0.001
	SYN547897	1.7	0.002	2.6	0.001
	SYN508272	N/A	N/A	9.6	0.003
	NOA449410	N/A	N/A	3.1	0.001
	Unassigned ⁵	13.8	0.015	32.3	0.012
	Other fractions ⁶	20.5	0.021	2.7	0.001
	Unextracted ⁷	4.3	0.004	8.4	0.003
	Losses/gains on fractionation ⁸	10.6 (Loss)	0.009 (Loss)	6.6 (Loss)	0.002 (Loss)
Total		100.0	0.101	100.0	0.032

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - The components of the TRR that were derived from chromatographic analysis.
- 4 - Conjugated material found exclusively as the 2,4,6 TCP sulphate conjugate.
- 5 - Unassigned radiocomponents chromatographed by HPLC
Phenyl label: comprising at least 5 discrete components, no single one of which >3.7% TRR (>0.004 mg eq/kg)
Pyrazole label: comprising at least 9 discrete components, no single one of which >6.4% TRR (>0.002 mg eq/kg)
- 6 - Extractable residue fractions which were not analysed. No single fraction comprised > 6.0% TRR (>0.006 mg eq/kg) in either radiolabelled experiment.
- 7 - Radioactivity remaining in the debris after extraction with hexane/aqueous acetonitrile. No further work was carried out on the debris.
- 8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Storage stability investigations

The applicant included some storage stability analyses (chromatographic comparisons obtained in the metabolism study context) to compare HPLC analysis generated at the initial analysis (within 6 months) time period with HPLC determinations done in the later period of the study (circa 2.5 years). This was done for representative samples of fat and egg yolk.

The phenyl egg yolk extract comparison, with a single main peak was similar before and after storage. The pyrazole egg yolk extract with a number of main peaks and fat extracts (phenyl and pyrazole) were more complex samples (with the peaks not all completely resolved). The chromatograms (though not identical before and after storage with some small retention time shifts, and lack of full resolution of each of the peaks to baseline), do not overall indicate particular concerns regarding the storage stability of the residues over the course of the study. The chromatograms for these comparisons do not involve any labelling of the peak identity as the comparisons are done using the first initial HPLC analyses as the main comparison point; at this stage of initial metabolic profiling, the identity of the metabolites is not known. It is difficult to fully assess when the chromatograms do not show the same peak shape in the 'before' and 'after' chromatograms. In a letter response to an HSE query, the applicant explained that initial radio-HPLC analysis of the livestock profiles in both hen and goat studies were carried out using a generic chromatography system in order to ascertain the initial complexity of the metabolic pathway as well as obtain an initial storage stability profile.

HSE considers that it is not ideal that all the poultry metabolism samples were stored frozen for 4 months prior to extraction and analysis, given that storage stability of residues in the metabolism context is something that requires consideration, if overall the metabolism studies last for longer than 6 months.

The applicant has noted that in the poultry metabolism the residues were generally low with the exception of parent and 2,4,6-TCP. These residues are covered in the freezer storage stability studies reported (in section B.7.1.2 and in vol 1 2.7.1). In these 'cold' (non-radiolabelled) studies, residues of parent pydiflumetofen and 2,4,6 – TCP (free and conjugated) were shown to be stable in various animal matrices over frozen storage for at least 2 years (parent) and at least 1 year (2,4,6 –TCP).

The 'cold' (non-radiolabelled) storage stability studies also demonstrated stability of: SYN508272 and SYN548264 in milk for at least a year, and SYN548263 in bovine kidney for at least a year.

Some degradation of the metabolite SYN547897 was observed in the 'cold' (non radiolabelled) storage stability work which tested stability for up to a one-year period in liver and kidney. This metabolite was only stable in bovine liver up to ~9.5 months and in bovine kidney for up to 11 months. These periods are greatly exceeded by the maximum storage period in the metabolism study for poultry; the metabolite was identified in the poultry metabolism study, but was not found in significant quantities and was not studied further in the feeding study for poultry. The storage stability comparisons in poultry (in the metabolism context) were for fat and egg yolk only. A discussion of the non-inclusion of this metabolite with regards to the residue definition for livestock is given in Volume 1, section 2.7.3.

Enantiomer ratio

There are two enantiomers of the parent compound (pydiflumetofen). In the dose capsules for the phenyl and pyrazole labelled active, the isomer ratio of [¹⁴C]-SYN546969/[¹⁴C]-SYN546968 was found to be 50.8/49.2 and 50.6/49.4 respectively when studied with chiral HPLC.

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur.

Unlike the plant metabolism studies (where the main component of the residue was parent pydiflumetofen), the enantiomer ratio in animal commodities was not studied in metabolism (or feeding) studies. See discussion in Vol 1 section, 2.7.2.

Metabolic pathway

The proposed metabolic pathway of pydiflumetofen in laying hens is shown in Figure 7-5. In total, seven metabolites were identified. The main biotransformation steps to pydiflumetofen leading to these metabolites include; N-demethylation of the pyrazole ring, N-demethoxylation of the amide nitrogen, monohydroxylation of the benzyl methylene functionality and monohydroxylation of the trichlorophenyl ring. Conjugation of the residues is also a main feature of the residues formed from pydiflumetofen (see below).

Conclusion

Pydiflumetofen was administered orally to twelve laying hens in two radiolabelled forms (phenyl and pyrazole labels) for fourteen consecutive days (nominal dose of 30 mg as equivalents/kg, dry matter intake).

Approximately 103.4 % and 88.0 % of the administered dose were recovered in total for the phenyl and pyrazole label, respectively. The main fraction was excreted via excreta accounting for approximately 99.1 % (phenyl label) and 84.3 % (pyrazole label). Radioactive residues associated with edible portions (egg and tissues) accounted for up to <0.1% of the administered dose for both labels.

In eggs, the level of radioactive residues for the phenyl label increased to a plateau at 6 and 10 days with a concentration of 0.064 mg eq/kg and 0.344 mg eq/kg, in egg white and yolk respectively. There was no sample available for day 14 for the phenyl label. For the pyrazole label, a plateau of 0.062 mg eq/kg (egg white) and 0.116 mg eq/kg (egg yolk) was reached after 7 days.

In the edible matrices, the highest TRR concentrations were calculated for liver (0.210 – 0.404 mg eq/kg) and egg yolk (0.106 – 0.358 mg eq/kg). For all other matrices, the TRR was in a range from 0.027 – 0.101 mg eq/kg (phenyl label) and from 0.021 – 0.052 mg eq/kg (pyrazole).

Samples of liver, egg yolk, egg white and muscle were extracted with acetonitrile and water. Fat samples were extracted with a mixture of acetonitrile, water and hexane and subsequently with acetonitrile and water. The extractable TRR levels for both labels were generally high, ranging from 87.0 % TRR to 98.8 % TRR, except for egg yolk (pyrazole label; 81.2% TRR) and liver (51.7% TRR and 52.5 % TRR for the phenyl and pyrazole labels, respectively). Radioactive residues in the RRR obtained after the initial extraction of egg yolk (pyrazole label) and liver (both labels) was 18.7 – 48.3 % TRR, which were further investigated.

To quantify and identify radioactive components, thin layer chromatography (1D and 2D) was performed on samples and the plates were subject to bioimage analysis to detect the radioactive areas. Reverse phase HPLC was also performed on all types of samples to quantify and identify the residues. Chromatographic comparison of the residues was done with synthetic reference standards of pydiflumetofen and its metabolites.

The main observations from the identification work are the following:

- The majority of radioactivity was observed in excreta (84.3-99.1% TRR)
- Total radioactive residues in egg yolk achieved a plateau concentration at approximately 0.344 mg eq/kg (phenyl) and 0.116 mg eq/kg (pyrazole) after 10 and 7 days of dosing respectively
- Parent (pydiflumetofen) was found in egg yolk, egg white and all tissues at levels ranging from 0.001 mg eq/kg to 0.025 mg eq/kg (0.5% TRR to 46.5% TRR). In liver and egg white, parent was the major component.
- Seven metabolites were identified from the metabolism of pydiflumetofen:
 - Phenolic metabolite 2, 4, 6-trichlorophenol (formed by cleavage at the benzylic methylene location) and its sulphate ester conjugate. The 2, 4, 6-TCP found was attributed to the sulphate conjugate (not found as free 2, 4, 6-TCP). The sulphate conjugate of 2,4,6-TCP was a main component in all matrices, except liver where it was not found.
 - Alkyl hydroxy metabolite SYN547948
 - Phenolic metabolite SYN547897
 - N-desmethoxy metabolite SYN545547
 - Pyrazole N-desmethyl metabolite SYN547891
 - Pyrazole amide metabolite SYN508272
 - Pyrazole carboxylic acid metabolite NOA449410
- The most prominent metabolites (≥10% TRR) of pydiflumetofen identified in tissues and eggs were:

- 2,4,6-TCP sulphate conjugate (egg yolk: 67.8% TRR; 0.242 mg eq/kg, muscle: 48.4% TRR; 0.013 mg eq/kg, fat: 26.5% TRR; 0.027 mg eq/kg, egg white: 14.5% TRR; 0.008 mg eq/kg)
- SYN508272 (muscle: 46.3% TRR; 0.010 mg eq/kg, egg white: 34.3% TRR; 0.018 mg eq/kg)
- NOA449410 (egg white: 15.4% TRR; 0.008 mg eq/kg)
- To a varying extent, conjugation of metabolites was a feature, not only for the 2,4,6-TCP. The conjugates are expected to be either glucuronide or sulphate conjugates (see below).

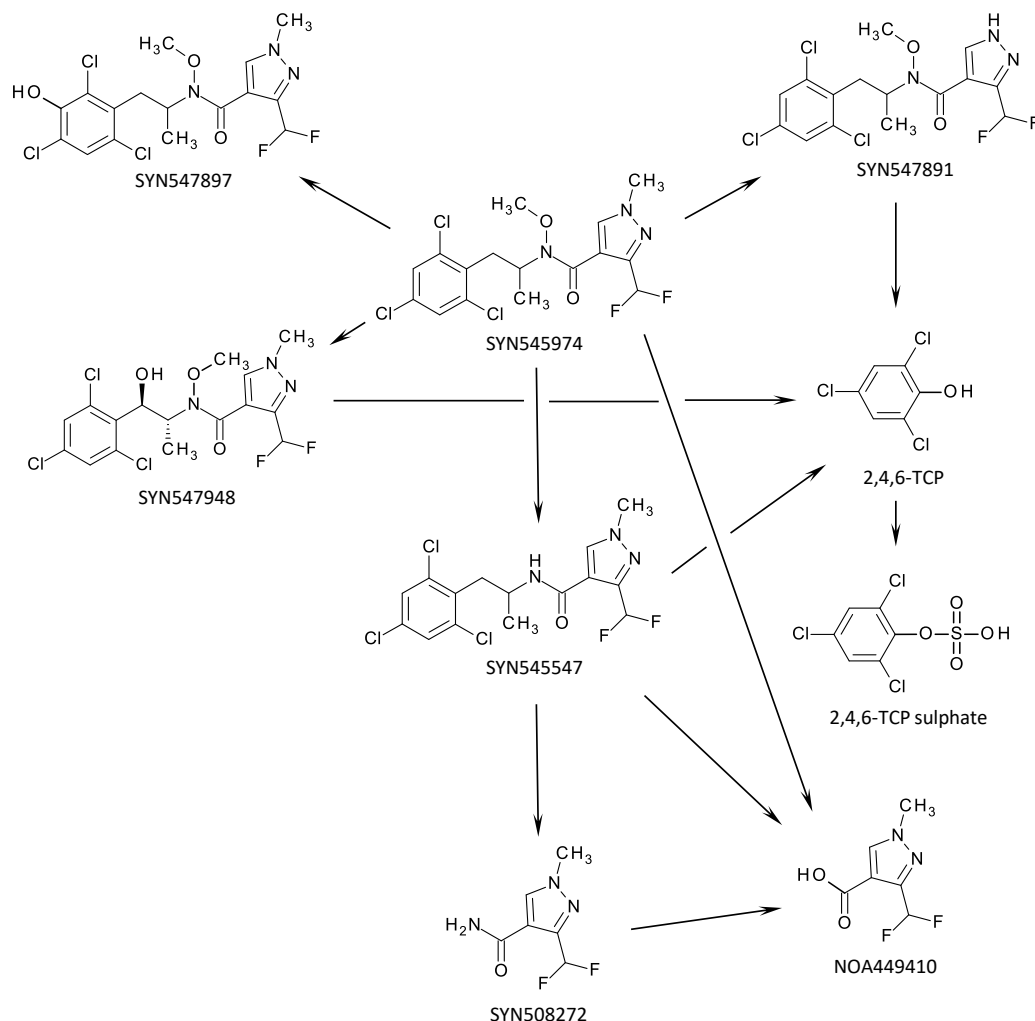
The transformation steps in the metabolic pathway for these are:

- N-demethylation of the pyrazole ring
- N-demethoxylation of the amide nitrogen
- monohydroxylation of the benzyl methylene functionality
- monohydroxylation of the trichlorophenyl ring
- cleavage at the benzylic methylene, N-alkyl and amide linkages between the phenyl and pyrazole rings
- Conjugation of metabolites to form their glucuronide and/or sulphate ester analogues.

Reference standards that were analysed for but not detected:

- SYN547893
- SYN547892
- SYN547890
- SYN547895
- SYN547894
- SYN548266
- SYN545720
- SYN547949 – two enantiomers of the compound
- SYN548263
- SYN548264

Regarding conjugates, a reference standard was available for the sulphate conjugate of 2,4,6-TCP. Other reference standards for conjugates were not available, however the presence of conjugates for the other metabolites was determined by the deconjugation behaviour when samples were incubated with β -glucuronidase. It was observed that this enzyme had both glucuronidase and sulphatase activity and was therefore able to cleave glucuronide and sulphate conjugates. Therefore, although presence as conjugates was known, the specific type of the conjugates has not been determined.

Figure 7-5 Proposed metabolic pathway of pydiflumetofen (SYN545974) in laying hen

B.7.2.3. Lactating ruminants

In this section the relationship between the actual doses used in the study, and the anticipated use rates (the N rate) following the assessment of the uses considered in this evaluation, are expressed considering the following various assessment scenario following presentation to the Expert Committee on Pesticides (ECP) in the process of seeking Independent Scientific Advice (ISA) and taking account of both rotational crop and primary crop residues. The fate parameters (on estimation of residues in soil), affect the rotational crop residues which in turn feed into the animal dietary burden. Two different scenarios have been assessed in this residues evaluation—'Tier 1 10 year use' and 'Tier 2 long term use'. These are The approach is further explained in Vol 1 section 2.7.7 (rotational crops) and Vol 1 section 2.7.5 (animal dietary burden).

Report: K-CA 6.2.3/01, (2015)
Title: SYN545974 – Metabolism of [¹⁴C]-SYN545974 in the Lactating Goat
Report No: Report No. 33963
Document No: Document No. VV-414236 (Syngenta File No. SYN545974_10284)
Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

	OECD guideline 503 for testing of chemicals “Metabolism in Livestock”, 08-Jan-2007.
Guideline deviations:	None
GLP:	Yes

Materials and methods

Materials

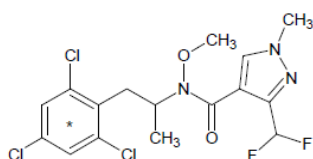
1. C-label pydiflumetofen

Description: [Phenyl-U-¹⁴C]-SYN545974 (Spec. Activity of a.s. 5.7905 MBq/mg)

Lot/Batch #: RDR-XV-94

Radiochemical Purity: 99.1%

Structures:
(* marks position of radiolabel)



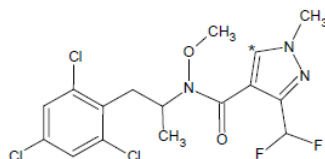
2. C-label pydiflumetofen

Description: [Pyrazole-5-¹⁴C]-SYN545974 (Spec. Activity of a.s. 5.06 MBq/mg)

Lot/Batch #: 5271GAR001-4

Radiochemical Purity: 99.2%

Structures:
(* marks position of radiolabel)



Methods

The metabolism of pydiflumetofen (SYN545974) was investigated in lactating goats (breed *Saanen*) following repeated oral administration of pydiflumetofen labelled either at the [phenyl] or the [pyrazole] position, as shown in the above structural diagrams. The experiments with both labels were carried out simultaneously.

A total of 2 goats were dosed with pydiflumetofen radiolabelled at either the [phenyl] or the [pyrazole] position. The planned dose rate was 100 mg as equivalents/kg, dry matter intake. Doses were prepared based on a daily dry matter intake of 2 kg, this was equivalent to 204.1 mg [phenyl] and 196.4 mg [pyrazole] per capsule per day. As the dry matter consumption was lower than the assumed (at 0.694 – 1.619 kg feed), the achieved dose rates were approximately 143.6 and 204.6 mg as equivalents/kg dry feed respectively (4.6 mg as equivalents/kg bodyweight) for 7 consecutive days (1 goat per label). The dosing rate of 4.6 mg as equivalents/kg bodyweight represents 55N 22N (Tier 1 10 year use) and 28N (Tier 2 term use). Details of the study outline are summarized in Table 7-35. The specific activity of the [phenyl] and [pyrazole] radiolabelled pydiflumetofen were adjusted to 2.15 MBq/mg and 1.90 MBq/mg respectively using non-radiolabelled pydiflumetofen. The test items were prepared in gelatine capsules and orally administered once daily, after morning milking. The actual dose was calculated based on the food consumption (dry weight).

Dose capsules were prepared on the day before the first dose, and all capsules were sealed and stored at *ca* -20 °C. Additional capsules were prepared so that stability and radiochemical purity tests could be done. Prior to the preparation of the actual dose formulations of phenyl and pyrazole labelled [¹⁴C]- pydiflumetofen, stability tests were carried out on dose formulations (in solvent and in capsules) over an 8-day period to ensure stability over the daily dosing period at *ca* -20 °C. For each radiolabel, a capsule was analysed prior to the first dose and following the final dose to determine the radiochemical purity by HPLC. The capsule that was analysed after the final dosing was stored at *ca* -20 °C until analysis.

Table 7-35 Dosing of lactating goats with pydiflumetofen

Radiolabel	Animal No.	Treatment period (days)	Nominal daily dose	Actual daily dose ^{1, 2}		N rate (Tier 1 10-year use)	N rate (Tier 2 long term use)	Time of sacrifice (hours) ³
			[mg/kg feed]	[mg/kg dry matter]	[mg/kg bw/d]			
Phenyl	1	7	100	143.6	4.6	55N 22N	28N	11
Pyrazole	1	7	100	204.6	4.6	55N 22N	28N	11

¹ Mean value of day 1-6² Using the mean animal weight over the dosing period³ Hours after last dose

Just prior to the start of dosing the goats were examined by a veterinarian and deemed acceptable for the study. The appearance and general health of the goats continued to be assessed twice daily for the duration of the study.

Urine was collected prior to the first dose and at 24hr intervals up until sacrifice. The weight was recorded, and the samples mixed before aliquots were taken and radioassayed. Sub samples were taken and stored at *ca* -20 °C alongside remaining bulk samples. Samples of faeces were collected prior to dose administration and at each 24 h period until sacrifice and mixed with a solution of methanol: water. The weights of the individual samples were recorded. After the addition of methanol: water (1:1, v/v), addition of acetone (volume approximately equal to the original faeces weight) and homogenisation, the weight of the subsequent homogenates were recorded. Aliquots of each sample were taken for radioassay measurements by combustion / LSC. All samples were then stored in a freezer at *ca* -20 °C following collection.

Following the final urine and faeces collection, the cage was washed with methanol: water (1:1, v/v). Once soaked for at least 24 hours, the samples were mixed, and aliquots taken for radioassay by LSC. Samples were stored at ambient temperature.

Milk was collected twice daily (am and pm) during the acclimatisation and the 7-day dosing period. The total amount of milk was weighed each time and then the individual samples were thoroughly mixed, and aliquots were taken. On days 4 – 7, 20% of the milk by weight was taken as a subsample from both the AM and PM collection. During this time period, the subsample from the PM collection was stored at *ca* 4°C overnight and mixed with the following morning's subsample. Each 24-hour composite sample was used to make cream and skimmed milk using physical separation via centrifugation. Both the cream and skimmed milk were radioassayed for each 24-hour period.

The goats were sacrificed approximately 11 hours after the administration of the final dose. Edible tissues (liver, kidneys, fat (peritoneal, perirenal and subcutaneous) and muscle (Flank and Loin), GI tract (plus contents), blood (*ca* 30 mL), carcass and bile were removed postmortem and weighed. All samples were then stored at *ca* -20°C.

Analysis/general procedures

Samples of liver, kidney, peritoneal fat, perirenal fat, subcutaneous fat, flank muscle and loin muscle were homogenized prior to analysis. The tissues were homogenised frozen with the aid of dry ice to form a fine powder. Samples of milk were also homogenised with acetonitrile and hexane. Following processing and analysis each tissue sample was stored at *ca* -20 °C.

Where radioactive residues in muscle (flank and loin) and fat (tissues were perirenal, subcutaneous and peritoneal) were ≥ 0.01 mg eq/kg and of similar magnitude, composite samples for each label (phenyl and pyrazole) were prepared by combining the respective tissues. For muscle, the flank and loin muscle were combined in a ratio of 1-1.5:2 and fat samples combined in the ratio 1:1.2:2.3 (Perirenal:Subcutaneous:Peritoneal). The total radioactive residues in milk were calculated for each 24-hour period. A plateau was reached after 2 days for the phenyl label and 6 days for the pyrazole. The average residue for days 3, 4, 5 and 6 was taken to give the TRR for milk for the phenyl label and the day 6 result for pyrazole. As described above, sub-samples from days 4 – 7 were separated to give skimmed milk and cream.

Samples of liver, kidney and muscle were extracted two times with acetonitrile: water (4:1, v/v) and once with acetonitrile: water (1:1, v/v). Samples of fat were extracted two times with acetonitrile: water (4:1, v/v) + hexane and once with acetonitrile: water (1:1, v/v). Samples of milk were extracted with acetonitrile and hexane. For the first extraction, the nominal expected moisture content of the liver, kidney and muscle was accounted for when calculating the volume of water required. After centrifugation, the respective acetonitrile and water extracts (liver, kidney and muscle) or acetonitrile, water and hexane (fat and milk) were removed from the solid tissue debris and adjusted to a defined volume with appropriate solvents. These aliquots then underwent LSC analysis, and the solid residue left after solvent extraction was subjected to combustion analysis.

To quantify and identify radioactive components, thin layer chromatography (1D and 2D) was performed on samples, and the plates were subject to bioimage analysis to detect the radioactive areas. Reverse phase HPLC was also performed on all types of samples to quantify and identify residues. Where required, organic solvent/water partition and enzyme hydrolysis were performed prior to chromatographic analysis.

If required, enzyme hydrolysis was performed using β -glucuronidase, which also had sulphatase activity and was therefore able to cleave glucuronide and sulphate conjugates. Aliquots of the aqueous partition fraction from organic solvent/water partitioning were adjusted in pH prior to the addition of β -glucuronidase and then samples were incubated overnight in a shaking water bath at 37°C. After this, the enzyme was denatured, and a liquid-liquid partition was performed via shaking.

Chromatographic comparison of the residues was done with synthetic reference standards of pydiflumetofen and its metabolites.

Characterisation of unextracted residues

Significant levels of radioactivity in liver (phenyl: 49.7% TRR; 3.471 mg eq/kg, pyrazole: 52.6% TRR, 4.643 mg eq/kg) and kidney (phenyl: 16.6% TRR; 0.287 mg eq/kg, pyrazole: 9.2% TRR, 0.215 mg eq/kg) remained unextracted in the debris following solvent extraction. For both commodities, the unextracted residues were investigated further by solubilisation either with the surfactant, sodium dodecyl sulphate (SDS), or proteolytic enzyme hydrolysis.

Subsamples of liver and kidney for both labels were treated with SDS solution to create a micellar suspension of proteins which were then precipitated out of solution and the aliquot was centrifuged. For liver and kidney, the resultant protein pellet accounted for approximately half and a third respectively of the radioactivity from the original debris. Chromatographic analysis of the radioactivity remaining in the supernatant fraction was not possible due to the high levels of surfactant.

To a separate set of subsamples of liver and kidney, protease was added to the aliquots and incubated. The residues released by hydrolysis were separated into soluble and insoluble material and the soluble material was radioassayed. Using 1D-TLC it was determined that the soluble fraction of liver extract was comprised of a complex mixture of polar un-assigned species either on – or close to – the origin of the plate for the phenyl label or with species on – or close to – the origin with minor components further up the plate for pyrazole. Using a more chromatographically forcing solvent system led to more separation from the origin. Using this system, it was observed that for phenyl there were a minimum of 8 unassigned resolvable regions on the chromatogram, no region accounted for more than 7.4% TRR (0.52 mg eq./kg). The pyrazole label showed a similar profile with no region accounting for more than 6.5% TRR (0.57 mg eq./kg). The extracts of kidney also underwent 1D-TLC, the extract was comprised of a complex mixture of polar un-assigned species either on – or close to – the origin of the plate for the phenyl label or with species on – or close to – the origin with minor components further up the plate for pyrazole. For phenyl there were a minimum of 5 unassigned regions on the chromatogram, no region accounted for more than 5.0% TRR (0.087 mg eq./kg), for pyrazole there were 5, with no region accounting for more than 3.1% TRR (0.073 mg eq./kg).

Storage stability investigation:

The ruminant study was conducted over a period of around 2.5 years (time between necropsy and final analytical work). Samples were kept frozen over the period of storage. The samples were stored for 4 months frozen prior to extracting the residues. The initial analyses of the samples (metabolic profiling) were done within the 4-to-6-month period.

OECD Guidelines (OECD Guidelines (503, livestock metabolism) indicate that metabolism studies should be completed within an analysis period of six months, or otherwise be appropriately supported by storage stability investigations performed in the context of the metabolism studies.

The applicant included some storage stability analyses (chromatographic comparisons obtained in the metabolism study context) to compare HPLC analysis generated at the initial analysis (within 6 months) time period with HPLC determinations done in the later period of the study. This was done and chromatographic comparisons were presented in the report for representative samples of milk and fat. Since the report also discussed storage stability investigations for liver samples (and as some possible instability of metabolite residues was reported in the ‘cold’ - non radiolabelled storage stability studies, see the discussion relating to metabolite SYN547897 in liver/kidney in section B.7.1.2 and Vol 1 section 2.7.1), HSE also asked to be provided with the metabolism storage stability chromatographic comparisons for liver determined at the time of the ruminant metabolism study. The applicant provided these from the raw data.

Results and discussion

All mg/kg levels reported are in mg/kg parent equivalents.

Total radioactive residue (TRR)

The overall recovery of radioactive residues is provided in Table 7-36 as % administered dose. The recoveries were good, at 96.0% for the phenyl labelled pydiflumetofen and 94.7% for the pyrazole label.

The main fraction was excreted via urine and faeces accounting to approximately 31.5 and 52.7% respectively (phenyl label) and 29.9 and 46.4% (pyrazole label). GI contents were 9.9% for the phenyl label and 16.6% for pyrazole. Radioactive residues associated with edible portions (milk and tissues) accounted for <0.1% to 0.4% of the administered dose for both labels.

Table 7-36 Recovery of radioactive material (results expressed as % dose administered)

Matrix	% of dosed radioactivity recovered	
	[phenyl- ¹⁴ C]-pydiflumetofen	[pyrazole- ¹⁴ C]-pydiflumetofen
Urine	31.5	29.9
Faeces	52.7	46.4
GI contents	9.9	16.6
Milk	<0.1	<0.1
Liver	0.4	0.4
Kidney	<0.1	<0.1
Flank muscle	<0.1	<0.1
Loin muscle	<0.1	<0.1
Fat (peritoneal, perirenal and subcutaneous)	<0.1	<0.1
Bile	0.1	0.1
Cage wash	1.4	1.3
Total	96.0	94.7

GI – Gastrointestinal tract

The extractable TRR levels were generally high, ranging from 86.0 % TRR to 98.8 % TRR, except for liver (50.4% TRR and 47.4% TRR for the phenyl and pyrazole labels, respectively) and kidney (83.4% TRR and 90.7% TRR for the phenyl and pyrazole labels, respectively). Radioactive residues in the RRR (residual radioactive residue) obtained after the initial extraction of liver and kidney was 9.2 – 52.6 % TRR, which were further investigated. The RRR of all other relevant matrices were at or below 14.0 % TRR (0.014 mg eq/kg) for the pyrazole label. For the phenyl label, all matrices were at or below 6.1% (0.008 mg eq/kg).

In the edible matrices, the highest TRR concentrations were calculated for liver (6.984 – 8.827 mg eq/kg) and kidney (1.730 – 2.341 mg eq/kg). For all other matrices, the TRR was in the range 0.102 to 0.221 mg eq/kg (phenyl label) and 0.132 – 0.279 mg eq/kg (pyrazole).

Table 7-37 Summary of total radioactive residues and extractability in tissue and milk samples from goats treated with [¹⁴C]- pydiflumetofen

Radiolabel	Sample	Extractable radioactivity		Non-extractable radioactivity		TRR	TRR ⁽²⁾
		%TRR	mg eq/kg	%TRR	mg eq/kg	mg eq/kg	mg eq/kg
[Phenyl-U- ¹⁴ C]-pydiflumetofen	Milk (79 h)	92.3	0.112	7.7	0.009	N/A	0.122
	Liver	50.4	3.520	49.7	3.471	N/A	6.984
	Kidney	83.4	1.443	16.6	0.287	N/A	1.730
	Muscle ²	86.0	0.087	14.0	0.014	0.101	0.102
	Fat ³	98.8	0.219	1.1	0.002	0.205	0.221
[Pyrazole-5- ¹⁴ C]-pydiflumetofen	Milk (127 h)	93.9	0.124	6.1	0.008	N/A	0.132
	Liver	47.4	4.184	52.6	4.643	N/A	8.827
	Kidney	90.7	2.123	9.2	0.215	N/A	2.341
	Muscle ²	94.3	0.130	5.7	0.008	0.117	0.138
	Fat ³	97.6	0.272	2.4	0.007	0.240	0.279

1 – mg eq/kg calculated from combustion/LSC analysis of composite samples

2 – mg eq/kg calculated directly from radioactivity extracted, radioactivity in the debris and specific activity.

2 – Composite muscle samples (includes flank and loin muscle combined in the ratio 1-1.5 : 2 by weight).

3 – Composite fat samples (includes perirenal, subcutaneous and peritoneal fat combined in the ratio 1:1.2:2.3 by weight).

The concentration of radioactive residues in milk are provided in Table 7-38. Milk samples were obtained by combining samples over a 24-hr period for 7 consecutive days. For the phenyl label, the level of radioactive residues increased to a plateau after 2 days with a concentration of 0.091 mg eq/kg (by averaging residues for days: 3, 4, 5 and 6). For the pyrazole label, a plateau of 0.138 mg eq/kg was reached after 5 days.

Table 7-38 Summary of Total Radioactive Residues in Milk from a Goat dosed with [Pyrazole-¹⁴C] and [Phenyl-U-¹⁴C]- pydiflumetofen

Day	Residues (mg eq/kg)	
	[Phenyl-U- ¹⁴ C]	[Pyrazole-5- ¹⁴ C]
1 (24h)	0.093	0.076
2 (48h)	0.110	0.101
3 (72h)	0.106	0.105
4 (96h)	0.091	0.123
5 (120h)	0.079	0.138
6 (144h)	0.087	0.126
7 ¹ (156h)	0.107	0.151

¹ not taken into account for plateau determination since it is not a full 24-hour period

The extracted radioactivity was analysed by chromatography. The identified residues are given in Table 7-39 and Table 7-40 for the phenyl & pyrazole label, respectively. The full distribution of residues, including identified, characterised and none extracted residues are summarised in the tables following (Table 7-41 to Table 7-46).

Table 7-39 Summary of the identified components in samples of lactating goats treated with [phenyl-¹⁴C]- pydiflumetofen

Component	Milk		Liver		Kidney		Muscle		Fat	
	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)
pydiflumetofen	15.7	0.019	8.2	0.570	0.8	0.014	24.4	0.025	67.2	0.149

Hydroxy SYN545974 ^a	-	-	-	-	-	-	-	-	8.6	0.019
2,4,6-TCP (as conjugates*)	43.2 (42.2 ¹)	0.052 (0.051 ¹)	0.5 (0.5)	0.037 (0.037)	1.2 (1.2)	0.021 (0.021)	9.0 (6.1)	0.009 (0.006)	-	-
SYN547948 (as conjugates*)	2.2	0.003	2.6 (0.9)	0.180 (0.064)	0.9 (N/D)	0.016 (N/D)	3.8	0.004	5.3	0.012
SYN548263 (as conjugates*)	-	-	-	-	-	-	-	-	-	-
SYN547897 (as conjugates*)	-	-	1.9 (N/D)	0.136 (N/D)	2.9 (2.9)	0.050 (0.050)	1.8	0.002	-	-
SYN548264 (as conjugates*)	-	-	-	-	-	-	-	-	-	-
SYN545547 (as conjugates*)	-	-	3.4 (2.7)	0.239 (0.188)	7.4 (7.4)	0.128 (0.128)	-	-	-	-
SYN547891 (as conjugates*)	-	-	1.4 (0.6)	0.100 (0.041)	-	-	-	-	-	-
SYN508272 (as conjugates*)	-	-	-	-	-	-	-	-	-	-
NOA449410 (as conjugates*)	-	-	-	-	-	-	-	-	-	-

* If applicable

¹ sulphate conjugate^a : hydroxy SYN545974- hydroxylated pydiflumetofen was identified as one of the metabolites; the specific location of the OH group was not known.Table 7-40 Summary of the identified components in samples of lactating goats treated with [pyrazole-¹⁴C]-pydiflumetofen

Component	Milk		Liver		Kidney		Muscle		Fat	
	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)	% TRR	(mg eq/kg)
pydiflumetofen	8.7	0.011	2.0	0.179	0.5	0.011	13.4	0.018	73.8	0.206
Hydroxy SYN545974 ^a	-	-	-	-	-	-	-	-	10.2	0.028
2,4,6-TCP (as conjugates*)	-	-	-	-	-	-	-	-	-	-
SYN547948 (as conjugates*)	0.7	0.001	1.9 (N/D)	0.170 (N/D)	0.7 (N/D)	0.016 (N/D)	1.1	0.002	3.3	0.009
SYN548263 (as conjugates*)	14.2	0.019	-	-	16.6 (14.6)	0.389 (0.342)	4.9	0.007	4.3	0.012
SYN547897 (as conjugates*)	-	-	3.0 (N/D)	0.268 (N/D)	2.7 (1.9)	0.063 (0.045)	1.2	0.002	-	-
SYN548264 (as conjugates*)	28.7	0.038	-	-	0.8 (0.8)	0.019 (0.019)	0.6	0.001	-	-
SYN545547 (as conjugates*)	-	-	1.8 (1.6)	0.160 (0.139)	N/D (N/D)	N/D (N/D)	-	-	-	-
SYN547891 (as conjugates*)	-	-	0.4 (N/D)	0.038 (N/D)	-	-	-	-	-	-
SYN508272 (as conjugates*)	11.0	0.014	-	-	1.5 (0.7)	0.036 (0.017)	17.7	0.024	1.0	0.003
NOA449410 (as conjugates*)	2.6	0.003	2.9 (1.7)	0.248 (0.146)	11.7 (9.1)	0.275 (0.214)	3.6	0.005	-	-

* If applicable

¹ sulphate conjugate^a : hydroxy SYN545974- hydroxylated pydiflumetofen was identified as one of the metabolites; the specific location of the OH group was not known.

Characterisation and identification

Pydiflumetofen and metabolites were identified and quantified using reversed phase HPLC and co-chromatography experiments with radiolabelled and non-radiolabelled reference samples. The identity of unknown metabolites of pydiflumetofen were investigated by LC-MS in milk and fat for the phenyl label and fat for the pyrazole label. The presence of 2,4,6-TCP sulphate conjugate was also confirmed using LC-MS (using a reference standard specifically for the sulphate conjugate).

*Distribution of pydiflumetofen and metabolites in milk and tissues*Milk

Two (phenyl label) and five (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in milk. For the phenyl label, the metabolites 2,4,6-TCP (sulphate conjugate given in brackets) and SYN547948 were found in milk; they accounted for 0.052 mg eq/kg or 43.2% TRR (0.051 mg eq/kg and 42.2% TRR for the sulphate conjugate) and 0.003 mg eq/kg or 2.2% TRR respectively. Parent compound pydiflumetofen accounted for 0.019 mg eq/kg (15.7% TRR). For the pyrazole label, the metabolites SYN548263, SYN548264 and SYN508272 were the most prevalent, accounting for 0.019 mg eq/kg or 14.2% TRR, 0.038 mg eq/kg or 28.7% TRR and 0.014 mg eq/kg or 11.0% TRR respectively. Parent compound pydiflumetofen and metabolites SYN547948 and NOA449410 ranged from 0.001 to 0.011 mg eq/kg (0.7 – 8.7% TRR).

There were also unassigned residues present. For the phenyl label this was 0.020 mg eq/kg or 16.3% TRR and was comprised of at least 7 components, none of which accounted for more than 0.009 mg eq/kg (7.4% TRR). For pyrazole, these unassigned radiocomponents accounted for 0.025 mg eq/kg or 18.3% TRR and again was comprised of at least 7 components, none of which accounted for more than 0.009 mg eq/kg (7.1% TRR).

Other fractions not chromatographed accounted for 0.008 mg eq/kg or 6.3% TRR for the phenyl label and 0.002 mg eq/kg or 1.7% TRR for the pyrazole label. For the phenyl label this consisted of four fractions and for pyrazole, three fractions. For both radiolabels, none of the fractions accounted for more than 0.004 mg eq/kg (3.5% TRR).

Liver

Five (both phenyl and pyrazole label) metabolites plus the unchanged parent compound (pydiflumetofen) were identified in liver. For the phenyl label, the metabolites SYN547891, SYN547948, SYN547897, SYN545547 and 2,4,6-TCP were found between 0.037 – 2.39 mg eq/kg (0.5 – 3.4 % TRR). Parent compound pydiflumetofen was found at 0.570 mg eq/kg (8.2 % TRR). For the pyrazole label, SYN547891, SYN547948, SYN547897, SYN545547 and NOA449410 were found along with the parent compound between 0.038 and 0.268 mg eq/kg (0.4 – 3.0% TRR).

After extraction with aqueous acetonitrile there was a significant amount of unextracted radioactivity in debris; 3.471 mg eq/kg (49.7% TRR) for the phenyl label and 4.643 mg eq/kg (52.6% TRR) pyrazole label. The RRR of the phenyl label was further examined by protease solubilization. Organosoluble unassigned residues accounted for 0.220 mg eq/kg (3.1% TRR) for the phenyl label and 0.678 mg eq/kg (7.9% TRR) for the pyrazole, post-enzyme hydrolysis led to an increase in residues to 0.696 mg eq/kg (10.0% TRR) and 0.925 mg eq/kg (10.5% TRR) for phenyl and pyrazole respectively. Post enzyme hydrolysis, at least 12 components were present for the phenyl label, none of which accounted for more than 0.147 mg eq/kg (2.1% TRR). At least 9 components were available for pyrazole, accounting for no more than 0.195 mg eq/kg (2.2% TRR) each. The levels of water-soluble residues post-enzyme hydrolysis were 0.293 mg eq/kg (4.2% TRR) and 0.212 mg eq/kg (2.4% TRR) for phenyl and pyrazole respectively. This was made up of 12 components for phenyl and 14 for pyrazole, comprising of no more than 0.063 mg eq/kg (0.9% TRR) and 0.062 mg eq/kg (0.7% TRR) respectively.

Kidney

Four (phenyl label) and seven (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in kidney. For the phenyl label, the metabolite SYN545547 accounted for the main portion at 0.128 mg eq/kg or 7.4% TRR. Parent compound pydiflumetofen and metabolites SYN547948, SYN547897 and 2,4,6-TCP ranged from 0.014 to 0.050 mg eq/kg (0.8 – 2.9% TRR). For the pyrazole label, the metabolites SYN548263 and NOA449410 accounted for the main portion with 0.389 mg eq/kg or 16.6% TRR and 0.275 mg eq/kg or 11.7% TRR, respectively. Parent compound pydiflumetofen and metabolites SYN547948, SYN547897,

SYN545547, SYN548264 and SYN508272 ranged from 0.011 to 0.063 mg eq/kg (0.5 – 2.7% TRR). Unassigned, chromatographed species, derived from initial extraction with aqueous acetonitrile were present at 0.109 mg eq/kg (6.3% TRR) for the phenyl label and 0.112 mg eq/kg (4.8% TRR) for the pyrazole label.

After extraction with aqueous acetonitrile there was a significant amount of unextracted radioactivity in debris; 0.287 mg eq/kg (16.6%) and 0.215 mg eq/kg (9.2 %) TRR for the phenyl and pyrazole label respectively. The RRR of the phenyl label was further examined by protease solubilization.

Organosoluble unassigned residues accounted for 0.068 mg eq/kg (3.9% TRR) for the phenyl label and 0.128 mg eq/kg (5.5% TRR) for the pyrazole. Post-enzyme hydrolysis led to an increase in residues to 0.331 mg eq/kg (18.9% TRR) and 0.380 mg eq/kg (16.3% TRR) for phenyl and pyrazole respectively. Post enzyme hydrolysis, at least 10 components were present for the phenyl label, none of which accounted for more than 0.098 mg eq/kg (5.6% TRR). At least 11 components were available for pyrazole, accounting for no more than 0.126 mg eq/kg (5.4%TRR) each. The levels of water-soluble residues post-enzyme hydrolysis were 0.260 mg eq/kg (15.0% TRR) and 0.150 mg eq/kg (6.4% TRR) for phenyl and pyrazole respectively. This was made up of 10 components for phenyl and 12 for pyrazole, comprising of no more than 0.087 mg eq/kg (5.0% TRR) and 0.047 mg eq/kg (2.0% TRR) respectively.

Over the course of analysis, it was calculated that 0.396 mg eq/kg (23.2%) and 0.519 mg eq/kg (22.1%) TRR for the phenyl and pyrazole label respectively were lost.

Muscle

Three (phenyl label) and six (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in muscle. For the phenyl label, the parent compound pydiflumetofen accounted for the main portion at 0.025 mg eq/kg or 24.4% TRR. The metabolites 2,4,6-TCP, SYN547948 and SYN547897 ranged from 0.002 to 0.009 mg eq/kg (1.8 – 9.0% TRR). For the pyrazole label, the metabolite SYN508272 and parent (pydiflumetofen) accounted for the main portions at 0.024 mg eq/kg (17.7%) and 0.018 mg eq/kg (13.4%) respectively. The metabolites SYN547948, SYN547897, SYN548263, SYN548264 and NOA449410 ranged in magnitude from 0.001 to 0.007 mg eq/kg (0.6 – 4.9% TRR).

Unassigned, chromatographed species, derived from initial extraction with aqueous acetonitrile were present at 0.042 mg eq/kg (39.7%) for the phenyl label and 0.052 mg eq/kg (36.4%) for the pyrazole label. At least 9 components were present for the phenyl label, none of which accounted for more than 0.008 mg eq/kg (7.4%). At least 23 components were available for pyrazole, accounting for no more than 0.008 mg eq/kg (5.6%) each.

After extraction with aqueous acetonitrile, 0.014 mg eq/kg (14.0%) of the phenyl label and 0.008 mg eq/kg (5.7%) of the pyrazole label remained unextracted. No further extraction procedures were undertaken.

Fat

Two (phenyl label) and four (pyrazole label) metabolites and unchanged parent compound (pydiflumetofen) were identified in samples of fat. For both the phenyl and pyrazole label, parent compound pydiflumetofen accounted for the main portion at 0.149 mg eq/kg (67.2%) and 0.206 mg eq/kg (73.8%) TRR respectively. For the phenyl label, the two metabolites hydroxy SYN545974 and SYN5479488 were found at 0.019 mg eq/kg (8.6%) and 0.012 mg eq/kg (5.3%) respectively. For the pyrazole label, the metabolites hydroxy SYN545974, SYN547948, SYN548263 and SYN508272 ranged from 0.003 – 0.028 mg eq/kg (1.0 – 10.2% TRR).

Chromatographed but unassigned fractions accounted for 0.024 mg eq/kg or 10.9% TRR for the phenyl label and 0.009 mg eq/kg or 3.4% TRR for the pyrazole label. For the phenyl label this consisted of at least 4 fractions, none of which accounted for more than 0.008 mg eq/kg (3.7% TRR). For the pyrazole label, at least 2 fractions were present, none of which accounted for more than 0.005 mg eq/kg (1.9% TRR).

Other fractions not chromatographed accounted for 0.004 mg eq/kg or 1.9% TRR for the phenyl label and 0.007 mg eq/kg or 2.5% TRR for the pyrazole label. No single fraction comprised more than 0.004 mg eq/kg (1.9%) or 0.006 mg eq/kg (2.0%) respectively. After extraction with aqueous acetonitrile, 0.002 mg eq/kg (1.1%) of the phenyl label and 0.007 mg eq/kg (2.4%) of the pyrazole label remained unextracted. No further extraction procedures were undertaken.

Table 7-41 Total Radioactive Residue (TRR) in Cream and Skimmed Milk from Goats Dosed with [¹⁴C]-pydiflumetofen

Sample	Timepoint (h)	Concentration (mg/kg)		Cream/Skimmed Milk Ratio
		Skimmed Milk	Cream	
[Phenyl- ¹⁴ C]-pydiflumetofen	PreDose	N/S	N/S	N/S
	24 h	N/S	N/S	N/S
	48 h	N/S	N/S	N/S
	72 h	N/S	N/S	N/S
	96 h	0.076	0.338	4.45
	120 h	0.066	0.309	4.68
	144 h	0.076	0.345	4.54
	156 h	0.087	0.494	5.68
[Pyrazole- ¹⁴ C]-pydiflumetofen	PreDose	N/S	N/S	N/S
	24 h	N/S	N/S	N/S
	48 h	N/S	N/S	N/S
	72 h	N/S	N/S	N/S
	96 h	0.116	0.298	2.57
	120 h	0.131	0.297	2.27
	144 h	0.118	0.292	2.47
	156 h	0.138	0.474	3.43

N/S - Not Sampled

Table 7-42 Summary of the characterisation and identification of components in milk from goats treated with [¹⁴C]-pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.122 ¹		0.132 ¹	
TRR by direct quantification mg eq/kg		0.132 ²		0.140 ²	
TRR for chromatography, %		77.4		84.4	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed	pydiflumetofen	15.7	0.019	8.7	0.011
	2,4,6-TCP (sulphate conjugate)	43.2 (42.2)	0.052 (0.051)	N/A (N/A)	N/A (N/A)
	SYN547948	2.2	0.003	0.7	0.001
	SYN548263	N/A	N/A	14.2	0.019
	SYN548264	N/A	N/A	28.7	0.038
	SYN508272	N/A	N/A	11.0	0.014
	NOA449410	N/A	N/A	2.6	0.003
	Unassigned ³	16.3	0.020	18.3	0.025
	Other fractions ⁴	6.3	0.008	1.7	0.002
	Unextracted ⁵	7.7	0.009	6.1	0.008
	Losses/gains on fractionation ⁶	8.6 (Loss)	0.011 (Loss)	7.8 (Loss)	0.011 (Loss)
	Total	100.0	0.122	100.0	0.132

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.

2 - The radioactive residue determined by direct quantification employing combustion/LSC.

3 - Unassigned radiocomponents from HPLC for phenyl and pyrazole label, respectively.

Phenyl label: comprising at least 7 discrete components, no single one of which >7.4% TRR (>0.009 mg eq/kg)

Pyrazole label: comprising at least 7 discrete components, no single one of which >7.1% TRR (>0.009 mg eq/kg)

- 4 - Extractable residues in 4 fractions for phenyl and 3 fractions for pyrazole radiolabelled experiments were not analysed (associated residues too low for analysis). No single fraction comprised > 3.5% TRR (>0.004 mg eq/kg) in either radiolabelled experiment.
- 5 - Radioactivity remaining in the debris after extraction with hexane/aqueous acetonitrile. No further work was carried out on the debris (associated residues too low for analysis).
- 6 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-43 Summary of the characterisation and identification of components in liver from goats treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		6.984 ¹		8.827 ¹	
TRR by direct quantification mg eq/kg		6.967 ²		9.372 ²	
TRR for chromatography, %		42.4		39.1	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed	pydiflumetofen	8.2	0.570	2.0	0.179
	SYN547891 (as conjugates)	1.4 (0.6)	0.100 (0.041)	0.4 (N/D)	0.038 (N/D)
	SYN547948 (as conjugates)	2.6 (0.9)	0.180 (0.064)	1.9 (N/D)	0.170 (N/D)
	SYN547897 (as conjugates)	1.9 (N/D)	0.136 (N/D)	3.0 (N/D)	0.268 (N/D)
	SYN545547 (as conjugates)	3.4 (2.7)	0.239 (0.188)	1.8 (1.6)	0.160 (0.139)
	2,4,6-TCP (as conjugates)	0.5 (0.5)	0.037 (0.037)	N/A (N/A)	N/A (N/A)
	NOA449410 (as conjugates)	N/A (N/A)	N/A (N/A)	2.9 (1.7)	0.248 (0.146)
	Unassigned in pre-enzyme hydrolysis organo soluble ³	3.1	0.220	7.9	0.678
	Unassigned in post enzyme hydrolysis organo soluble ⁴	10.0	0.696	10.5	0.925
	Unassigned in post enzyme hydrolysis water soluble ⁵	4.2	0.293	2.4	0.212
	Unassigned in other fractions ⁶	7.0	0.489	6.5	0.574
	Unextracted ⁷	49.7	3.471	52.6	4.643
	Losses/gains on fractionation ⁸	8.0 (Loss)	0.553 (Loss)	8.1 (Loss)	0.0732 (Loss)
Total		100.0	6.984	100.0	8.827

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - Unassigned radiocomponents in pre-enzyme hydrolysis organo-soluble fraction, chromatographed by HPLC.
Phenyl label: comprising at least 3 discrete components, no single one of which >2.1% TRR (>0.147 mg eq/kg).
Pyrazole label: comprising at least 14 discrete components, no single one of which >1.0% TRR (>0.085 mg eq/kg).
- 4 - Unassigned radiocomponents in post enzyme hydrolysis organo-soluble fraction, chromatographed by HPLC
Phenyl label: comprising at least 12 discrete components, no single one of which >2.1% TRR (>0.147 mg eq/kg).
Pyrazole label: comprising at least 9 discrete components, no single one of which >2.2% TRR (>0.195 mg eq/kg).
- 5 - Post enzyme hydrolysis water soluble fraction analysed by 1D-TLC.
Phenyl label: comprising at least 12 discrete components, no single one of which >0.9% TRR (>0.063 mg eq/kg).
Pyrazole label: comprising at least 14 discrete components, no single one of which >0.7% TRR (>0.062 mg eq/kg).
- 6 - Single acetonitrile: water (1:1, v/v) fraction analysed by 2D-TLC.
Phenyl and pyrazole labels: radioactivity located on the origin. In both experiments the radioactivity located on the origin was characterised to be similar to that present in the liver debris.
- 7 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. The nature of this residue was characterised further by SDS extraction procedure and protease digestion experiment and shown to comprise a complex mixture of unassigned radiocomponents.
- 8 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-44 Summary of the characterisation and identification of components in kidney from goats treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		1.730 ¹		2.341 ¹	
TRR by direct quantification mg eq/kg		1.701 ²		2.280 ²	
TRR for chromatography, %		57.5		67.4	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed	pydiflumetofen	0.8	0.014	0.5	0.011
	SYN547948 (as conjugates)	0.9 (N/D)	0.016 (N/D)	0.7 (N/D)	0.016 (N/D)
	SYN547897 (as conjugates)	2.9 (2.9)	0.050 (0.050)	2.7 (1.9)	0.063 (0.045)
	SYN545547 (as conjugates)	7.4 (7.4)	0.128 (0.128)	N/D (N/D)	N/D (N/D)
	2,4,6-TCP (as conjugates)	1.2 (1.2)	0.021 (0.021)	N/A (N/A)	N/A (N/A)
	SYN548263 (as conjugates)	N/A (N/A)	N/A (N/A)	16.6 (14.6)	0.389 (0.342)
	SYN548264 (as conjugates)	N/A (N/A)	N/A (N/A)	0.8 (0.8)	0.019 (0.019)
	SYN508272 (as conjugates)	N/A (N/A)	N/A (N/A)	1.5 (0.7)	0.036 (0.017)
	NOA449410 (as conjugates)	N/A (N/A)	N/A (N/A)	11.7 (9.1)	0.275 (0.214)
	Unassigned in pre-enzyme hydrolysis organo soluble ³	3.9	0.068	5.5	0.128
	Unassigned in post enzyme hydrolysis organo soluble ⁴	18.9	0.331	16.3	0.380
	Unassigned in post enzyme hydrolysis water soluble ⁵	15.0	0.260	6.4	0.150
	Unassigned derived from acetonitrile water (1:1 v/v) extract ⁶	6.3	0.109	4.8	0.112
	Other fractions ⁷	2.9	0.050	1.2	0.028
	Unextracted ⁸	16.6	0.287	9.2	0.215
Losses/gains on fractionation ⁹		23.2 (Loss)	0.396 (Loss)	22.1 (Loss)	0.519 (Loss)
Total		100.0	1.730	100.0	2.341

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

-
- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
 - 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
 - 3 - Unassigned radiocomponents in pre-enzyme hydrolysis organo-soluble fractions, which were either chromatographed by HPLC.
Phenyl label: comprising at least 2 discrete components, no single one of which >1.0% TRR (>0.017 mg eq/kg).
Pyrazole label: comprising at least 12 discrete components, no single one of which >0.8% TRR (>0.019 mg eq/kg).
or chromatographed by 1D-TLC.
Phenyl label only: comprising at least two discrete components, no single one of which >1.0% TRR (>0.017% TRR)
 - 4 - Unassigned radiocomponents in post enzyme hydrolysis organo-soluble fraction which chromatographed by HPLC.
Phenyl label: comprising at least 10 discrete components, no single one of which >5.6% TRR (>0.098 mg eq/kg).
Pyrazole label: comprising at least 11 discrete components, no single one of which >5.4% TRR (>0.126 mg eq/kg).
 - 5 - Post enzyme hydrolysis water soluble fraction
Phenyl label: analysed by 1D-TLC and comprising and at least 10 discrete components, no single one of which >5.0% TRR (>0.087 mg eq/kg).
Pyrazole label: analysed by 1D-TLC and comprising at least 12 discrete components, no single one of which .2.0% TRR (>0.047 mg eq/kg).
 - 6 - Initial concentrated acetonitrile: water (1:1, v/v) fraction analysed by 2D-TLC.
Phenyl and pyrazole labels: radioactivity located on the origin. In both experiments the radioactivity located on the origin was characterised to be similar to that present in the liver debris.
 - 7 - Extractable residues in 3 fractions (phenyl) and 2 fractions (pyrazole) that were produced during analysis and were individually too low for analysis. No single fraction comprised > 1.6% TRR (≥ 0.028 mg eq/kg) in either radiolabelled experiment.
 - 8 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. The nature of this residue was characterised further by SDS extraction and a protease digestion experiment and shown to comprise a complex mixture of unassigned radiocomponents.
 - 9 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-45 Summary of the characterisation and identification of components in muscle from goats treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.102 ¹		0.138 ¹	
TRR by direct quantification mg eq/kg		0.101 ²		0.117 ²	
TRR for chromatography, %		78.7		79.2	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed	pydiflumetofen	24.4	0.025	13.4	0.018
	2,4,6-TCP (sulphate conjugate)	9.0 (6.1)	0.009 (0.006)	N/A N/A	N/A N/A
	SYN547948	3.8	0.004	1.1	0.002
	SYN547897	1.8	0.002	1.2	0.002
	SYN548263	N/A	N/A	4.9	0.007
	SYN548264	N/A	N/A	0.6	0.001
	SYN508272	N/A	N/A	17.7	0.024
	NOA449410	N/A	N/A	3.6	0.005
	Unassigned ³	39.7	0.042	36.4	0.052
	Other fractions ⁴	3.0	0.003	1.3	0.002
	Unextracted ⁵	14.0	0.014	5.7	0.008
	Losses/gains on fractionation ⁶	4.3 (Loss)	0.003 (Loss)	14.1 (Loss)	0.017 (Loss)
	Total	100.0	0.102	100.0	0.138

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

- 1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.
- 2 - The radioactive residue determined by direct quantification employing combustion/LSC.
- 3 - Unassigned radiocomponents by HPLC for phenyl and pyrazole label, respectively.
Phenyl label: comprising at least 9 discrete components, no single one of which >7.4% TRR (>0.008 mg eq/kg).
Pyrazole label: comprising at least 23 discrete components, no single one of which >5.6% TRR (>0.008 mg eq/kg).
- 4 - Extractable residues in 1 fraction per radiolabelled experiment that were not chromatographed as the residues were too low for analysis. No single fraction comprised >3.0% TRR (>0.003 mg eq/kg) in either radiolabelled experiment.
- 5 - Radioactivity remaining in the debris after extraction with aqueous acetonitrile. The nature of phenyl residue was characterised further by SDS analysis.
- 6 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Table 7-46 Summary of the characterisation and identification of components in fat from goats treated with [¹⁴C]- pydiflumetofen

		[Phenyl-U- ¹⁴ C]		[Pyrazole-5- ¹⁴ C]	
TRR by summation mg eq/kg		0.221 ¹		0.279 ¹	
TRR by direct quantification mg eq/kg		0.205 ²		0.240 ²	
TRR for chromatography, %		92.1		96.0	
Origin of component	Component	% TRR	mg eq/kg	% TRR	mg eq/kg
Chromatographed	pydiflumetofen	67.2	0.149	73.8	0.206
	Hydroxy SYN545974	8.6	0.019	10.2	0.028
	SYN547948	5.3	0.012	3.3	0.009
	SYN548263	N/A	N/A	4.3	0.012
	SYN508272	N/A	N/A	1.0	0.003
	Unassigned ³	10.9	0.024	3.4	0.009
	Other fractions ⁴	1.9	0.004	2.5	0.007
	Unextracted ⁵	1.1	0.002	2.4	0.007
	Losses/gains on fractionation ⁶	5.0 (Loss)	0.011 (Loss)	-0.9 (Gain)	-0.002 (Gain)
Total		100.0	0.221	100.0	0.279

N/D = Not detected.

N/A = Not applicable to this radiolabelled experiment.

1 - TRR determined by summation of radioactivity present in the extracts and debris following solvent extraction.

2 - The radioactive residue determined by direct quantification employing combustion/LSC.

3 - Unassigned radiocomponents in HPLC.

Phenyl label: comprising at least 4 discrete components, no single one of which >3.7% TRR (>0.008 mg eq/kg).

Pyrazole label: comprising at least 2 discrete components, no single one of which >1.9% TRR (>0.005 mg eq/kg).

4 - Extractable residues in 3 fractions that were produced during processing and were too low for analysis.

Phenyl label: No single fraction comprised >1.9% TRR (>0.004 mg eq/kg).

Pyrazole label: No single fraction comprised >2.0% TRR (>0.006 mg eq/kg).

5 - Radioactivity remaining in the debris after extraction with hexane/aqueous acetonitrile. No further work was carried out on the debris.

6 - The net cumulative incremental losses or gains during analysis. Calculated as 100 % - sum of all components.

Storage stability investigations

The applicant included some storage stability analyses (chromatographic comparisons obtained in the metabolism study context) to compare HPLC analysis generated at the initial analysis (within 6 months) time period to HPLC determinations done in the later period of the study (circa 2.5 years). This was done for representative samples of milk, fat, and liver.

The chromatograms in the study report for these comparisons do not involve any labelling of the peak identity as the comparisons are done using the first initial HPLC analyses as the main comparison point; at this stage of initial metabolic profiling, the identity of the metabolites is not known. In a letter response to an HSE query, the applicant explained that initial radio-HPLC analysis of the livestock profiles in both hen and goat studies were carried out using a generic chromatography system in order to ascertain the initial complexity of the metabolic pathway as well as obtain an initial storage stability profile.

The phenyl milk and fat extracts (and the pyrazole fat extract) were generally similar before and after storage; the fat samples had a single main peak. The pyrazole milk extracts were more complex to consider, as there were some additional peaks that were present at the later retention times (26 to 27 mins) in a 6-month sample that were not present in a 5 month 'initial' sample. A difference in the peak(s) was also observed in this region in the 2.5-year sample. When this was raised in a query with the applicant, they noted that this was an anomaly in the 5-month sample (a peak was missing that was shown to be present in the TLC work and that this peak is likely to be the parent pydiflumetofen residue). This region did have a residue in both the 6 month and 2.5-year timepoint HPLC analysis of the pyrazole milk extract.

HSE also asked about further information on the storage stability investigations performed (in the metabolism context) in the liver since some metabolites were found in kidney and liver that were not found in milk/fat in the ruminants. The report had noted that liver samples were worked on for storage stability, but the chromatographic comparisons were not available in the report. The applicant provided the liver chromatograms from the raw data. The chromatograms were difficult to compare, and some differences were noted; the chromatograms were not identical before and after storage with retention time shifts, and lack of full resolution of some of the peaks to baseline. The applicant also provided some analyses with named peaks in a different HPLC system to support the view that the main difference in the chromatography in the liver storage stability samples (initial analysis compared to analysis at 2.5 years) was due to instability and degradation of the peak SYN547897. This seems reasonable since, some instability of this metabolite was observed before the one-year timepoint in the 'cold' (non radiolabelled) storage stability work (see below).

The applicant remarked that overall, (aside from the clear differences noted for the liver), storage stability profiles presented can be considered sufficiently comparable with the caveat that complex samples analysed on different HPLC systems and columns at different time intervals will show some inconsistency in peak resolution and retention time. HSE agrees that is difficult to fully assess when the chromatograms do not show the same peak shape in the 'before' and 'after' chromatograms.

HSE considers that it is not ideal that all the ruminant metabolism samples were stored frozen for 4 months prior to extraction and analysis, given that storage stability of residues in the metabolism context is something that requires consideration, if overall the metabolism studies last for longer than 6 months.

In terms of context, the applicant observed that the major residue in both hen and goat studies are parent SYN554974 and the 2,4,6-TCP metabolite which was replicated in the 'cold' (non-radiolabelled) ruminant and poultry feeding studies. These residues are covered in the freezer storage stability studies reported (in section B.7.1.2 and in vol 1 2.7.1). In these 'cold' (non-radiolabelled) freezer stability studies, residues of parent pydiflumetofen and 2,4,6 – TCP (free and conjugated) were shown to be stable in various animal matrices over frozen storage for at least 2 years (parent) and at least 1 year (2,4,6 –TCP).

The applicant also noted that additional lower-level metabolites <10% TRR were also analysed in the ruminant feeding studies in order to demonstrate a thorough investigation of the residue of concern. HSE acknowledges this and also recognises that one of the feeding studies involved a very quick analysis in order to ensure stability of the metabolite SYN547897 in the feeding study in liver/kidney, since the 'cold' (non-radiolabelled) residues stability study had demonstrated some degradation of the metabolite SYN547897 which tested freezer stability for up to a one-year period in liver and kidney. This metabolite was only stable in bovine liver up to ~9.5 months and in bovine kidney for up to 11 months. These periods are greatly exceeded by the maximum storage period in the metabolism study for the ruminant study; the metabolite was investigated and analysed particularly quickly in a ruminant feeding study.

The 'cold' (non-radiolabelled) storage stability studies also demonstrated stability of: SYN508272 and SYN548264 in milk for at least a year, and SYN548263 in bovine kidney for at least a year.

Enantiomer ratio

There are two enantiomers of the parent compound (pydiflumetofen). In the dose capsules for the phenyl and pyrazole labelled active, the isomer ratio of [¹⁴C]-SYN546969/[¹⁴C]-SYN546968 was found to be 49.75/50.25 and 51.39/48.61 respectively when studied with chiral HPLC.

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur.

Unlike the plant metabolism studies (where the main component of the residue was parent pydiflumetofen), the enantiomer ratio in animal commodities was not studied in metabolism (or feeding) studies. See discussion in Vol 1 section, 2.7.2.

Metabolic pathway

The proposed metabolic pathway of pydiflumetofen in lactating ruminants is shown in Figure 7-6. In total, seven metabolites were identified. The main biotransformation steps to pydiflumetofen leading to these metabolites include; N-demethylation of the pyrazole ring, N-demethoxylation of the amide nitrogen, monohydroxylation of the benzyl methylene functionality, monohydroxylation of the trichlorophenyl ring. Conjugation of the residues is also a main feature of the residues formed from pydiflumetofen (see below).

Conclusion

Pydiflumetofen was administered orally to two lactating goats in two radiolabelled forms (phenyl and pyrazole labels) for seven consecutive days (nominal dose of 100 mg as equivalents/kg, dry matter intake).

Approximately 96.0 % and 94.7 % of the administered dose were recovered in total for the phenyl and pyrazole label, respectively. The main fraction was excreted via excreta accounting to approximately 84.2 % (phenyl label) and 76.3 % (pyrazole label). Radioactive residues associated with edible portions (milk and tissues) accounted for <0.1% to 0.4% of the administered dose for both labels.

In milk, the level of radioactive residues for the phenyl label increased to a plateau of 0.091 mg eq/kg at day 2 of the study. For the pyrazole label, a plateau of 0.138 mg eq/kg was reached after 5 days. Sub-samples from days 4-7 were separated to give skimmed milk and cream.

In the edible matrices, the highest TRR concentrations were calculated for liver (6.984 – 8.827 mg eq/kg) and kidney (1.730 – 2.341 mg eq/kg). For all other matrices, the TRR was in a range from 0.102 to 0.221 mg eq/kg (phenyl label) and from 0.132 – 0.279 mg eq/kg (pyrazole).

Samples of liver, kidney and muscle were extracted with acetonitrile and water. Fat and milk samples were extracted with a mixture of acetonitrile, water and hexane and subsequently with acetonitrile and water. The extractable TRR levels were generally high, ranging from 83.4 % TRR to 98.8 % TRR, except for liver (50.4% TRR and 47.4% TRR for the phenyl and pyrazole labels, respectively). Radioactive residues in the RRR obtained after the initial extraction of liver and kidney was 9.2 – 52.6 % TRR, which were further investigated.

To quantify and identify radioactive components, thin layer chromatography (1D and 2D) was performed on samples and the plates were subject to bioimage analysis to detect the radioactive areas. Reverse phase HPLC was also performed on all types of samples to quantify and identify the residues. Chromatographic comparison of the residues was done with synthetic reference standards of pydiflumetofen and its metabolites.

The main observations from the identification work are the following:

- The majority of radioactivity was observed in excreta (76.3 - 84.2% TRR).
- Total radioactive residues in milk achieved a plateau concentration at approximately 0.091 mg eq/kg (phenyl) and 0.138 mg eq/kg (pyrazole) after 2 days (averaging residues for days: 3, 4, 5 and 6) and 5 days of dosing, respectively.
- Parent (pydiflumetofen) was found in milk and all tissues at levels ranging from 0.011 mg eq/kg to 0.570 mg eq/kg (0.5% TRR to 73.8% TRR). In fat, parent was the major component.
- Six of the eleven identified metabolites from the metabolism of pydiflumetofen accounted for ≥10% TRR:
 - 2,4,6-TCP sulphate conjugate (milk: 42.2% TRR; 0.051 mg eq/kg). The 2, 4, 6-TCP found was attributed to the sulphate conjugate (not found as free 2, 4, 6-TCP). The sulphate conjugate of 2,4,6-TCP was a main component in milk (and also a metabolite in muscle).
 - SYN548264 (milk: 28.7% TRR; 0.038 mg eq/kg).
 - SYN508272 (muscle: 17.7% TRR; 0.024 mg eq/kg, milk: 11.0% TRR; 0.014 mg eq/kg).
 - SYN548263 (kidney: 16.6% TRR; 0.389 mg eq/kg, milk: 14.2% TRR; 0.019 mg eq/kg).
 - Hydroxy pydiflumetofen (fat: ≤10.2% TRR; ≤0.028 mg eq/kg).
 - NOA449410 (kidney: 11.7 % TRR; 0.275 mg eq/kg)

- To a varying extent, except for hydroxy pydiflumetofen, conjugation of metabolites was a feature, not only for the 2,4,6-TCP. The conjugates are expected to be either glucuronide or sulphate conjugates (see below).

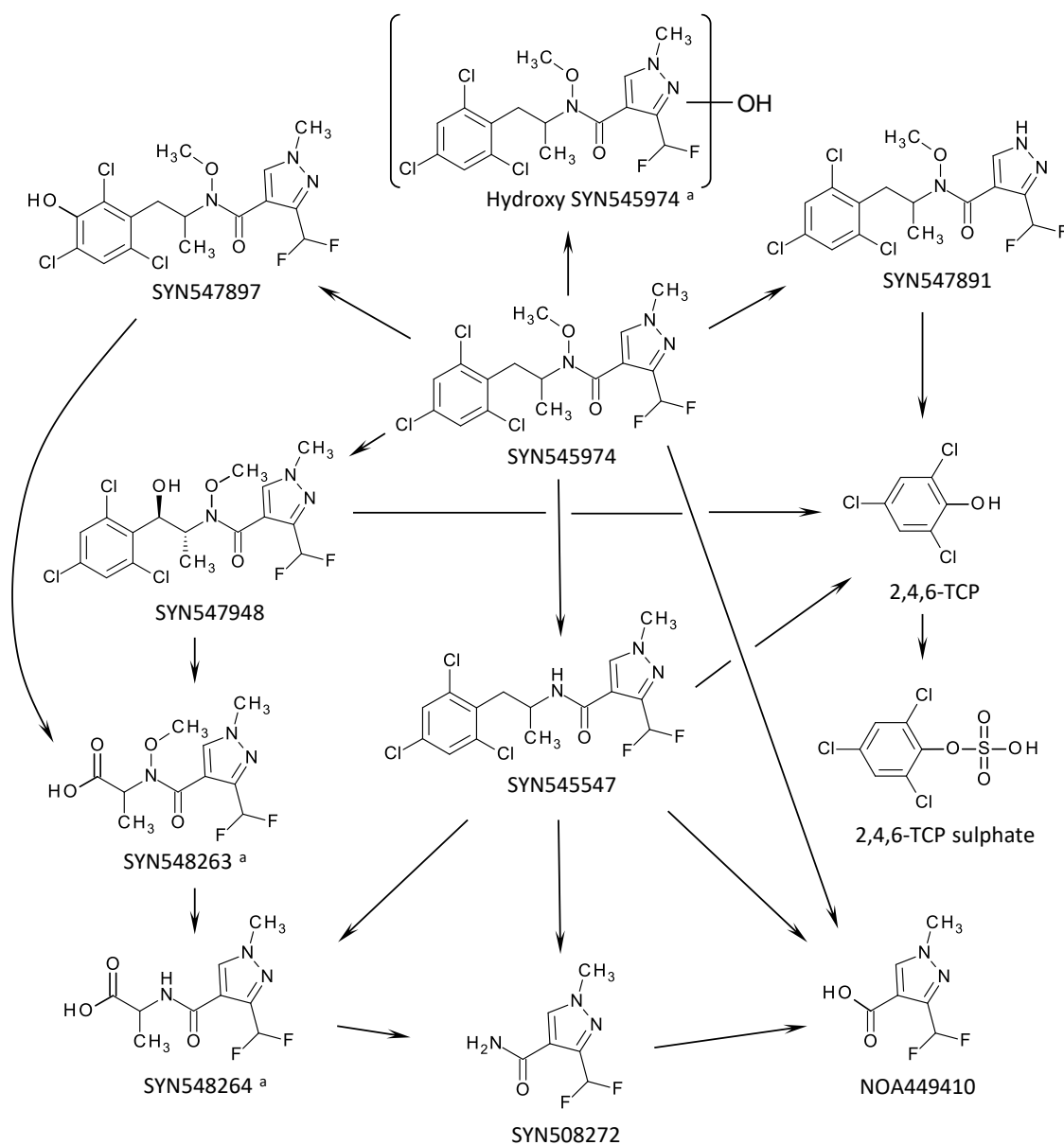
The transformation steps in the metabolic pathway for these are:

- N-demethylation of the pyrazole ring
- N-demethoxylation of the amide nitrogen
- monohydroxylation of the benzyl methylene functionality
- monohydroxylation of the trichlorophenyl ring
- cleavage at the benzylic methylene, N-alkyl and amide linkages between the phenyl and pyrazole rings
- Conjugation of metabolites to form their glucuronide and/or sulphate ester analogues (all except hydroxy pydiflumetofen). In all instances conjugates were characterised as glucuronide/sulphate due to the nature of the enzyme used to hydrolyse them with the exception of the 2,4,6-trichlorophenol sulphate in milk and muscle of the phenyl label experiment which was formally identified.

Reference standards that were analysed for but not detected:

- SYN547893
- SYN547892
- SYN547890
- SYN547895
- SYN547894
- SYN548266
- SYN545720
- SYN547949 - two enantiomers of the compound

Regarding conjugates, a reference standard was available for the sulphate conjugate of 2,4,6-TCP. Other reference standards for conjugates were not available, however the presence of conjugates for the other metabolites was determined by the deconjugation behaviour when samples were incubated with β -glucuronidase. It was observed that this enzyme had both glucuronidase and sulphatase activity and was therefore able to cleave glucuronide and sulphate conjugates. Therefore, although presence as conjugates was known, the specific type of the conjugates had not been determined.

Figure 7-6 Proposed metabolic pathway of pydiflumetofen (SYN545974) in lactating goats

B.7.2.4. Pigs

As the metabolic pathway observed in hens and goats is similar to the metabolism observed in rats (Vol 3 CA B6, Section 6.1), an assessment of the metabolism in pigs is not required.

B.7.2.5. Fish

At present there is no agreed guidance on how to conduct fish metabolism studies to determine the residue definition for risk assessment and enforcement and there are no agreed guidance documents on how to conduct a fish feeding study. The EU guidance: SANCO/10181/2013– rev. 5, 12 June 2019, Guidance Document For Applicants On Preparing Dossiers For The Approval Of A Chemical New Active Substance And For The Renewal Of Approval Of A Chemical Active Substance According To Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 states:

“In some cases, agreed test methods or guidance documents are not yet available for particular data requirements. In these cases, the non-submission of particular studies required by the EU legislation should be thoroughly justified and statements (often referred to as ‘position papers’) must be substantiated with data or information provided by the applicant in the dossier. Applicants should follow on a routine basis the current developments, e.g. activities of the European Food Safety Authority for guidance documents and in particular publications in the Official Journal and the updates of the Commission Communications 2013/C 95/01 and 2013/C 95/02”

It is noted that the data requirements under Regulation (EU) No 283/2013 make it clear that bioaccumulation studies can be considered to address this data requirement. However, at the PRAS meeting in December 2017, for the expert discussion on Spinosad, it was agreed by the experts and EFSA that the use of such a study could not be considered at this time as it was not clear how the study design was applicable to assessing residues for consumer exposure and agreement on the approach to the dietary assessment for fish was required.

Guidance on residues in fish (metabolism studies and feeding studies) has been under development in the EU. The OECD programme on residue guidelines has not yet considered guidelines applicable to fish.

Since no agreed guidance is available at this time, it is considered that the above requirements do not need to be addressed in the current evaluation.

B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

The present dossier supports the use of the representative formulation A21857B (Emulsifiable Concentrate (EC) formulation containing 62.5 g/L pydiflumetofen) in Great Britain. The representative uses at active substance approval are for cereal crops (wheat, durum wheat, barley, rye, triticale, oat and spelt) and oilseed rape.

MRL work is being conducted in parallel with the new active substance review. As part of this work, a proposed GAP on carrots and associated root crops is being considered as a future GB use. The intended GAPs for carrots and associated root crops are for the formulation A19649H (Suspension Concentrate (SC) formulation containing 200 g/L pydiflumetofen). An assessment for this future GB use is also presented within this dossier.

The requested GAPs are presented in Table 7-47 below; the critical GAPs (cGAPs) have been identified and are highlighted in bold.

Table 7-47 Requested GAPs for GB uses

Use- No.	Region	Crop	Application				PHI (days)
			Method / kind	Growth stage of crop	Number of applications (interval - days)	Rate per application (g a.s./ha)	
Representative Uses							
1	UK	Spring and winter barley	Spray	BBCH 30-59 (or 41-59)	1	166	n.a.
2	UK	Spring and winter barley and oat	Spray	BBCH 55-65	1	200	n.a.
3	UK	Spring and winter wheat, durum wheat and spelt	Spray	BBCH 30-69 (or 41-69)	1	166	n.a.
4	UK	Spring and winter wheat, durum wheat, spelt, rye and triticale	Spray	BBCH 61-69	1	200	n.a.
5	UK	Spring and winter oilseed rape (OSR) ^(a)	Spray	BBCH 57-69	1	200	n.a.
MRL Application							
6	UK	Root crops (carrot, parsley root, parsnip)	Spray	BBCH 14-49 (or BBCH 21-49)	2 (14)	70	14

n.a – not applicable (PHI is covered by the time remaining between application and harvest).

(a) One application every 3 years.

In support of the representative uses, the notifier has provided magnitude of residue trials in the following crops: wheat, barley and oilseed rape. The use on cereal crops is considered first. The uses on barley and oats are covered by one cGAP assessment (see section B.7.3.1). Barley residue field trial data can be extrapolated to support the proposed use on oats. The uses on wheat, durum wheat, rye, triticale and spelt are covered by a second cGAP assessment (see section B.7.3.2). Wheat residue field trial data can be extrapolated to support the other crops (wheat, durum wheat, spelt, rye and triticale) with this cGAP (see Table 7-47). The use on oilseed rape is considered in section B.7.3.3.

In support of the MRL work supporting the future GB root crop use, the notifier has provided magnitude of residue trials in carrots. The use on carrots (and other root crops) is considered in section B.7.3.4.

Formulations:

The representative formulation at active substance approval is A21857B (EC formulation). In the residue trials on wheat, barley and OSR presented in the sections below, three formulations were used:

- A21857B, i.e., the representative formulation,
- A17573A, an EC formulation containing 100 g/L pydiflumetofen, and
- A19649B, an SC formulation containing 200 g/L pydiflumetofen.

Hence, some of the residue trials have been conducted using different formulation types. In accordance with SANCO 7525/VI/95 Rev. 10.3, 'experience shows that EC, WP, WG, and SC formulations usually produce comparable residues (especially if the last application is more than seven days prior to harvest)'. The proposed

GAPs result in an interval between application and harvest that is >7 days. Therefore, it is considered that residue trials conducted with SC and EC formulations are likely to produce comparable residue levels.

The residue trials on carrots were conducted with the formulation A19649B, whereas the intended GAP for use on carrots (not the representative formulation for the GB new active substance assessment) is for formulation A19649H. Both formulations are SC formulations containing 200 g/L pydiflumetofen and therefore it is considered that A19649B and A19649H are likely to produce comparable residue levels.

Residue Definitions:

The following residue definitions are being considered in this assessment:

Residue definition for risk assessment purposes (RD-RA): parent pydiflumetofen

Residue definition for enforcement purposes (RD-Enf): parent pydiflumetofen

Note that the MRLs required for the proposed GAPs have been outlined on the basis of the OECD calculator. Please refer to the Volume 1 for a detailed consideration of the MRLs.

Basic core acceptability criteria:

The basic criteria core acceptability is listed below:

Trials details

Crop variety	
Location, position and year of trial	-acceptable spread of location/season
Formulations used	-formulation reported/as proposed
Application/dilution rate	-reported/as specified on label
Maximum number of treatments	-reported/applicable
Method of application	-reported/applicable
Growth stage of the crop at treatment or Pre-harvest interval	-appropriate to proposed GAP
Geo-climate information	-reported/applicable
Residue level (control and treated)	

Analytical aspects

Method specified and submitted
 Storage of samples prior to analysis (conditions and time period)
 Limit of determination at an acceptable level
 Acceptable recovery (means 70-110%).

The residue trial values 'selected' for use to derive residue end-points (e.g. STMR and HR) and for values to input into the OECD MRL calculator are emboldened and underlined in the onward trial summaries.

B.7.3.1. Cereals: Barley and Oat

The requested GAPs for winter and spring barley and oat are presented below (Table 7-48). Two GAPs have been identified in winter and spring barley and oats. The cGAP for spring and winter barley and oats consists of one spray application at a rate of 1 x 200 g a.s./ha when the crop has reached the growth stage BBCH 55-65 (Use No. 2). The cGAP is highlighted in bold.

Residue trial data for barley have been submitted in support of these GAPs. According to SANCO 7525/IV/95 rev 10.3 (June 2017), it is possible to extrapolate residue data from barley (0500010) to oat (0500050), (allowed for applications after the edible part has formed).

Table 7-48 Requested GAPs and cGAP for barley and oat (in bold)

Use-No.	Member states/zones	Crop	Application				PHI (days)
			Method / kind	Growth stage of crop	Number of applications	Rate per application (g a.s./ha)	
1	UK	Spring and winter barley	Spray	BBCH 30-59 (or 41-59)	1	166	n.a.
2	UK	Spring and winter cereals (barley and oat)	Spray	BBCH 55-65	1	200	n.a.

n.a – not applicable (PHI is covered by the time remaining between application and harvest).

A summary of the trials submitted to support the cGAP on barley is given in Table 7-49.

Barley and oat are major crops. A minimum of eight residue trials that reflect the agronomic and climatic conditions of GB are required. The applicant has submitted 8 trials relevant to GB. As the applicant has provided 8 trials, and a minimum of 8 trials are required, this is sufficient to meet the data requirements.

It should be noted that the submitted dossier includes additional residue trials that were conducted with application rates that are not relevant to the cGAP (KCA 6.5.3/7, Report No. S13-02518). These trials were conducted to produce grain samples for processing studies, as reported in section 7.5 (magnitude of residues in processed commodities). These trials have not been considered further in this section.

The submitted dossier also includes residue trials data from southern Europe, e.g. southern France, Spain, Italy and Greece (KCA 6.3.14/2, Report No. 38049). These trials were not evaluated as they do not reflect the agronomic and climatic conditions of the UK and no additional information was provided to support the use of these trials.

Table 7-49 Number of residue trials relevant to GB and vegetation period

Crop	Season	Number of trials	Countries	Reference
Barley	2016	8	FR (north), DE, PL, HU, UK	KCA 6.3.14/1 Report No. 38034
Total number of trials		8		

Report:	KCA 6.3.14/1; [REDACTED], 2017
Title:	SYN545974 – Residue Study on Barley in North France, Germany, Poland, Hungary and the UK in 2016.
Report No.:	38034
Document No.:	VV-467584
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	Minor deviations were noted: <ul style="list-style-type: none">• Trial 6 was overdosed by 10.5% and Trial 7 was overdosed by 18.5%. Even with these deviations, the applications rates are still within 25% of the target rate• Minor deviations to the storage temperature were noted for four trials. These deviations occurred either prior to transport loading or during transport, but the temperature did not exceed -15°C. These deviations are not considered to have had any impact on the outcome of the trials.
GLP/GEP:	Yes

During the 2016 growing season, 8 field trials on barley were conducted at eight independent locations: two in northern France, two in Germany, one in Poland, one in Hungary and two in the United Kingdom. The trials were to determine the residue level of pydiflumetofen (SYN545974) in or on raw agricultural commodity (RAC).

Pydiflumetofen was applied to barley as A21857B, an EC formulation containing 62.5 g a.s./L. For all trials, plot 1 served as the control plot (C1). At all trials, A21857B was applied to plot 2 (P2) as a foliar spray once at a rate equivalent to 200 g a.s./ha at BBCH 65-67. Four of the trials (trials 1-4) had an additional plot, plot 3 (P3), which was treated with one application of 200 g a.s./ha at the earlier BBCH of 33-34.

Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no unusual events were noted. Rainfall was reported on the same day as application for the critical plot (P2) for three of the eight trials, however, the rainfall did not occur within 2 hours of the application. OECD Test Guideline 509 states that application should not be made when rainfall is expected shortly after application. This is not considered to have had a marked impact on the outcome of the trials as the resulting residue results are comparable.

For the at harvest trials, treated samples of at least 1 kg of barley grain and 0.5 kg of barley straw were collected at normal commercial harvest (NCH). For the decline trials, samples of at least 1 kg of barley whole plant were collected at 0, 7, 14, 27-28 and 41-42 days after last application (DALA). Untreated samples were collected at 14 DALA for whole plant and at NCH for grain and straw. All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 5 hours of sampling and stored at or below -18°C for a maximum of 11 months from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 11 months in barley grain, straw and whole plant is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 4 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The specimens were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A, quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for wheat grain, wheat straw and cereal forage within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). Within the analytical phases in Report No. 38034 and Report No. S13-02518 (KCA1 6.5.3/7), there are sufficient method validation data to meet the requirements for a reduced validation dataset for barley matrices. Example chromatograms show the method is specific and no significant interference (>30% of the LOQ)

was observed. Therefore, the method is considered satisfactorily validated for barley matrices in accordance with SANCO 3029/99 rev. 4. Extraction efficiency was satisfactorily addressed for the method.

A limited number of procedural recoveries for SYN544974 were determined concurrently with the sample analysis to check the accuracy of the residue analysis. Data were reported at two fortification levels per matrix: LOQ and 1000 x LOQ. The determined recoveries are reported in Table 7-50 and are in the acceptable range of 70 – 110%. While only a limited number of procedural recoveries were reported in report 38034, it is noted that the same analytical method was used to determine residues of pydiflumetofen across all submitted residue trials, with acceptable procedural recovery levels across all of the trials. Sufficient procedural recoveries have been performed across the submitted trials in barley matrices.

Table 7-50 Procedural recoveries for pydiflumetofen in barley whole plant, grain and straw.

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Barley whole plant	2	0.01	107, 109	108	
		2	10.0	105, 107	106	
		4	Overall			2
	Barley grain	2	0.01	100, 96	98	
		2	10.0	96, 97	97	
		4	Overall			2
	Barley straw	2	0.01	82, 86	84	
		2	10.0	84, 80	82	
		4	Overall			3

The trial design at plot P2 is representative of the cGAP for barley and oat, i.e. 1 x 200 g a.s./ha at BBCH 65. Results from plot P3 (earlier latest growth stage of application) are presented for information purposes only.

Barley whole plant samples were taken at 4 time intervals: 0, 7, 14, 27-28 and 41-42 days. The results show an overall decline in residue levels from day 0 to day 42.

Barley straw and wheat grain samples were taken at NCH, which occurred at 29 – 50 DALA.

The residues of pydiflumetofen are summarised as follows:

Barley Whole Plant

- 3.54 – 4.78 mg/kg at 0 DALA
- 1.42 – 4.69 mg/kg at 6-8 DALA
- 0.55 – 2.75 mg/kg at 14 DALA
- 0.19 – 1.96 mg/kg at 27-29 DALA
- 0.33 – 1.79 mg/kg at 42 DALA

Barley Grain

- 0.06 – 0.32 mg/kg at NCH

Barley Straw

- 0.57 – 2.72 mg/kg at NCH

No residues of pydiflumetofen were found in any of the control samples above the limit of quantification of 0.01 mg/kg.

Residues of pydiflumetofen found in barley matrices from the individual trials are summarised in Table 7-51.

Table 7-51 Residues of pydiflumetofen in barley whole plant, grain and straw

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling [BBCH]	Crop Part	DALA	pydiflumetof en [mg/kg]
680534 Trial 1 Northern France (Senlis-le- Sec, 80300)	P2 ^b	003	1 x 200 (at BBCH 65)	89	Grain	NCH (42 DALA)	0.12
		009		89	Straw	NCH (42 DALA)	1.19
	P3 ^b	005	1 x 200 (at BBCH 33)	89	Grain	NCH (80 DALA)	<0.01
		011		89	Straw	NCH (80 DALA)	1.07
	C1	0.01	Control	89	Grain	NCH [†]	<0.01
		0.07		89	Straw	NCH [†]	<0.01
680534 Trial 2 United Kingdom (Borrowby, YO7 4QY)	P2	003	1 x 200 (at BBCH 65)	89	Grain	NCH (48 DALA)	0.13
		009		89	Straw	NCH (48 DALA)	1.27
	P3 ^b	005	1 x 200 (at BBCH 33)	89	Grain	NCH (75 DALA)	<0.01
		011		89	Straw	NCH (75 DALA)	1.76 ^a
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680534 Trial 3 Poland (Kopienica, 42-674)	P2	003	1 x 200 (at BBCH 65)	89	Grain	NCH (29 DALA)	0.08
		009		89	Straw	NCH (29 DALA)	1.85
	P3 ^b	005	1 x 200 (at BBCH 33)	89	Grain	NCH (53 DALA)	<0.01
		011		89	Straw	NCH (53 DALA)	0.30
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680534 Trial 4 Hungary (Bezenye, 9223)	P2 ^b	003	1 x 200 (at BBCH 65)	89	Grain	NCH (42 DALA)	0.10
		009		89	Straw	NCH (42 DALA)	1.13
	P3	005	1 x 200 (at BBCH 33)	89	Grain	NCH (62 DALA)	<0.01
		011		89	Straw	NCH (62 DALA)	2.14 ^a
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680534 Trial 5 Northern France (Versigny, 60440)	P2 ^b	001	1 x 200 (at BBCH 65-67)	65-67	Whole plant	0 DALA	4.78
		003		69-71		7 DALA	2.91
		007		77-79		14 DALA	2.75
		009		38-85		27 DALA	1.96
		011		86-87		42 DALA	1.79
		015		89	Grain	NCH (49 DALA)	0.32
	C1	019		89	Straw	NCH (49 DALA)	2.72
		005	Control	77-79	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (49 DALA)	<0.01
		017		89	Straw	NCH (49 DALA)	<0.01
680534 Trial 6 Germany (Offenthal, 63303)	P2 ^b	001	1 x 200 (at BBCH 65)	65	Whole plant	0 DALA	3.54
		003		69		7 DALA	4.69
		007		71		14 DALA	0.93
		009		77-83		28 DALA	0.75
		011		87		41 DALA	1.59
		015		89	Grain	NCH (50 DALA)	0.10
		019		89	Straw	NCH (50 DALA)	1.20
	C1	005	Control	71	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (50 DALA)	<0.01
		017		89	Straw	NCH (50 DALA)	<0.01
680534 Trial 7 Germany (Oberursel, 61440)	P2 ^b	001	1 x 200 (at BBCH 65)	65	Whole plant	0 DALA	3.53
		003		65-69		7 DALA	1.42
		007		71		14 DALA	0.55
		009		77-83		28 DALA	0.19
		011		85-87		41 DALA	0.24
		015		89	Grain	NCH (50 DALA)	0.08
		019		89	Straw	NCH (50 DALA)	0.27

680534 Trial 8 United Kingdom (Ormiston, EH35 5NL)	C1	005	Control	71	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (50 DALA)	<0.01
		017		89	Straw	NCH (50 DALA)	<0.01
	P2 ^b	001	1 x 200 (at BBCH 65)	65	Whole plant	0 DALA	4.53
		003		69		7 DALA	2.16
		007		73		14 DALA	0.90
		009		83		28 DALA	0.48
		011		85		41 DALA	0.33
		015		89	Grain	NCH (52 DALA)	<u>0.06</u>
		019		89	Straw	NCH (52 DALA)	<u>0.57</u>
	C1	005	Control	73	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (52 DALA)	<0.01
		017		89	Straw	NCH (52 DALA)	<0.01

NCH = Normal Commercial Harvest

Plot C1: Untreated, Plot P2: Treated with formulation A21857B at BBCH 65, Plot P3: Treated with formulation A21857B at BBCH 33.

[†] Control samples taken at same day after last application as treated plots.

^aThe four barley trials that have comparative data (4 trials) for application at BBCH 33 compared to BBCH 65, show that the residues are considerably higher at the later application timing with regard to residues in grain. Indeed, all of the later application trials had positive residues of pydiflumetofen in grain, at 0.06 to 0.32 mg/kg (conversely following the earlier application, all the grain residues were <0.01 mg/kg). The results in straw across both of the GAPs (based on different timings) were more variable (two of the earlier application trials had higher residues for straw and two of the later application trials had higher residues for straw). Based on the grain results, the evaluation has concluded that 1 x 200 g as/ha for BBCH 55-65 represents the critical GAP. The highest straw residue was observed for a trial conducted at the later application timing. Therefore the ‘underlining’ of the trials has kept to the trials that reflect this critical GAP.

[This is noted since the application rate in the trials of 200 g as/ha is also within +/-25% of the GAP for 1 x 166 g as/ha for a possible earlier application at early growth stage (after BBCH 30). The intended GAP timing for the 1 x 200 g as/ha GAP is later BBCH 55-65].

In wheat trials (see section B.7.3.2) there were also comparative data (4 trials) for application at BBCH 33 (or BBCH 37) compared to BBCH 69 and these trials always had higher residues in grain and straw for the later application timing. In four of the wheat trials there were also comparative data (4 trials) for application at BBCH 33 (or BBCH 37) compared to BBCH 69 and these trials always had same or higher residues in both grain and straw for the later application timing.

It is concluded 1 x 200 g as/ha for BBCH 55-65 represents the cGAP.

^bFor a number of trials, rainfall was reported within 24 hours of product application for at least one of the two treated plots (P2 or P3). However, no trials recorded rainfall within 2 hours of application at any of the plots where rainfall was recorded within 24 hours. The rainfall observed in some of the trials is not considered to have a marked impact on the study. Details of the rainfall recorded are presented in the Table 7-52 below. There was no clear correlation between rainfall and residue levels in grain/straw based on the results from P2 plots that represent the cGAP. The results with the HR for grain and straw had some rain (within the 2 to 24 hr period after application). The two trials with the lowest residue results (those at cGAP, trials 7 and 8) did experience some rainfall. It is not possible to derive a robust conclusion on the correlation between rainfall and residues based on the results from the P3 plots. This is because the dataset is small (only 4 trials) the results in grain were all <LOQ. However, it is noted that this trial does not represent the cGAP.

Table 7-52 Observation of rainfall in the barley trials and residue levels at harvest

Trial Number	Plot number	Application (g a.s./ha)	Rainfall on day of product application (>2 hours after application) (mm)	Rainfall on day after product application (within 24 hours) (mm)	Total rainfall, 2-24 hrs (mm)	Crop part	Pydiflumetofen (mg/kg)
1	P2	1 x 200	-	11.8	11.8	Grain	0.12

		(at BBCH 65-67)				Straw	1.19
2			-	-	-	Grain	0.13
						Straw	1.27
3			-	-	-	Grain	0.08
						Straw	1.85
4			-	0.5	0.5	Grain	0.10
						Straw	1.13
5			-	5.0	5.0	Grain	0.32
						Straw	2.72
6			0.8	-	0.8	Grain	0.10
						Straw	1.20
7			2.8	-	2.8	Grain	0.08
						Straw	0.27
8			0.2	1.2	1.4	Grain	0.06
						Straw	0.57
1			-	See next column	0.2 ¹	Grain	<0.01
						Straw	1.07
2			-	See next column	0.25 ¹	Grain	<0.01
						Straw	1.76
3			0.2	28.8	29	Grain	<0.01
						Straw	0.30
4			-	-	-	Grain	<0.01
						Straw	2.14

¹rainfall reported as falling within 24 hours of application but actual day of rainfall not recorded.

Table 7-53 Summary of available residues trials on barley at cGAP

Crop	Situation	<u>RD-Enf – pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	<u>RD-RA – pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	HR (RD-RA) (mg/kg)	STMR (RD-RA) (mg/kg)
Barley grain (extrapolated to oat grain)	Outdoor	0.06, 2 x 0.08, 2 x 0.10, 0.12, 0.13, 0.32	0.06, 2 x 0.08, 2 x 0.10, 0.12, 0.13, 0.32	0.32	0.10
Barley straw (extrapolated to oat straw)	Outdoor	0.27, 0.57, 1.13, 1.19, 1.20, 1.27, 1.85, 2.72	0.27, 0.57, 1.13, 1.19, 1.20, 1.27, 1.85, 2.72	2.72	1.195

B.7.3.2. Cereals: Wheat, Durum Wheat, Rye, Spelt and Triticale

The requested GAPs for winter and spring wheat, durum wheat, rye, spelt, triticale are presented below (Table 7-54). Two GAPs have been identified in winter and spring wheat, durum wheat, spelt, rye and triticale. The cGAP for wheat, durum wheat, spelt, triticale and rye consist of one spray application at a rate of 200 g a.s./ha at BBCH 61-69 (Use No. 4). The cGAP is highlighted in bold.

Residue trial data for wheat have been submitted in support of these GAPs. According to SANCO 7525/VI/95 rev. 10.3 (June 2017), it is possible to extrapolate residue data from wheat (0500090) to rye (0500070), (allowed for applications after the edible part has formed). Durum wheat, spelt, and triticale are Part 1B commodities on the GB MRL Statutory Register, for which the same MRL applies as wheat (Part 1A commodity). Therefore, these crops are also supported by the submitted residue trials on wheat.

Table 7-54 Requested GAPs and identification of cGAPs (in bold)

Use-No.	Region	Crop	Application				PHI (days)
			Method / kind	Growth stage of crop	Number of applications	Rate per application (g a.s./ha)	
3	UK	Spring and winter wheat, durum wheat and spelt	Spray	BBCH 30-69 (or 41-69)	1	166	n.a.
4	UK	Spring and winter wheat, durum wheat, spelt, rye and triticale	Spray	BBCH 61-69	1	200	n.a.

n.a – not applicable (PHI is covered by the time remaining between application and harvest).

A summary of the trials submitted to support the cGAP on wheat and rye is given in Table 7-55.

Wheat and rye are major crops. A minimum of eight residue trials that reflect the agronomic and climatic conditions of the UK are required. The applicant has submitted 12 trials relevant to the UK. As the applicant has provided 12 trials, and a minimum of 8 trials are required, this is sufficient to meet the data requirements.

It should also be noted the submitted dossier includes eight residue trials data from southern Europe, e.g. southern France, Spain, Italy and Greece (KCA 6.3.15/2, Report No. 38050). These trials were not evaluated as they do not reflect the agronomic and climatic conditions of the UK and no additional information was provided to support the use of these trials.

In four of the wheat trials there were also comparative data (4 trials) for application at BBCH 33 (or BBCH 37) compared to BBCH 69 and these trials always had same or higher residues in both grain and straw for the later application timing.

It is concluded 1 x 200 g a.s./ha for BBCH 61-69 represents the critical GAP.

Table 7-55 Number of residue trials relevant to the UK and vegetation period

Crop	Season	Number of trials	Countries	Reference
Wheat	2013	4	FR (north), UK	KCA 6.3.15/3 Report No. S13-02516
	2016	8	FR (north), DE, PL, CZ, UK	KCA 6.3.15/1 Report No. 38051

Total number of trials	12	-	
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Report:	KCA 6.3.15/3; [REDACTED], [REDACTED], [REDACTED], [REDACTED], 2017
Title:	SYN545974 – Residue Study on Wheat in Northern France and the United Kingdom in 2013.
Report No.:	S13-02516
Document No.:	Document No. VV-467692, A17573A_10005
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	Minor deviations were noted: <ul style="list-style-type: none"> There was an increase in the storage temperature during transportation of samples from all trials. The temperature never exceeded -15 °C and the samples did not thaw. Samples marked for retention were shipped in place of samples marked for shipping (Trial S13-02516-03). These deviations are not considered to have had any impact on the outcome of the trials.
GLP/GEP:	Yes

During the 2013 growing season, 4 field trials on wheat were conducted at four independent locations: two in Northern France and two in the United Kingdom. These trials were to determine the residue level of pydiflumetofen in or on RAC. Additionally, two of the trials contained plots treated to produce samples for processing studies. Details of the results from these plots can be found in section 7.5 (magnitude of residues in processed commodities).

Pydiflumetofen was applied to wheat as A17573A, an EC formulation containing 100 g a.s./L. Plot 1 for each trial served as the control plot (C1). A17573A was applied to plot 2 (P2) as a foliar spray. Two applications were made: application 1 consisted of 125 g a.s./ha at BBCH 43 – 65 and application 2 consisted of 150 g a.s./ha at BBCH 69. An application interval of 14-19 days was observed. A17573A was applied to plot 3 (P3) as a foliar spray once at a rate equivalent to 200 g a.s./ha at BBCH 69. Two of the trials had an additional plot, plot 4 (P4), to which A17573A was applied as a foliar spray. Two applications were made: application 1 consisted of 375 g a.s./ha at BBCH 69 and application 2 consisted of 450 g a.s./ha at BBCH 77-79. An application interval of 14-21 days was observed. Grain samples from plot 4 were collected for processing studies reported in section 7.5.

Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no adverse conditions were reported.

Treated samples of whole plant comprising 12 plants with a total weight of at least 1 kg were collected at 0, 7, 14 and 21 DALA. Treated samples of at least 1 kg of wheat grain and at least 0.5 kg of wheat straw were collected at NCH. In the trials, NCH occurred at 42 – 52 DALA. Untreated samples of whole plant comprising 12 plants with a total weight of at least 1 kg were collected from the control plot C1 immediately before the last application (0 days before last application, DBLA). Untreated samples of grain and straw were collected at NCH (42 – 52 DALA). All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 5 hours of sampling and were stored at or below -18 °C for a maximum of 15 months from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 15 months in wheat grain, straw and whole plant is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 17 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The specimens were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A, quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for wheat grain, wheat straw and cereal forage matrices within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). The method is acceptably validated in accordance with SANCO 3029/99 rev. 4 with an LOQ of 0.01 mg/kg. Extraction efficiency was satisfactorily addressed for the method.

A limited number of procedural recoveries for SYN544974 were determined concurrently with the sample analysis to check the accuracy of the residue analysis. Data were reported at three fortification levels per matrix: LOQ, 10 x LOQ and at least 50 x LOQ. The determined recoveries are reported in Table 7-56 and are in the acceptable range of 70 – 110%. While only a limited number of procedural recoveries were reported in S13-02516, it is noted that the same analytical method was used to determine residues of pydiflumetofen across all submitted residue cereal trials with acceptable procedural recovery levels across all of the trials. Sufficient procedural recoveries have been performed across the submitted trials in wheat matrices.

Table 7-56 Procedural recoveries for pydiflumetofen in wheat whole plant, grain and straw.

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Wheat whole plant	2	0.01	77, 97	87	-
		2	0.10	76, 85	82	-
		1	5.0	92	-	-
		5	overall	76-97	85	11
	Wheat straw	1	0.01	86	-	-
		1	0.10	102	-	-
		1	8.0	92	-	-
		3	overall	86-102	93	8.7
	Wheat grain	1	0.01	96	-	-
		1	0.10	91	-	-
		2	0.50	69, 80	74.5	-
		4	overall	69-80	84	14.3

The trial design at plot P3 is representative of the cGAP for wheat and rye, i.e. 1 x 200 g a.s./ha at BBCH 69. Results from plot P2 (2 underdosed individual applications) and plot P4 (2 overdosed individual applications) are presented for information purposes only.

Wheat whole plant samples were taken at 4 time intervals: 0, 7, 14 and 21 days. The results show the decline of residues over the whole time interval.

Wheat straw and wheat grain samples were taken at NCH, which occurred at 42 – 52 DALA.

The residues of pydiflumetofen are summarised as follows:

Wheat Whole Plant

- 2.6 – 4.2 mg/kg at 0 DALA
- 1.1 – 3.9 mg/kg at 7 DALA
- 0.62 – 2.3 mg/kg at 14 DALA
- 0.55 – 2.3 mg/kg at 21 DALA

Wheat Grain

- 0.02– 0.03 mg/kg at NCH

Wheat Straw

- 1.0 – 4.0 mg/kg at NCH

Residues of pydiflumetofen were found in two control samples: one in wheat grain and one in wheat straw from trial S13-02516-01 at 42 DALA. Residues were detected at the limit of quantification level of 0.01 mg/kg. No explanation is provided for the origin of SYN545974 in these two samples or for how contamination may have occurred. However, the contamination of these control samples is not considered detrimental to the results as no residues were found in any of the other control samples above the limit of quantification of 0.01 mg/kg, including control grain or straw samples at NCH.

Residues of pydiflumetofen found in wheat matrices from the individual trials are summarised in Table 7-57.

Table 7-57 Residues of pydiflumetofen in wheat whole plant, grain and straw

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling [BBCH]	Crop Part	DALA	pydiflumetofe n [mg/kg]
S13-02516-01 Northern France (Mespuits, 91150)	P2	003	1 x 125; 1 x 150 (app 1 at BBCH 43 – 45, app 2 at BBCH 69)	69	Whole plant	0	2.4
		007		71		7	0.70
		011		73-75		14	0.46
		015		77		21	0.48
		025		87	Straw	42	1.3
		038		89		52 (NCH)	1.2
		023		87	Grain	42	0.05
		036		89		52 (NCH)	<0.01
	P3	005	1 x 200 (at BBCH 69)	69	Whole plant	0	2.9
		009		71		7	1.1
		013		73-75		14	0.76
		017		77		21	0.65
		029		87	Straw	42	2.0
		027		89		52 (NCH)	2.2
		042		87	Grain	42	0.02
		040		89		52 (NCH)	0.02
	P4	044	1 x 375; 1 x 450 (app 1 at BBCH 69, app 2 at BBCH 77)		Grain	31 (NCH)	0.22
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		021	Control**	87	Straw	42	0.01
		033	Control	89		52 (NCH)	<0.01
		019	Control**	87	Grain	42	0.01* (0.01, 0.01)
		031	Control	89		52 (NCH)	<0.01
S13-02516-02 United Kingdom (Melbourne, DE73 8AG)	P2	003	1 x 125; 1 x 150 (app 1 at BBCH 65, app 2 at BBCH 69)	69	Whole plant	0	2.0
		007		75		7	1.3
		011		79-83		14	0.81
		015		85		21	0.59
		037		89	Straw	44 (NCH)	2.7
		035		89	Grain	44 (NCH)	0.03
	P3	005	1 x 200 (at BBCH 69)	69	Whole plant	0	2.6
		009		75		7	1.3
		013		79-83		14	0.62
		017		85		21	0.73
		041		89	Straw	44 (NCH)	3.0
		039		89	Grain	44 (NCH)	0.03
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		033		89	Straw	44 (NCH)	<0.01

		031		89	Grain	44 (NCH)	<0.01
S13-02516-03 United Kingdom (Church Lawford, CV23 9HD)	P2	003	1 x 125; 1 x 150 (app 1 at BBCH 57 – 59, app 2 at BBCH 69)	69	Whole plant	0	4.5
		007		75		7	3.9
		011		79		14	2.2
		015		83		21	2.0
		025		87-89	Straw	46	3.8
		039		89-92		51 (NCH)	3.5
		023		87-89	Grain	46	0.02
		037		89-92		51 (NCH)	0.02
	P3	005	1 x 200 (at BBCH 69)	69	Whole plant	0	4.2
		009		75		7	3.9
		013		79		14	2.3
		017		83		21	2.3
		029		87-89	Straw	46	4.0
		043		89-92		51 (NCH)	3.6
		027		87-89	Grain	46	0.02
		041		89-92		51 (NCH)	0.03
	P4	045	1 x 375; 1 x 450 (app 1 at BBCH 69, app 2 at BBCH 79)		Grain	37 (NCH)	0.26
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		021		87-89	Straw	46	<0.01
		033		89-92		51 (NCH)	<0.01
		019		87-89	Grain	46	<0.01
		031		89-92		51 (NCH)	<0.01
S13-02516-04 Northern France (Yevre la Ville, 45300)	P2	003	1 x 125; 1 x 150 (app 1 at BBCH 45-51, app 2 at BBCH 69)	69	Whole plant	0	2.5
		007		73		7	1.3
		011		75-77		14	0.56
		015		77-83		21	0.50
		037		89	Straw	42 (NCH)	0.60
		035		89	Grain	42 (NCH)	0.02
	P3	005	1 x 200 (at BBCH 69)	69	Whole plant	0	2.6
		009		73		7	1.6
		013		75-77		14	0.75
		017		77-83		21	0.55
		041		89	Straw	42 (NCH)	1.0
		039		89	Grain	42 (NCH)	0.02
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		033		89	Straw	42 (NCH)	<0.01
		031		89	Grain	42 (NCH)	<0.01

Plot C1: Untreated, Plot P2-P4: Treated with formulation A17573A

DBLA – Days before last application; DALA – Days after last application; NCH – Normal commercial harvest
No correction of results for either control residues or recovery values has been performed.

* Mean of two results (duplicate analyses from single sample)

** Low level of pydiflumetofen residues were found in the control grain and straw samples at 42 DALA in trial S13-02516-01 (0.01 mg/kg). However, no residues were found at NCH (52 DALA) in either grain or straw samples. Therefore, overall, it is considered that this contamination had no impact on the level of residues found in the treated samples and consequently no impact on the study.

Report:	KCA 6.3.15/1; [REDACTED], 2017
Title:	SYN545974 – Residue Study on Wheat in North France, Germany, Poland, the Czech Republic and the UK in 2016.
Report No.:	38051
Document No.:	VV-467609
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	Minor deviations were noted.: <ul style="list-style-type: none">• The cut height of the crop during sampling was not recorded (Trial 2 and Trial 5),• The distance between plots was not confirmed as being greater than 10 m (Trial 5),• The storage temperature during transportation rose to -5 °C before declining back to -13 °C (some samples from Trial 6). It is noted that the results from this trial are comparable with the results from the others. These deviations are not considered to have had any impact on the outcome of the trials.
GLP/GEP:	Yes

During the 2016 growing season, 8 field trials on wheat were conducted at eight independent locations: two in northern France, two in Germany, one in Poland, one in the Czech Republic and two in the United Kingdom. The trials were to determine the residue level of pydiflumetofen in or on RAC.

Pydiflumetofen was applied to wheat as A21857B, an EC formulation containing 62.5 g a.s./L. For all trials, plot 1 served as the control plot (C1). At all trials, A21857B was applied to plot 2 (P2) as a foliar spray once at a rate equivalent to 200 g a.s./ha at BBCH 67-69. Four of the trials (trials 1-4) had an additional plot, plot 3 (P3), which was treated with one application of 200 g a.s./ha at the earlier BBCH of 33-37.

Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no unusual events were noted. Rainfall was reported on the day of application for five of the eight trials; however, the rainfall did not occur within 2 hours of the application. OECD Test Guideline 509 states that application should not be made when rainfall is expected shortly after application. This is not considered to have had a marked impact on the outcome of the trials as the resulting residue results are comparable.

For the at harvest trials, treated samples of at least 1 kg of wheat grain and 0.5 kg of wheat straw were collected at NCH. For the decline trials, samples of at least 1 kg of whole plant were collected at 0, 6-8, 14, 27-29 and 42 DALA. It is noted that for trials 6 and 7, whole plant samples were not collected at the 42 DALA timepoint as this coincided with NCH. Untreated samples were collected at 14 DALA for whole plant and at NCH for grain and straw. All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 5 hours of sampling and stored at or below -18°C for a maximum of 11 months from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 11 months in wheat grain, straw and whole plant is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 6 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The specimens were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A, quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for wheat grain, wheat straw and cereal forage matrices within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). The method is acceptably validated in accordance with SANCO 3029/99 rev. 4 with an LOQ of 0.01 mg/kg. Extraction efficiency was satisfactorily addressed for the method.

A limited number of procedural recoveries for SYN544974 were determined concurrently with the sample analysis to check the accuracy of the residue analysis. Data were reported at two fortification levels per matrix: LOQ and 1000 x LOQ. The determined recoveries are reported in Table 7-58 and are in the acceptable range of 70 – 110%. While only a limited number of procedural recoveries were reported in report 38051, it is noted that the same analytical method was used to determine residues of pydiflumetofen across all submitted residue cereal trials with acceptable procedural recovery levels across all of the trials. Sufficient procedural recoveries have been performed across the submitted trials in wheat matrices.

Table 7-58 Procedural recoveries for pydiflumetofen in wheat whole plant, grain and straw.

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Wheat whole plant	2	0.01	86, 89	88	--
		2	10.0	92, 90	91	--
		4	overall	86-92	89	3
	Wheat grain	2	0.01	96, 108	102	--
		2	10.0	89, 95	92	--
		4	overall	89-108	93	8.7
	Wheat straw	2	0.01	109, 92	101	--
		2	10.0	99, 87	93	--
		4	overall	87-109	84	14.3

The trial design at plot P2 is representative of the cGAP for wheat and rye, i.e. 1 x 200 g a.s./ha at BBCH 69. Results from plot P3 (earlier latest growth stage of application) are presented for information purposes only.

Wheat whole plant samples were taken at 4 time intervals: 0, 6-8, 14, 27-29 and 42 days. The results show an overall decline in residue levels from day 0 to day 42.

Wheat straw and wheat grain samples were taken at NCH, which occurred at 42 – 56 DALA.

The residues of pydiflumetofen are summarised as follows:

Wheat Whole Plant

- 2.77 – 5.11 mg/kg at 0 DALA
- 0.46 – 0.75 mg/kg at 6-8 DALA
- 0.25 – 0.43 mg/kg at 14 DALA
- 0.14 – 0.44 mg/kg at 27-29 DALA
- 0.09 – 0.29 mg/kg at 42 DALA

Wheat Grain

- <0.01 – 0.05 mg/kg at NCH

Wheat Straw

- 0.28 – 2.39 mg/kg at NCH

No residues of pydiflumetofen were found in any of the control samples above the limit of quantification of 0.01 mg/kg.

Residues of pydiflumetofen found in wheat matrices from the individual trials are summarised in Table 7-59.

Table 7-59 Residues of pydiflumetofen in wheat whole plant, grain and straw

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling [BBCH]	Crop Part	DALA	pydiflumetof en [mg/kg]
680571 Trial 1 Northern France (Douai, 59500)	P2 ^a	003	1 x 200 (at BBCH 69)	89	Grain	NCH (44 DALA)	0.05
		009		89	Straw	NCH (44 DALA)	0.86
	P3 ^a	005	1 x 200 (at BBCH 33)	89	Grain	NCH (78 DALA)	<0.01
		011		89	Straw	NCH (78 DALA)	0.25
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680571 Trial 2 United Kingdom (Ellerton, YO42 4PX)	P2 ^a	003	1 x 200 (at BBCH 69)	89	Grain	NCH (56 DALA)	<0.01
		009		89	Straw	NCH (56 DALA)	0.41
	P3 ^a	005	1 x 200 (at BBCH 33)	89	Grain	NCH (87 DALA)	<0.01
		011		89	Straw	NCH (87 DALA)	0.21
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680571 Trial 3 Poland (Gosciecin, 47270)	P2	003	1 x 200 (at BBCH 69)	89	Grain	NCH (45 DALA)	0.02
		009		89	Straw	NCH (45 DALA)	2.39
	P3 ^a	005	1 x 200 (at BBCH 37)	89	Grain	NCH (67 DALA)	<0.01
		011		89	Straw	NCH (67 DALA)	0.42
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680571 Trial 4 Czech Republic (Uhersky Ostroh, 68724)	P2	003	1 x 200 (at BBCH 69)	89	Grain	NCH (41 DALA)	<0.01
		009		89	Straw	NCH (41 DALA)	0.88
	P3	005	1 x 200 (at BBCH 33)	89	Grain	NCH (75 DALA)	<0.01
		011		89	Straw	NCH (75 DALA)	0.85
	C1	001	Control	89	Grain	NCH [†]	<0.01
		007		89	Straw	NCH [†]	<0.01
680571 Trial 5 Northern France (Bresles, 60510)	P2 ^a	001	1 x 200 (at BBCH 67-69)	67-69	Whole plant	0 DALA	2.77
		003		70		7 DALA	0.46
		007		75		14 DALA	0.25
		009		85		28 DALA	0.25
		011		87		42 DALA	0.29
		015		89	Grain	NCH (50 DALA)	0.04
		019		89	Straw	NCH (50 DALA)	0.40
	C1	005	Control	75	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (50 DALA)	<0.01
		017		89	Straw	NCH (50 DALA)	<0.01
680571 Trial 6 Germany (Offheim, 65555)	P2 ^a	001	1 x 200 (at BBCH 69)	69	Whole plant	0 DALA	4.98
		003		73-75		7 DALA	0.55
		007		77		14 DALA	0.30
		009		87		28 DALA	0.39
		015		89	Grain	NCH (42 DALA)	0.04
		019		89	Straw	NCH (42 DALA)	0.84
	C1	005	Control	77	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (42 DALA)	<0.01
		017		89	Straw	NCH 42 DALA)	<0.01
680571 Trial 7 Germany (Neurott, 69124)	P2	001	1 x 200 (at BBCH 69)	69	Whole plant	0 DALA	5.11
		003		71		6 DALA	0.75
		007		75-77		14 DALA	0.43
		009		83-85		27 DALA	0.44
		015		89	Grain	NCH (42 DALA)	0.03
		019		89	Straw	NCH (42 DALA)	0.94
	C1	005	Control	75-77	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (42 DALA)	<0.01

		017		89	Straw	NCH (42 DALA)	<0.01
680571 Trial 8 United Kingdom (Dunshalt, KY14 7EP)	P2 ^a	001	1 x 200 (at BBCH 69)	69	Whole plant	0 DALA	3.04
		003		71		7 DALA	0.59
		007		75		14 DALA	0.29
		009		77		28 DALA	0.14
		011		85		42 DALA	0.09
		015		89	Grain	NCH (55 DALA)	0.01
		019		89	Straw	NCH (55 DALA)	0.28
	C1	005	Control	75	Whole plant	14 DALA	<0.01
		013		89	Grain	NCH (55 DALA)	<0.01
		017		89	Straw	NCH (55 DALA)	<0.01

NCH = Normal Commercial Harvest

Plot C1: Untreated, Plot P2: Treated with formulation A21857B at BBCH 67-69, Plot P3: Treated with formulation A21857B at BBCH 33-37.

[†] Control samples taken at same day after last application as treated plots.

^aFor six of the eight trials, rainfall was reported within 24 hours of product application, however none of the affected trials recorded rainfall within 2 hours of application. The rainfall amount ranged from 2.0 mm to 15.4 mm within 2-24 hours of product application. This is not considered to have largely impacted the study when considering the residue results. Trial 5 represented (for the cGAP trials) the highest rainfall on the day of application and this led to a relatively low (in the range) result for straw, but a high (in the range) result for grain. Details of the rainfall recorded are presented below:

Trial Number	Plot number	Application (g a.s./ha)	Rainfall on day of product application (mm) (>2 hrs after application)	Rainfall on day after application (mm)	Total rainfall, 2-24 hrs (mm)
1	P2	1 x 200 (at BBCH 65-67)	1.4	0.6	2.0
2			2.3	1.5	3.8
5			10.9	-	10.9
6			5.8	0.8	6.6
8			2.4	3.4	5.8
1	P3	1 x 200 (at BBCH 33)	15.4	-	15.4
2			-	8.9	8.9
3			3.0	1.2	4.2

Table 7-60 Summary of available residues trials on wheat at cGAP

Crop	Situation	<u>RD-Enf - pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	<u>RD-RA - pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	HR (RD-RA) (mg/kg)	STMR (RD-RA) (mg/kg)
Wheat grain (extrapolated to rye, triticale, durum wheat and spelt grain)	Outdoor	2 x < 0.01, 0.01, 3 x 0.02, 3 x 0.03, 2 x 0.04, 0.05	2 x < 0.01, 0.01, 3 x 0.02, 3 x 0.03, 2 x 0.04, 0.05	0.05	0.025
Wheat straw (extrapolated to rye, triticale, durum wheat and spelt straw)	Outdoor	0.28, 0.40, 0.41, 0.84, 0.86, 0.88, 0.94, 1.0, 2.2, 2.39, 3.0, 4.0	0.28, 0.40, 0.41, 0.84, 0.86, 0.88, 0.94, 1.0, 2.2, 2.39, 3.0, 4.0	4.0	0.910

B.7.3.3. Oilseed Rape

The requested GAP for oilseed rape is presented below (Table 7-61). The cGAP for oilseed rape consists of one spray application at a rate of 1 x 200 g a.s./ha at up to BBCH 57-69. It is noted that the cGAP for oilseed rape is for one application every three years.

Table 7-61 cGAP for oilseed rape

Use-No.	Member states/zones	Crop	Application				PHI (days)
			Method / kind	Growth stage of crop	Number of applications	Rate per application (g a.s./ha)	
5	UK	Spring and winter oilseed rape (OSR)^(a)	Spray	BBCH 57-69	1	200	n.a.

n.a – not applicable (PHI is covered by the time remaining between application and harvest).

^(a) 1 application every 3 years.

A summary of the trials submitted to support the cGAP on oilseed rape is given in Table 7-62.

As oilseed rape is a major crop, a minimum of eight residue trials that reflect the agronomic and climatic conditions of the UK are required. In total, there are 8 residue trials relevant to the UK. Eight trials are sufficient to address the requirements for the minimum number of residue trials.

The submitted dossier also includes eight residue trials from southern Europe, e.g. southern France, Spain and Italy (KCA 6.3.13/1, Report No. CEMR-6532 and KCA 6.3.13/3, Report No. S13-02260). These trials were not evaluated as they do not reflect the agronomic and climatic conditions of the UK and no additional information was provided to support the use of these trials.

Table 7-62 Number of residue trials relevant to the UK and vegetation period

Crop	Season	Number of trials	Countries	Reference
Oilseed rape	2013	4	FR (north), UK	KCA 6.3.13/4 Report No. S13-02259
	2014	4	FR (north), DE, UK	KCA 6.3.13/2 Report No. CEMR-6531
Total number of trials		8	-	

Report:	KCA 6.3.13/4; [REDACTED], [REDACTED] 2016
Title:	SYN545974 – Residue Study on Oilseed Rape in the United Kingdom and Northern France in 2013
Report No.:	S13-02259
Document No.:	VV-415279, A19649B_10230
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	No significant deviations were noted. For all four trials, fluctuations in temperature during storage or transportation were noted, however, the temperature did not exceed -12°C and samples remained frozen throughout. These minor deviations are not considered to have impacted the results of this study.
GLP/GEP:	Yes

During the 2013 growing season, four field trials on oilseed rape were conducted at four independent locations: two in northern France and two in the United Kingdom. The trials were to determine the residue level of pydiflumetofen in or on RAC.

Pydiflumetofen was applied to oilseed rape as A19649B, an SC formulation containing 200 g a.s./L. For all trials, plot 1 served as the control plot (C1). At all trials, A19649B was applied to plot 2 (P2) as a foliar spray once at a rate equivalent to 200 g a.s./ha at growth stage BBCH 67 – 71. Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no adverse conditions were reported.

Treated samples of at least 1 kg of whole plant were collected at 0, 7, 14, 21 and 42 DALA. Treated samples of at least 0.5 kg of oilseed rape (seed) were collected at NCH (51 – 62 DALA). For all trials, untreated samples of whole plant were collected from the control plot, C1, immediately before the last application (DBLA). Untreated samples of seed were collected at NCH (51 – 62 DALA). All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 6 hours of sampling and stored at or below -18°C for a maximum of 8 months from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 8 months in oilseed rape seed and whole plant is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 4 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The samples were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A, quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for oilseed rape (seeds) within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). The method is acceptably validated for oilseed rape seed in accordance with SANCO 3029/99 rev. 4 with an LOQ of 0.01 mg/kg.

Method validation data for oilseed rape whole plant are not available within Report No. S14-05352. However, method validation data on the comparable matrix of cereal forage are available in that report. Within the analytical phases in Report No. S13-02259 and Report No. CEMR-6531, there are sufficient method validation data to meet the requirements for a reduced validation dataset for oilseed rape whole plants. Example chromatograms show the method is specific and no significant interference (>30% of the LOQ) was observed. Therefore, the method is considered validated for oilseed rape whole plants in accordance with SANCO 3029/99 rev. 4. Extraction efficiency was satisfactorily addressed for the method.

A limited number of procedural recoveries for SYN544974 were determined concurrently with the sample analysis to check the accuracy of the residue analysis. Data were reported at two fortification levels per matrix: LOQ and 10 x LOQ. The determined recoveries are reported in Table 7-63 and the mean recoveries are in the acceptable range of 70 – 110%. While only a limited number of procedural recoveries were reported in report S13-02259, it

is noted that the same analytical method was used to determine residues of pydiflumetofen across all submitted cereal and oilseed rape residue trials, with acceptable procedural recovery levels across all of the trials [some other acceptable procedural recoveries were reported in the other report for oilseed rape (trials), report CEMR-6531, see evaluation below]. Sufficient procedural recoveries have been performed across the submitted trials in oilseed matrices.

Table 7-63 Procedural recoveries for pydiflumetofen in oilseed rape whole plant and seed.

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Oilseed Rape (seed)	1	0.01	105	-	-
		1	0.10	75	-	-
		2	overall	75-105	90	na
	Oilseed Rape (whole plant)	2	0.01	119, 93	106	-
		2	0.10	105, 99	102	-
		1	5.0	98	-	-
		5	overall		103	9.7

The trial design at plot P2 is representative of the cGAP for OSR, i.e. 1 x 200 g a.s./ha at BBCH 69.

OSR whole plant samples were taken at 4 time intervals: 0, 7, 14, 21 and 42 days. The results show an overall decline in residue levels from day 0 to day 42.

OSR seed samples were taken at NCH, which occurred at 51 – 62 DALA.

The residues of pydiflumetofen are summarised as follows:

OSR Whole Plant

- 2.0 – 3.5 mg/kg at 0 DALA
- 0.84 – 1.9 mg/kg at 7 DALA
- 0.55 – 2.1 mg/kg at 14 DALA
- 0.27 – 1.1 mg/kg at 21 DALA
- 0.07 – 0.48 mg/kg at 42 DALA

OSR Seed

- < 0.01 – 0.03 mg/kg at NCH

No residues of pydiflumetofen were found in any of the control samples above the limit of quantification of 0.01 mg/kg.

Residues of pydiflumetofen found in oilseed rape matrices from the individual trials are summarised in Table 7-64.

Table 7-64 Residues of pydiflumetofen in oilseed rape whole plant and seed.

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling (BBCH)	Crop Part	DALA	pydiflumetofen [mg/kg]
S13-02259-01 United Kingdom	P2	003	1 x 200 (at BBCH 67 – 69)	67-69	Whole plant	0 DALA	3.2
		005		71-73		7 DALA	1.4
		007		71-74		14 DALA	0.67
		009		79		21 DALA	0.65
		011		85-89		42 DALA	0.23

(Sancton, YO43 4RJ)	C1	015		89	Seed	NCH (51 DALA)	0.03
		001	Control	67-69	Whole plant	0 DBLA	<0.01
		013		89	Seed	NCH (51 DALA)	<0.01
S13-02259-02 United Kingdom (Borrowash, NG10 5NP)	P2	003	1 x 200 (at BBCH 69)	69	Whole plant	0 DALA	2.0
		005		69-72		7 DALA	1.9
		007		73-75		14 DALA	1.5
		009		74-76		21 DALA	1.1
		011		83-87		42 DALA	0.48
		015		89	Seed	NCH (57 DALA)	<0.01
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		013		89	Seed	NCH (57 DALA)	<0.01
S13-02259-03 Northern France (Scherwiller, 67750)	P2	003	1 x 200 (at BBCH 69)	69	Whole plant	0 DALA	2.9
		005		69-72		7 DALA	0.84
		007		69-72		14 DALA	0.55
		009		79		21 DALA	0.31
		011		79-80		42 DALA	0.07
		015		89	Seed	NCH (62 DALA)	0.02
	C1	001	Control	69	Whole plant	0 DBLA	<0.01
		013		89	Seed	NCH (62 DALA)	<0.01
S13-02259-04 Northern France (Rouvres Saint Jean, 45300)	P2	003	1 x 200 (at BBCH 69 – 71)	69-71	Whole plant	0 DALA	3.5
		005		69-72		7 DALA	1.7
		007		76		14 DALA	2.1
		009		76-77		21 DALA	0.27
		011		80		42 DALA	0.18
		015		89	Seed	NCH (61 DALA)	<0.01
	C1	001	Control	69-71	Whole plant	0 DBLA	<0.01
		013		89	Seed	NCH (61 DALA)	<0.01

DBLA – Days before last application; DALA – Days after last application; NCH – Normal commercial harvest
Plot C1: Untreated, Plot P2: Treated with formulation A19649B

Report:	KCA 6.3.13/2; [REDACTED], [REDACTED] 2017
Title:	SYN545974 – Residue Study on Oilseed Rape and Processed Products in Northern France, Germany and United Kingdom in 2014
Report No.:	CEMR-6531
Document No.:	VV-468119, A19649B_10334
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	Minor deviations were noted: <ul style="list-style-type: none"> • The untreated control samples taken from trial GBU-14-18587 FR01 were taken before the application of the product to the treated plots, and not after the application, as specified in the plan. • During transport of samples from trials GN-14-18587-FR02 and GB-14-18587-DE03, the temperature was not maintained at -18°C. However, the temperature did not exceed -14°C and the samples remained frozen throughout shipment. These deviations are not considered to have had any impact on the outcome of these trials.
GLP/GEP:	Yes

During the 2014 growing season, 4 field trials on oilseed rape were conducted at four independent locations: two in northern France, one in Germany and one in the United Kingdom. The trials were to determine the residue level of pydiflumetofen in or on RAC. Two of the trials contained plots treated to produce samples for processing studies. Details of the results from these plots can be found in section 7.5 (magnitude of residues in processed commodities).

Pydiflumetofen was applied to oilseed rape as A19649B, an SC formulation containing 200 g a.s./L. For all trials plot 1 served as the control plot (C1). At all trials, A19649B was applied to plot 2 (P2) as a foliar spray once at a rate equivalent to 200 g a.s./ha at or just before growth stage BBCH 69. For two of the trials, A19649B was applied to plot 3 (P3) as a foliar spray once at a rate equivalent to 400 g a.s./ha at growth stage BBCH 73 – 75. Treated seed samples were collected at normal commercial harvest from P3 for processing studies reported in section 7.5.

Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no adverse conditions were reported. Rainfall was reported within 5 hours of application during trial GBU-14-18587-DE03; however, it was reported that the application spray droplets were dry before the rainfall started. OECD Test Guideline 509 states that application should not be made when rainfall is expected shortly after application. This is not considered to have had a marked impact on the outcome of the trial.

Treated samples of at least 12 units weighing a minimum of 1 kg of whole plant were collected at 0 and 7 days after application (DAA). Treated samples of at least 0.5 kg oilseed rape seed were collected at NCH, (63–78 DAA) in accordance with OECD No. 509. For all trials, untreated samples of whole plant were collected from the control plot, C1, immediately after the application (0 DAA) and untreated samples of seed were collected at NCH (63–78 DAA). All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 11 hours of sampling and stored at or below -18°C for a maximum of 148 days (approximately 5 months) from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 148 days (approximately 5 months) in oilseed rape seed and whole plant is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 2 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The samples were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A, quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for oilseed rape (seeds) within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). The method is acceptably validated for oilseed rape seed in accordance with SANCO 3029/99 rev. 4 with an LOQ of 0.01 mg/kg.

Method validation data for oilseed rape whole plant are not available within Report No. S14-05352. However, method validation data on the comparable matrix of cereal forage are available in that report. Within the analytical phases in Report No. S13-02259 and Report No. CEMR-6531, there are sufficient method validation data to meet the requirements for a reduced validation dataset for oilseed rape whole plants. Example chromatograms show the method is specific and no significant interference (>30% of the LOQ) was observed. Therefore, the method is considered validated for oilseed rape whole plants in accordance with SANCO 3029/99 rev. 4. Extraction efficiency was satisfactorily addressed for the method.

A limited number of procedural recoveries for SYN544974 were determined concurrently with the sample analysis to check the accuracy of the residue analysis. Data were reported at three fortification levels per matrix: LOQ, 10 x LOQ and at least 100 x LOQ. The determined recoveries are reported in Table 7-65 and the overall mean recoveries are in the acceptable range of 70 – 110%. While only a limited number of procedural recoveries were reported in report CEMR-6531, it is noted that the same analytical method was used to determine residues of pydiflumetofen across all submitted cereal and oilseed rape residue trials, with acceptable procedural recovery levels across all of the trials [some other acceptable procedural recoveries were reported in the other report for oilseed rape (trials), report S13-02259, see evaluation above]. Sufficient procedural recoveries have been performed across the submitted trials in oilseed rape matrices.

Table 7-65 Procedural recoveries for pydiflumetofen in oilseed rape whole plant and seed.

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Oilseed Rape (seed)	1	0.01	87	-	-
		1	0.1	110	-	-
		1	1.0	114	-	-
		3	overall		104	14.1
	Oilseed Rape (whole plant)	1	0.01	113	-	-
		1	0.1	116	-	-
		1	5.0	96	-	-
		3	overall		108	10.0

The trial design at plot P2 is representative of the cGAP for OSR, i.e. 1 x 200 g a.s./ha at BBCH 69. Results from plot P3 (exaggerated application rates and later growth stage at application) are presented for information purposes only.

OSR whole plant samples were taken at 2 time intervals: 0 and 7 days. The results show an overall decline in residue levels from day 0 to day 7.

OSR seed samples were taken at NCH, which occurred at 60 – 78 DAA.

The residues of pydiflumetofen are summarised as follows:

OSR Whole Plant

- 2.52 - 3.53 mg/kg at 0 DALA
- 0.90 - 1.92 mg/kg at 7 DALA

OSR Seed

- < 0.01 – 0.04 mg/kg at NCH

No residues of pydiflumetofen were found in any of the other control samples above the limit of quantification of 0.01 mg/kg.

Residues of pydiflumetofen found in oilseed rape matrices from the individual trials are summarised in Table 7-66.

Table 7-66 Residues of pydiflumetofen in oilseed rape whole plant and seed.

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling (BBCH)	Crop Part	DAA	pydiflumetofe n [mg/kg]
GBU-14-18587 FR01 Northern France (Perthes, 08300)	P2	003	1 x 200 (at BBCH 69)	69	Whole plant	0	2.52
		005		69-71		7	1.80
		009		89	Seed	NCH (78 DAA)	0.01
	P3	011	1 x 400 (at BBCH 73 – 75)	89	Seed	NCH (58 DAA)	0.13
	C1	001	Control	69	Whole plant	0 DBA [†]	<0.01
		007		89	Seed	NCH (78 DAA)	<0.01
GBU-14-18587 FR02 Northern France (Dame Marie les Bois, 37110)	P2	003	1 x 200 (at BBCH 69)	69	Whole plant	0	3.53
		005		71		7	1.92
		009		89	Seed	NCH (63 DAA)	0.01
	P3	011	1 x 400 (at BBCH 73)	89	Seed	NCH (49 DAA)	0.05
	C1	001	Control	69	Whole plant	0 DAA	<0.01
		007		89	Seed	NCH (63 DAA)	<0.01
GBU-14-18587 DE03 Germany [‡] (Mulfingen-Oberer Railhof, 74673)	P2 [‡]	003	1 x 200 (at BBCH 69)	69	Whole plant	0	3.06
		005		69-71		7	0.90
		009		89	Seed	NCH (77 DAA)	<0.01
	C1	001	Control	69	Whole plant	0 DAA	<0.01
		007		89	Seed	NCH (77 DAA)	<0.01
GBU-14-18587 UK04 United Kingdom (Falcutt, NN13 5QY)	P2	003	1 x 200 (at BBCH 69)	69	Whole plant	0	3.10
		005		79		7	1.90
		009		89	Seed	NCH (60 DAA)	0.04
	C1	001	Control	69	Whole plant	0 DBA	<0.01
		007		89	Seed	NCH (60 DAA)	<0.01

DAA: Days after application, NCH: Normal Commercial Harvest, DBA: Days before application No correction of results for either control residues or recovery values has been performed.

[†]Note: in error, control sample at 0 DAA was collected at 0 days before application (0 DBA).

[‡]Note: rainfall occurred at around 5 hours after application. Spray deposits from the pesticide application were dry before the rainfall started. 8 mm of rainfall was recorded. The result from this trial GBU-14-18587-DE03 is considered to be comparable to the results from the other trials.

Table 7-67 Summary of available residues trials on oilseed rape at cGAP

Crop	Situation	<u>RD-Enf - pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	<u>RD-RA - pydiflumetofen</u> Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	HR (RD-RA) (mg/kg)	STMR (RD-RA) (mg/kg)
Oilseed rape (seeds)	Outdoor	3 x <0.01, 2 x 0.01, 0.02, 0.03, 0.04	3 x <0.01, 2 x 0.01, 0.02, 0.03, 0.04	0.04	0.01

B.7.3.4. Root Vegetables: Carrot, Parsnip and Parsley Root

MRL work is being conducted in parallel with the new active substance review for pydiflumetofen. As part of this MRL work, a proposed GAP on root crops (carrot, parsley root and parsnip) is being considered as a future GB use. It should be made clear that this proposal for root crops is distinct from the representative uses for pydiflumetofen in GB (cereals and OSR).

The requested GAPs for carrots, parsley roots and parsnips are presented below (Table 7-68). The cGAP consists of two spray applications to root crops at a rate of 2 x 70 g a.s./ha with a 14-day application interval at BBCH 14-49 and a 14-day PHI.

In support of the proposed GAPs for root vegetables, residue trials on carrots have been submitted. According to SANCO 7525/VI/95 rev. 10.3 (June 2017), it is possible to extrapolate residue data from carrot (commodity code 213020) to parsnip (commodity code 213060) and parsley root (commodity code 213070) for treatments made before and after the formation of the edible portion of the plant. Therefore, it is acceptable to extrapolate carrot residues data to support the proposed GAPs for parsnip and parsley root.

Table 7-68 Requested GAPs and cGAP for carrot (in bold)

Use-No.	Member states/zones	Crop	Application				PHI (days)
			Method / kind	Growth stage of crop	Number of applications (interval – days)	Rate per application (g a.s./ha)	
6	UK	Carrot	Spray	BBCH 14-49	2 (14)	70	14
		Parsley root		BBCH 21-49			
		Parsnip		BBCH 14-49			

A summary of the trials submitted to support the cGAP on carrot is given in Table 7-69.

As carrot is a major crop, a minimum of eight residue trials that reflect the agronomic and climatic conditions of the UK are required. In total, there are 8 residue trials relevant to the UK. Eight trials are sufficient to address the requirements for the minimum number of residue trials.

The submitted dossier also includes eight residue trials data from southern Europe, e.g. southern France, Spain, Greece and Italy (Report No. CEMR-7598). These trials were not evaluated as they do not reflect the agronomic and climatic conditions of the UK and no additional information was provided to support the use of these trials.

Table 7-69 Number of residue trials relevant to the UK and vegetation period

Crop	Season	Number of trials	Countries	Reference
Carrot	2016	8	FR (north), DE, PL UK	CEMR-7597
Total number of trials		8	-	

Report:	██████████, 2017
Title:	SYN545974 – Residue Study on Carrot in Northern France, Germany, Poland and the United Kingdom in 2016
Report No.:	CEMR-7597
Document No.:	Syngenta File No: A19649B_10342, VV-468307
Guidelines:	Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009. OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66).
Guideline deviations:	Minor deviations were noted: <ul style="list-style-type: none">• For six of the eight trials, fluctuations in temperature during transportation were noted, however, the temperature did not exceed -14.8°C and samples remained frozen throughout.• For trial VOI-16-26244-GB10, it is noted that no pesticide records exist for 2013 and 2014 as the field was purchased in 2015. These minor deviations are not considered to have impacted the results of this study.
GLP/GEP:	Yes

During the 2016 growing season, 8 field trials on carrot were conducted at eight independent locations: two each in northern France, Germany, Poland and the United Kingdom. The trials were to determine the residue level of pydiflumetofen in or on RAC.

Pydiflumetofen was applied to carrots as A19649B, an SC formulation containing 200 g a.s./L. For all trials, plot 1 served as the control plot (C1). At all trials, A19649B was applied to plot 2 (P2) as a foliar spray twice at a rate equivalent to 70 g a.s./ha in a spray volume of 300-600 L/ha with a 13–14-day application interval. Both applications were performed between growth stage BBCH 42-49.

Treated crops were described as being healthy throughout the duration of the trial. Climatic conditions were recorded throughout the trials and no adverse conditions were reported.

For decline trials, treated samples of at least 13 individual roots weighing a minimum of 2 kg of carrot root were collected immediately before the last application (0 DBLA) and at 0, 6-7, 9-10 DALA and at NCH, (14-15 DALA). For the at harvest trials, treated samples of at least 13 individual roots weighing a minimum of 2 kg of carrot root were collected at 0 DBLA and 13-14 DALA (NCH). For all trials, untreated samples of carrot root were collected from the control plot, C1, immediately before the last application (0 DBLA) and at NCH (13-14 DALA). All samples were collected in accordance with OECD 509 guidance.

Field specimens were deep frozen within 7 hours of sampling and stored at or below -18°C for a maximum of 5 months from sampling to analysis. The storage stability of pydiflumetofen in plant matrices has been demonstrated for up to 23 months, therefore the storage period of 5 months in carrot root is supported.

Extract solutions of field samples were stored refrigerated for a maximum of 3 days before analysis. The stability of pydiflumetofen in extracts was demonstrated by procedural recovery samples which were stored for under the same conditions and for the same period of time between extraction and analysis.

The specimens were analysed for residues of pydiflumetofen using LC-MS/MS method GRM061.03A quantifying the analyte with a limit of quantification (LOQ) of 0.01 mg/kg. Method validation data are available for the comparable matrix potato tuber within Report No. S14-05352 (KCA1 4.1.2/31, please refer to Volume 3 CA, B.5). Within the analytical phase in Report No. CEMR-7597, there are sufficient method validation data to meet the requirements for a reduced validation dataset for carrot roots. Example chromatograms show the method is specific and no significant interference (>30% of the LOQ) was observed. Therefore, the method is considered satisfactorily validated for carrot roots in accordance with SANCO 3029/99 rev. 4. Extraction efficiency was satisfactorily addressed for the method in carrots (section B.5.2.1).

Recovery rates for pydiflumetofen were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. Sufficient individual recovery rates were reported for carrot root with at least

two individual recoveries performed at each of three fortification levels. The recovery rates that were determined are reported in Table 7-70 and the mean values determined are in the acceptable range of 70 – 110%.

Table 7-70 Procedural recoveries for pydiflumetofen in carrot root

Analyte	Portion analysed	n	Fortification level (mg/kg)	Recovery (%)		
				Individual recoveries	Mean	RSD
pydiflumetofen	Carrot Root	3	0.01	77, 100, 97	91	13.7
		3	0.10	85, 107, 95	96	11.5
		2	1.0	90, 111	101	-
		8	overall	77-111	95	11.8

The trial design at plot P2 is representative of the cGAP for carrots, i.e. 2 x 70 g a.s./ha, 13 - 14-day application interval, 14-day PHI.

In the residue decline studies, root samples were taken at 5 time intervals: 0, 6-7, 9-10 and 14-15 days.

In the at harvest studies, root samples were taken at NCH (13-15 days).

The residues of pydiflumetofen are summarised as follows:

Carrot root

- 0.02 – 0.11 mg/kg at 0 DALA
- 0.02 – 0.04 mg/kg at 3 DALA
- 0.02 – 0.04 mg/kg at 6-7 DALA
- 0.02 – 0.04 mg/kg at 9- 10 DALA
- < 0.01 – 0.04 mg/kg at NCH (13 – 15 DALA)

No residues of pydiflumetofen were found in any of the control samples above the limit of quantification of 0.01 mg/kg.

Residues of pydiflumetofen found in carrot root from the individual trials are summarised in Table 7-71.

Table 7-71 Residues of pydiflumetofen in carrot root.

Trial no. Country	Plot	Sample no.	Number and nominal application rate (g a.s./ha)	Growth stage at sampling (BBCH)	Crop Part	DALA	pydiflumetofen [mg/kg]
VOI-16-26244 FR01 Northern France (Inchy en Artois, 62860)	P1	0.03	2 x 70 (app 1 at BBCH 45 – 46, app 2 at BBCH 47 – 48, 14-day interval)	47-48	Root	0 DBLA	0.02
		005		47-48		0 DALA	0.02
		007		47-48		3 DALA	0.03
		009		47-48		6 DALA	0.03
		011		48-49		10 DALA	0.03
		015		49		NCH (14 DALA)	0.02
	C1	001	Control	47-48		0 DBLA	<0.01
		013		49		NCH (14 DALA)	<0.01
VOI-16-26244 FR02 Northern France (Saint Martin de la Place, 49160)	P1	003	2 x 70 (app 1 at BBCH 46 – 47, app 2 at BBCH 49, 14-day interval)	47	Root	0 DBLA	0.01
		007		49		NCH (14 DALA)	0.04^(a)

	C1	001	Control	47		0 DBLA	<0.01
		005		49		NCH (14 DALA)	<0.01 ^{††}
VOI-16-26244 DE03 Germany (Monbrunn, 63897)	P1	003	2 x 70	42-45	Root	0 DBLA	0.04
		005	(app 1 at	42-45		0 DALA	0.11
		007	BBCH 42 –	42-46		3 DALA	0.02
		009	44, app 2 at	44-48		7 DALA	0.02
		011	BBCH 42 –	45-49		10 DALA	0.02
		015	45, 13-day interval)	45-49		NCH (15 DALA)	0.02
	C1	001	Control	42-45		0 DBLA	<0.01
		013		45-49		NCH (15 DALA)	<0.01
VOI-16-26244 DE04 Germany (Frankenhardt- Steinehaig, 74586)	P1	003	2 x 70	47-49	Root	0 DBLA	0.02
		007	(app 1 at BBCH 46 – 47, app 2 at BBCH 47 – 49, 13-day interval)	49		14 DALA	0.02^(b)
	C1	001	Control	47-49		0 DBLA	<0.01
		005		49		NCH (14 DALA)	<0.01 ^{††}
VOI-16-26244 PL05 Poland (Wola Ksiazeca, 63-220)	P1	003	2 x 70	49	Root	0 DBLA	0.04
		005	(app 1 at	49		0 DALA	0.05
		007	BBCH 47,	49		3 DALA	0.04
		009	app 2 at	49		7 DALA	0.04
		011	BBCH 49,	49		9 DALA	0.04
		015	14-day interval)	49		NCH (14 DALA)	0.04
	C1	001	Control	49		0 DBLA	<0.01
		013		49		NCH (14 DALA)	<0.01
VOI-16-26244 PL06 Poland (Stary Rzedków, 96-115)	P1	003	2 x 70	48	Root	0 DBLA	0.01
		007	(app 1 at BBCH 48, app 2 at BBCH 48, 14-day interval)	49		NCH (14 DALA)	0.03
	C1	001	Control	48		0 DBLA	<0.01
		005		49		NCH (14 DALA)	<0.01
VOI-16-26244 GB09 United Kingdom (Bormham, SN15 2HU)	P1	003	2 x 70	48-49	Root	0 DBLA	0.02
		005	(app 1 at	48-49		0 DALA	0.03
		007	BBCH 48 -	47-49		3 DALA	0.03
		009	49, app 2 at	48-49		7 DALA	0.05
		011	BBCH 48 -	48-49		9 DALA	0.04
		015	49, 13-day interval)	48-49		NCH (14 DALA)	0.03
	C1	001	Control	48-49		0 DBLA	<0.01
		013		48-49		NCH (14 DALA)	<0.01
VOI-16-26244 GB10 United Kingdom (Norton-sub- Hamdon, TA14 6SU)	P1	003	2 x 70	48-49	Root	0 DBLA	<0.01
		007	(app 1 at BBCH 47, 8 – 49, app 2 at BBCH 48 - 49, 13-day interval)	48-49		NCH (13 DALA)	<0.01
	C1	001	Control	48-49		0 DBLA	<0.01
		005		48-49		NCH (13 DALA)	<0.01

DBLA – Days before last application; DALA – Days after last application; NCH – Normal commercial harvest
Plot C1: Untreated, Plot P2: Treated with formulation A19649B

^(a) Mean result of two analysis, both 0.04 mg/kg. ^(b) Mean result of two analysis, both 0.02 mg/kg.

^{††}Mean result of two analysis, both <0.01.

Table 7-72 Summary of available residues trials on carrot root at cGAP

Crop	Situation	RD-Enf - pydiflumetofen Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	RD-RA - pydiflumetofen Residue levels (mg/kg) observed in the supervised residue trials relevant to the supported GAPs	HR (RD-RA) (mg/kg)	STMR (RD-RA) (mg/kg)
Carrot roots (extrapolated to parsley root and parsnip)	NEU Outdoor	<0.01, 3 x 0.02, 2 x 0.03, 2 x 0.04	<0.01, 3 x 0.02, 2 x 0.03, 2 x 0.04	0.04	0.025

B.7.4. FEEDING STUDIES

In this section the relationship between the actual doses used in the studies, and the anticipated use rates (the N rate) following the assessment of the uses considered in this evaluation, are expressed considering following various assessment scenario following presentation to the Expert Committee on Pesticides (ECP) in the process of seeking Independent Scientific Advice (ISA) and taking account of both rotational crop and primary crop residues. The fate parameters (on estimation of residues in soil), affect the rotational crop residues which in turn impact the animal dietary burden calculation. Two different scenarios have been assessed in this residues evaluation – ‘Tier 1 10 year use’ and ‘Tier 2 long term use’. These are The approach is further explained in Vol 1 section 2.7.7 (rotational crops) and Vol 1 section 2.7.5 (animal dietary burden).

B.7.4.1. Poultry

Report: K-CA 6.4.1/01, [REDACTED] (2015)
Title: SYN545974 – SYN545974-Magnitude of the Residues in Tissue and Eggs Resulting from the Feeding of Three Dose Levels to Poultry 2014
Report No: Report No. TK0103796 | GPL 140575 | 014-01423
Document No: Document No. VV-414618 (Syngenta File No. SYN545974_50189)
Guidelines: Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OECD guideline 505 for testing of chemicals “Residues in Livestock”, 08-Jan-2007.
Guideline deviations: None
GLP: Yes

Materials and methods

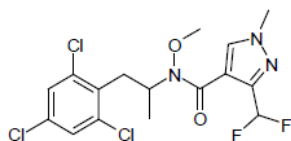
Materials

Pydiflumetofen

Description: SYN545974

Lot/Batch #: AMS 1432/1

Purity: 99.5% (nominal a.s. content in technical material)



Methods

The magnitude of residues of pydiflumetofen (SYN545974) in poultry tissue and eggs was investigated following the feeding of pydiflumetofen treated feed to poultry for a period of 28 days. Laying hens (breed *Tetra Brown*) were fed pydiflumetofen treated feed at three dosing levels of approximately 3 ppm, 9 ppm and 30 ppm (mg per kg) of dry matter (DM) feed.

A total of 54 hens were selected for the study, of these, 40 were fed with pydiflumetofen treated feed whilst 14 control hens received untreated feed. The 40 hens being fed with treated feed were split into a total of 4 dosing groups: 3 mg pydiflumetofen /kg DM (Group 2, low dose rate), 9 mg pydiflumetofen /kg DM (Group 3, mid dose rate), and 30 mg pydiflumetofen /kg DM (Group 4 and 5, high dose rate). Each of these four dosing groups were then further split into 3 replicate sub-groups each containing 3-4 hens. Details of the study outline are summarized in Table 7-73. After a quarantine period of 10 days following receipt, the hens were acclimatised for 14 days. During the initial 10 days of quarantine all hens were fed a custom feed, this custom food was then mixed with non-treated study feed at a decreasing ratio during the acclimation period until the hens were fed with only non-treated feed from study day (-9). Following the 28-day feeding phase, four of the control hens and ten of the high dose rate hens (group 5), began a 14-day depuration phase where they were fed non-treated feed. The non-depuration hens were sacrificed immediately after the 28-day feeding phase.

Animals were assessed for general health and food consumption. Overall, the animals remained healthy throughout the study, a consistent body weight was observed, and no issues were expected to impact the validity of the study.

Stock solutions of the test substance (pydiflumetofen) were prepared with ethanol for each treatment level and then mixed into premixes to give the stock feed batches. A control level was made by combining non-treated feed with ethanol only. The treatment levels were prepared so that they contained 110% of the target dose rates, they were therefore made at 3.3 mg/kg DM (Group 2, low dose rate), 9.9 mg/kg DM (Group 3, mid dose rate), and 33 mg/kg DM (Groups 4 and 5, high dose rate). The stock feed batches were prepared and stored frozen. At the start of each feeding period (week 1, 2, 3 and 4) the stock feed batch for that week was removed from the freezer and mixed with non-treated feed as subsamples. Level of feed was maintained in the feeders over the week and the feed weights were recorded at the start and finish of each period. From this, feed consumption per week and subsequently the average dose of pydiflumetofen (based on the nominal ppm of pydiflumetofen in the feed) received by each feed group over the course of the study were calculated. These are presented in Table 7-73.

Table 7-73 Consumption of pydiflumetofen by laying hens (the average feed consumption per day was 0.11 kg dm/hen/day¹)

Dose (mg/kg dm)	Group	Number of hens	Sub-Group Replicate IDs (# of Hens)	Average pydiflumetofen (mg/hen/day) ²	Average Body Weight (kg) ³	Actual Dose (mg/kg feed as dry matter)	Average Dose pydiflumetofen (mg/kg body weight/day) ⁴	N rate (Tier 1-10 year use)	N rate (Tier 2- long term use)
0	1	14	0103796-1A (3) 0103796-1B (3) 0103796-1C (4) 0103796-1D (4)	0	2.1	0	0	-	-
3	2 (low dose group)	10	0103796-2A (3) 0103796-2B (3) 0103796-2C (4)	0.33	2.1	3.3	0.16	3.5N 2.1N	2.5N

9	3 (mid dose group)	10	0103796-3A (3) 0103796-3B (3) 0103796-3C (4)	0.99	2.0	9.9	0.50	11.0N 6.6N	7.7N
30	4 (high dose group)	10	0103796-4A (3) 0103796-4B (3) 0103796-4C (4)	3.3	2.1	33	1.6	35.4N 24N	25N
30	5 (high dose group)	10	0103796-5A (3) 0103796-5B (3) 0103796-5C (4)	3.3	2.2	33	1.5	-	-

¹From table on consumption in the Biological Phase report (converted g to kg and corrected for 90% dry wt.)

²Nominal pydiflumetofen in feed (dose ppm) x average amount of feed consumed

³From table in Biological Phase Report, Average of the body weights of all hens per dose group (at necropsy)

⁴Calculated Average Dose (mg/kg bw/day) = mg pydiflumetofen consumed per day (mg/day)/average body weights (kg).

Eggs were collected (am and pm over a 24-hr period combined) on days 0, 1, 3, 7, 10, 14, 17 and 24 of the 28-day dosing period and the white and yolk weighed and combined for each day. On days 21 and 28, eggs were collected and the egg yolk and white were separated out. Composite egg white and yolk samples from each sub-group within the dose groups were prepared. During the depuration phase of group 5 (high dose rate) eggs were collected and separated into whites and yolks on days 31, 34, 38 and 42. The composite egg yolks and whites separated for each subgroup were not weighed on Days 21, 28, 31, 35, 38 or 42, however this is not expected to significantly impact the validity of the study. Samples were stored frozen until analysis and extraction, within 6.6 months of collection. As discussed below, storage stability data conducted separately demonstrates the stability of pydiflumetofen under frozen conditions for up to at least 24 months and 2,4,6 Trichlorophenol (free and conjugated) for up to at least 12 months in animal matrices.

Hens not selected for depuration were sacrificed after the 28 days of dosing, within 6 hours of the removal of treated food. Edible tissues (breast + thigh muscle, liver, kidney, skin with fat and abdominal and peritoneal fat) were removed postmortem and weighed. Following the depuration period, three of the high dose rate hens were sacrificed on days 31 (3 days post feed removal) and three on day 35 (7 days post feed removal), tissue collection as above. The remaining four (high dose rate) depuration hens and the four depuration control hens were sacrificed on day 42 (14 days post feed removal), tissue collection as above. Once weighed individually, tissues were chopped, combined by treatment group, transferred to containers, weighed again and frozen. The pooled tissue weights for each pool group (3 – 4 groups per dose group) from samples taken of breast plus thigh muscle is between 363 – 601 g whilst OECD 505 advises a minimum of 500 g to be taken. This is still considered acceptable, and the overall quantity of sample per dose group exceeds the value given in the guidance. As can be seen in the tables below, residues found in breast plus thigh muscle are <0.01 mg/kg for the highest dose group.

Analysis/general procedures

Samples of tissue and egg were homogenised frozen with the aid of dry ice to form a homogeneous consistency. For all matrices, the samples from the high dose rate group were analysed first and if residues above LOQ (0.01 mg/kg) were found, samples from the mid dose rate group for that matrix were analysed. The same was done for the low dose rate group if the mid dose rate dose group showed residues >LOQ. Samples of egg (white + yolks, whites and yolks) and kidney were analysed for pydiflumetofen and 2,4,6-TCP at all dose levels, whilst samples of liver, breast + thigh muscle, abdominal, peritoneal and skin with fat samples were only analysed from the high dose rate treated group.

Eggs and tissues were analysed for pydiflumetofen content using the method GRM061.06A (KCA1 4.1.2/29, please refer to Volume 3 CA, B.5.1.2), the LOQ for this method is 0.01 mg/kg. Eggs and tissues were analysed for conjugated 2,4,6-trichlorophenol content by GRM061.07A (KCA1 4.1.2/26, please refer to Volume 3 CA, B.5.1.2). As noted in section B.5.1.2, the method determines 2,4,6-trichlorophenol after enzyme hydrolysis 'deconjugating' the residue, and the method is validated for both free and conjugated 2,4,6-trichlorophenol. This method has been validated to an LOQ of 0.01 mg/kg in relevant matrices. All samples were analysed using LC-MS/MS.

No storage stability investigations were conducted within the study report. The storage period from sampling to analysis is in the range of 113 - 202 days (up to 6.6 months). Storage stability studies conducted separately to the feeding study demonstrate the stability of pydiflumetofen in animal matrices for at least up to 24 months when

stored at -20°C (██████████ (2016, final 2017)) and the stability of 2,4,6 Trichlorophenol for at least up to 12 months when stored at -18°C (██████████, ██████████. (2016)). See section B.7.1.2.

Once extracted, test samples were stored for a maximum of 6 days (control sample 9 days – tested for 2,4,6 – TCP) and is therefore covered by the stability of extracts given in reports no. GRM061.06A and GRM061.07A. Stability of extracts is further demonstrated through procedural recoveries; extracts of samples for procedural recovery analysis were stored for the same time under the same conditions. The mean procedural recoveries for pydiflumetofen and 2,4,6-TCP in whole egg (egg white + yolk), liver, kidney, breast + thigh muscle, skin + attached fat and abdominal and peritoneal fat were all acceptable at appropriate fortification levels (0.01 - 1.0 mg/kg). See also stability of residues in extracts in section B.7.1.3.

Results and discussion

Residues in egg samples

No residues of pydiflumetofen or 2,4,6-TCP were found in any non-treated control egg samples at levels at or above LOQ (0.01 mg/kg).

Table 7-74 Pydiflumetofen Residue Data for Whole Eggs (white + yolk)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day							
Target	Actual		0	1	3	7	10	14	17	24
Control		1 or 11	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		2B	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		2C	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mean	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	3A	ND	<0.01	<0.01	<0.01	<0.01	0.011	0.011	<0.01
		3B	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		3C	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mean	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	ND	<0.01	<0.01	<0.01	<0.01	0.011	0.011	<0.01
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	4A	ND	<0.01	0.027	0.024	0.021	0.019	0.021	0.01
		4B	ND	ND	0.027	0.024	0.026	0.025	0.019	0.026
		4C	ND	<0.01	0.026	0.023	0.017	0.017	0.027	0.021
		5A	ND	<0.01	0.026	0.022	0.023	0.017	0.015	0.016
		5B	ND	ND	0.023	0.024	0.016	0.015	0.017	0.019
		5C	ND	<0.01	0.023	0.025	0.020	0.025	0.019	0.02
		Mean	ND	<0.01	0.025	0.024	0.020	0.020	0.020	0.019
		Max	ND	<0.01	0.027	0.025	0.026	0.025	0.027	0.026

NA = not analysed (<LOQ for the previous dose group)

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Group 5 - Depuration

The data in Table 7-75 indicate that pydiflumetofen residues achieved a plateau level in whole eggs early during the study (study day 3 onwards). In comparison, data indicates that 2,4,6-TCP residues reached a plateau level in whole eggs slightly later than for pydiflumetofen (Table 7-75). A plateau was observed ~day 7 of the study.

Table 7-75 2,4,6-Trichlorophenol (TCP) Residue Data for Whole Eggs (white + yolk)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day							
Target	Actual		0	1	3	7	10	14	17	24
Control		1 or 11	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	2A	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		2B	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		2C	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mean	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	3A	ND	ND	<0.01	0.011	0.012	<0.01	<0.01	0.01
		3B	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		3C	ND	<0.01	<0.01	0.011	0.011	0.011	0.013	0.011
		Mean	ND	<0.01	<0.01	0.011	0.011	0.01	0.01	0.01
		Max	ND	<0.01	<0.01	0.011	0.012	0.011	0.013	0.01
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	4A	ND	<0.01	0.011	0.028	0.028	0.042	0.038	0.03
		4B	ND	<0.01	<0.010	0.020	0.018	0.021	0.026	0.020
		4C	ND	<0.01	0.013	0.024	0.024	0.028	0.025	0.030
		5A	ND	ND	0.015	0.037	0.036	0.042	0.038	0.043
		5B	ND	<0.01	<0.010	0.030	0.034	0.033	0.039	0.039
		5C	ND	<0.01	0.012	0.024	0.028	0.029	0.031	0.029
		Mean	ND	<0.01	0.012	0.027	0.028	0.033	0.033	0.032
		Max	ND	<0.01	0.015	0.037	0.036	0.042	0.039	0.043

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Eggs collected on days 21 and 28 for all dose groups and during the depuration phase (31, 34, 38 and 42), for group 5 (high dose rate) only, were separated into whites and yolks. The magnitude of residues of pydiflumetofen and 2,4,6-TCP in egg whites and yolks for these time periods are given in Tables 7-76, 7-77, 7-78 and 7-79.

Table 7-76 Pydiflumetofen Residue Data for Egg Whites (end of dosing phase and depuration)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day					
Target	Actual		21	28	31	35	38	42
Control		1 or 11	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-
		Max	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	2A	<0.01	<0.01	-	-	-	-
		2B	<0.01	<0.01	-	-	-	-
		2C	<0.01	<0.01	-	-	-	-
		Mean	<0.01	<0.01	-	-	-	-
		Max	<0.01	<0.01	-	-	-	-
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	3A	0.015	<0.01	-	-	-	-
		3B	<0.01	<0.01	-	-	-	-
		3C	<0.01	<0.01	-	-	-	-
		Mean	0.012	<0.01	-	-	-	-
		Max	0.015	<0.01	-	-	-	-
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	4A	0.038	0.016	-	-	-	-
		4B	0.027	0.028	-	-	-	-
		4C	0.022	0.029	-	-	-	-
		5A	0.019	0.034	<0.010	-	-	-

	long term use)	5B	0.023	0.021	<0.010	ND	-	-
		5C	0.031	0.03	<0.010	ND	ND	ND
		Mean	0.027	0.026	<0.010	ND	-	-
		Max	0.038	0.034	<0.010	ND	ND	ND

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-77 2,4,6-Trichlorophenol (TCP) Residue Data for Egg Whites (end of dosing phase and depuration)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day					
Target	Actual		21	28	31	35	38	42
Control		1 or 11	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-
		Max	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2-long term use)	2A	<0.01	<0.01	-	-	-	-
		2B	<0.01	<0.01	-	-	-	-
		2C	<0.01	<0.01	-	-	-	-
		Mean	<0.01	<0.01	-	-	-	-
		Max	<0.01	<0.01	-	-	-	-
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2-long term use)	3A	<0.01	<0.01	-	-	-	-
		3B	<0.01	<0.01	-	-	-	-
		3C	<0.01	<0.01	-	-	-	-
		Mean	<0.01	<0.01	-	-	-	-
		Max	<0.01	<0.01	-	-	-	-
30	33 (35.4N Tier 1-10 year, and 25N Tier 2-long term use)	4A	<0.01	<0.01	-	-	-	-
		4B	<0.01	<0.01	-	-	-	-
		4C	<0.01	<0.01	-	-	-	-
		5A	<0.01	<0.01	ND	-	-	-
		5B	<0.01	<0.01	<0.010	ND	-	-
		5C	<0.01	<0.01	ND	ND	ND	ND
		Mean	<0.01	<0.01	<0.010	ND	-	-
		Max	<0.01	<0.01	<0.010	ND	ND	ND

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-78 Pydiflumetofen Residue Data for Egg Yolks (end of dosing phase and depuration)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day					
Target	Actual		21	28	31	35	38	42
Control		1 or 11	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-
		Max	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2-long term use)	2A	<0.01	<0.01	-	-	-	-
		2B	<0.01	<0.01	-	-	-	-
		2C	<0.01	<0.01	-	-	-	-
		Mean	<0.01	<0.01	-	-	-	-
		Max	<0.01	<0.01	-	-	-	-
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2-long term use)	3A	<0.01	<0.01	-	-	-	-
		3B	<0.01	<0.01	-	-	-	-
		3C	<0.01	<0.01	-	-	-	-
		Mean	<0.01	<0.01	-	-	-	-
		Max	<0.01	<0.01	-	-	-	-
30	33 (35.4N Tier 1-10 year, and 25N Tier 2-long term use)	4A	<0.01	<0.01	-	-	-	-
		4B	<0.01	<0.01	-	-	-	-

	10 year, and 25N Tier 2 long term use)	4C	<0.01	0.010	-	-	-	-
		5A	<0.01	0.012	<0.010	-	-	-
		5B	<0.01	<0.01	<0.010	<0.010	-	-
		5C	0.012	0.011	<0.010	<0.010	<0.010	<0.010
		Mean	0.011	0.011	<0.010	<0.010	-	-
		Max	0.012	0.012	<0.010	<0.010	<0.010	<0.010

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-79 2,4,6-Trichlorophenol (TCP) Residue Data for Egg Yolks (end of dosing phase and depuration)

Dose Rate (mg pydiflumetofen /kg feed)		Group/Sub-group	Study Day					
Target	Actual		21	28	31	35	38	42
Control		1 or 11	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-
		Max	-	-	-	-	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	2A	<0.010	0.013	-	-	-	-
		2B	<0.010	<0.010	-	-	-	-
		2C	<0.010	<0.010	-	-	-	-
		Mean	<0.010	0.011	-	-	-	-
		Max	<0.010	0.013	-	-	-	-
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	3A	0.024	0.026	-	-	-	-
		3B	0.016	0.014	-	-	-	-
		3C	0.013	0.026	-	-	-	-
		Mean	0.017	0.022	-	-	-	-
		Max	0.024	0.026	-	-	-	-
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	4A	0.043	0.052	-	-	-	-
		4B	0.049	0.038	-	-	-	-
		4C	0.062	0.062	-	-	-	-
		5A	0.058	0.068	0.048	-	-	-
		5B	0.054	0.062	0.052	<0.010	-	-
		5C	0.055	0.041	0.034	<0.010	ND	ND
		Mean	0.054	0.054	0.044	<0.010	-	-
		Max	0.062	0.068	0.052	<0.010	ND	ND

NA = not analysed (<LOQ for the previous dose group)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Data obtained for eggs collected during the depuration phase indicate that residues of pydiflumetofen and 2,4,6-TCP are quickly eliminated after the removal of pydiflumetofen from the diet. Residues of both studied compounds return to levels <LOQ after day 35 of the study at the latest.

Residues in tissue samples

No residues of pydiflumetofen or 2,4,6-TCP were found in non-treated control tissue samples at levels at or above the LOQ (0.01 mg/kg).

Magnitude of residues of pydiflumetofen and 2,4,6-TCP found in poultry tissue samples collected following 28 days of dosing are summarized in the following tables.

Table 7-80 Residue Data for Hen Liver

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Group/Sub Group	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max

Control		28	11	ND	<0.01	<0.01	ND	<0.01	<0.01
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	28	2A	NA	-	-	NA	-	-
			2B	NA			NA		
			2C	NA			NA		
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	28	3A	NA	-	-	NA	-	-
			3B	NA			NA		
			3C	NA			NA		
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	28	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01 <0.01
			4B	<0.01			<0.01		
			4C	<0.01			<0.01		
			5A	<0.01			<0.01		<0.01
			5B	<0.01			<0.01		<0.01
			5C	<0.01			<0.01		<0.01
30	30	31	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		35	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		42	5C	<0.01	-	<0.01	<0.01	-	<0.01

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Table 7-81 Residue Data for Hen Kidney

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Group/Sub Group	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	<0.01	<0.01	<0.01	ND	<0.01	<0.01
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	28	2A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			2B	<0.01			<0.01		
			2C	<0.01			<0.01		
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	28	3A	<0.01	<0.01	<0.01	0.018	0.017	0.019
			3B	<0.01			0.013		
			3C	<0.01			0.019		

30	33 (35.4N 21N Tier 1 10 year and 25N Tier 2 long term use)	28	4A	<0.01	<0.01	<0.01	0.050	0.045	0.050
			4B	<0.01			0.037		
			4C	<0.01			0.049		
30		31	5A	<0.01	-	<0.01	<0.01	-	<0.01
30		35	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		42	5C	<0.01	-	<0.01	<0.01	-	<0.01

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Table 7-82 Residue Data for Hen Muscle (Breast + Thigh)

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Group/Sub Group	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	<0.01	<0.01	<0.01	ND	-	-
3	3.3 (3.5N 2.1N Tier 1 10 year and 2.5N Tier 2 long term use)	28	2A	NA	NA	NA	NA	NA	NA
			2B	NA			NA		
			2C	NA			NA		
9	9.9 (11.0N 6.6N Tier 1 10 year and 7.7N Tier 2 long term use)	28	3A	NA	NA	NA	NA	NA	NA
			3B	NA			NA		
			3C	NA			NA		
30	33 (35.4N 21N Tier 1 10 year and 25N Tier 2 long term use)	28	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			4B	<0.01			<0.01		
			4C	<0.01			<0.01		
30		31	5A	<0.01	-	<0.01	<0.01	-	<0.01
30		35	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		42	5C	<0.01	-	<0.01	<0.01	-	<0.01

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Table 7-83 Residue Data for Hen Skin with Fat

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Group/Sub Group	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	<0.01	<0.01	<0.01	ND	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	28	2A	NA	NA	NA	NA	NA	NA
			2B	NA			NA		
			2C	NA			NA		
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	28	3A	NA	NA	NA	NA	NA	NA
			3B	NA			NA		
			3C	NA			NA		
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	28	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			4B	<0.01			<0.01		
			4C	<0.01			<0.01		
30	33 (35.4N Tier 1-10 year, and 25N Tier 2 long term use)	31	5A	<0.01	-	<0.01	<0.01	-	<0.01
30		35	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		42	5C	<0.01	-	<0.01	<0.01	-	<0.01

NA = not analysed (<LOQ for the previous dose group)
 ND = not detectable (<0.00250 mg/kg)
 <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Table 7-84 Residue Data for Hen Abdominal plus Peritoneal Fat

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Group/Sub Group	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	<0.01	<0.01	<0.01	ND	-	-
3	3.3 (3.5N Tier 1-10 year, and 2.5N Tier 2 long term use)	28	2A	NA	NA	NA	NA	NA	NA
			2B	NA			NA		
			2C	NA			NA		
9	9.9 (11.0N Tier 1-10 year, and 7.7N Tier 2 long term use)	28	3A	NA	NA	NA	NA	NA	NA
			3B	NA			NA		
			3C	NA			NA		

	7.7N Tier (2 long term use)								
30	33 (35.4N 24N Tier 1-10 year, and 25N Tier 2 long term use)	28	4A	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			4B	<0.01			<0.01		
			4C	<0.01			<0.01		
30		31	5A	<0.01	-	<0.01	<0.01	-	<0.01
30		35	5B	<0.01	-	<0.01	<0.01	-	<0.01
30		42	5C	<0.01	-	<0.01	<0.01	-	<0.01

NA

= not analysed (<LOQ for the previous dose group)

ND

= not detectable (<0.00250 mg/kg)

<0.01

= <LOQ, lower limit of quantification (0.01 mg/kg)

The results demonstrate that no residues of pydiflumetofen or 2,4,6-TCP above LOQ are expected in poultry tissues of muscle (breast and thigh), liver and fat (skin with fat and abdominal plus peritoneal fat) at any feeding levels up to and including the high dose rate feeding group (30 mg/kg dm).

For kidney tissues, positive residues of 2,4,6-TCP were identified in the high dose rate and mid dose rate (9 mg/kg dm) feeding groups. Residues were between 0.037 – 0.05 mg/kg for the high dose rate group and 0.013 – 0.019 mg/kg for the mid dose rate group. Residues were all <LOQ for the low dose rate feeding group. Residues of pydiflumetofen were <LOQ in kidney for all feeding levels.

Conclusion

Pydiflumetofen:

Mean and highest residues of parent pydiflumetofen found in hen commodities from each dose group are summarised in Table 7-85 below.

Positive residues of pydiflumetofen were found in whole eggs (whites and yolks), egg whites, and egg yolks, at the highest dose and at the mid dose rate feeding level for both whole eggs and egg whites. For these samples, no residues >LOQ were identified at the low dose rate feeding level.

For all other hen commodities, no residues of pydiflumetofen >LOQ were identified at any of the feeding levels analysed.

Table 7-85 Mean and Highest Residues of pydiflumetofen in Hen Commodities

Matrix	pydiflumetofen Residue (mg/kg)							
	Group 2 (low dose rate, 3.3 mg/kg*)		Group 3 (mid dose rate, 9.9 mg/kg*)		Group 4 (highest dose rate, 33 mg/kg*)		Group 5 (high dose rate, 33 mg/kg depuration*)	
	Mean	Highest	Mean	Highest	Mean	Highest	Mean	Highest
Whole eggs (days 0 to 24)	<0.010	<0.010	<0.01	0.011	0.02	0.027	0.02	0.026
Egg Whites	<0.010	<0.010	0.010	0.015	0.027	0.038	0.026	0.034
Egg Yolks	<0.010	<0.010	<0.010	<0.010	<0.010	0.010	<0.010	0.012
Muscle	NA	NA	NA	NA	<0.01	<0.01	<0.010	<0.010
Liver	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010

Matrix	pydiflumetofen Residue (mg/kg)							
	Group 2 (low dose rate, 3.3 mg/kg*)		Group 3 (mid dose rate, 9.9 mg/kg*)		Group 4 (highest dose rate, 33 mg/kg*)		Group 5 (high dose rate, 33 mg/kg depuration*)	
	Mean	Highest	Mean	Highest	Mean	Highest	Mean	Highest
Kidney	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Skin + Fat	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010
Fat –abdominal + peritoneal	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010

NA – not analysed

* mg/kg - given on a dry matter basis

2,4,6-TCP (conjugated):

Mean and highest residues of 2,4,6-TCP (trichlorophenol) found in hen commodities from each dose group are summarised in Table 7-86. As noted in section B.5.1.2, the method determines 2,4,6-trichlorophenol after enzyme hydrolysis ‘deconjugating’ the residue, and the method is validated for both free and conjugated 2,4,6-trichlorophenol. We know from the metabolism data in poultry (section B.7.2.2) that these residues of 2,4,6-TCP are expected to be exclusively in the (sulphate) conjugated form. The method of analysis releases the residues in the free form, prior to chromatographic analysis. This step is achieved by enzyme de-conjugation using β -glucuronidase to give de-conjugated 2,4,6-TCP; this same approach was used in the metabolism study to release 2,4,6-TCP from the conjugated form.

Residues of 2,4,6-TCP were found in whole eggs (white and yolk) and egg yolk at the high dose rate and mid dose rate dose levels. Residues were still present in the yolk samples at the lowest dose level. In kidney tissues, residues of 2,4,6-TCP >LOQ were identified in the highest dose rate and mid dose rate feeding groups.

Table 7-86 Mean and Highest Residues of 2,4,6-Trichlorophenol (released from its conjugated form) in Hen Commodities

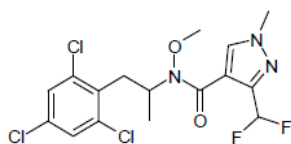
Matrix	2,4,6-Trichlorophenol Residue (mg/kg)							
	Group 2 (low dose rate, 3.3 mg/kg*)		Group 3 (mid dose rate, 9.9 mg/kg*)		Group 4 (highest dose rate, 33 mg/kg*)		Group 5 (high dose rate, 33 mg/kg depuration*)	
	Mean	Highest	Mean	Highest	Mean	Highest	Mean	Highest
Whole eggs (days 0 to 24)	<0.010	<0.010	0.010	0.013	0.021	0.04	0.03	0.04
Egg Whites	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Egg Yolks	0.011	0.013	0.02	0.026	0.05	0.06	0.057	0.068
Muscle	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010
Liver	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010
Kidney	<0.010	<0.010	0.017	0.019	0.045	0.050	<0.010	<0.010
Skin + Fat	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010
Fat –abdominal + peritoneal	NA	NA	NA	NA	<0.010	<0.010	<0.010	<0.010

NA – not analysed

* mg/kg - given on a dry matter basis

B.7.4.2. Ruminants

Report:	K-CA 6.4.2/01, [REDACTED] [REDACTED] [REDACTED] (2017)
Title:	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974
Report No:	Report No. 35775
Document No:	Document No. VV-414196 (Syngenta File No. SYN545974_10288)
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OECD guideline 505 for testing of chemicals “Residues in Livestock”, 08-Jan-2007.
Guideline deviations:	None
GLP:	Yes

Materials and methods*Materials*Pydiflumetofen**Description:** SYN545974**Lot/Batch #:** SMU2EP12007**Purity:** 98.5% (Active substance content in technical material)*Methods*

The magnitude of residues of pydiflumetofen (SYN545974) in ruminant tissue and milk was investigated following the administration of pydiflumetofen via gelatine capsules to ruminants for a period of 28 days. Non-pregnant, lactating, dairy cattle (breed *Ayrshire* and *Holstein/Friesian*) were fed gelatine capsules containing pydiflumetofen at three dosing levels of approximately 15 ppm, 45 ppm and 150 ppm (mg per kg) of dry matter (DM) feed.

A total of 11 dairy cows were selected for the study, of these, 9 were fed with pydiflumetofen via gelatine capsules whilst one control cow received gelatine dosing capsules containing no active substance. The 9 cows being given gelatine capsules containing pydiflumetofen were split into a total of 3 dosing groups: 15 mg pydiflumetofen /kg DM (Group 2, low dose rate), 45 mg pydiflumetofen /kg DM (Group 3, mid dose rate), and 150 mg pydiflumetofen /kg DM (Group 4, high dose rate). For each animal, the mean daily dose level achieved was within 6% of the target dose level. The mean daily dose levels per group, calculated on a body weight basis, was calculated as 0.40 (Group 2, low dose rate), 1.09 (Group 3, mid dose rate) and 4.32 (high dose rate) mg pydiflumetofen /kg body weight/day. The actual N rates (comparison to the anticipated livestock dietary intake) are presented below in Table 7-86. Details of the study outline are summarized in Table 7-87. The cattle were acclimatised for 21 – 22 days prior to dosing. The cattle were all fed commercially available concentrate twice daily and meadow hay and water were offered to the animals *ad libitum* per treatment group pen. Group hay and concentrate consumption were recorded daily during acclimatisation and dosing, and the moisture content of each diet type (hay and concentrate) was determined once during the acclimatisation period.

Animals were assessed for general health and food consumption, and weights of the animals recorded over the course of the study. Based on these assessments an adjustment was made to which cows were used as control cows

(on day 11, animal 11 replaced animal cow 1). A medicine was also administered to a control cow (animal 11); it was not considered that this would impact the results of the study. On a limited number of occasions, the initial dosing of the treated animal was not successful, but this was then managed as a follow up immediately post the initial attempt at dosing. These divergences are not expected to have a significant impact on the validity of the study.

Table 7-87 Consumption of pydiflumetofen by lactating cattle

Dose (mg/kg dm)	Group	Number of cows	Animal	Achieved dose of pydiflumetofen (mg/kg dm) ¹	Average Body Weight (kg) ²	Average Dose pydiflumetofen (mg/kg body weight/day) ³	N rate (Tier 1-10 year use)	N rate (Tier 2- long term use)
0	1	2	1/11	0	748	0	-	-
15	2 (low dose rate group)	3	2, 3, 4	15.68	656	0.40	4.1N 4.9N	2.4N
45	3 (mid dose rate group)	3	5, 6, 7	47.20	663	1.09	11.1N 5.3N	6.6N
150	4 (high dose rate group)	3	8, 9, 10	152.47	615	4.32	44.1N 21N	26N

¹Calculation of actual dose levels - based on amount of test item dispensed into each capsule and actual mean food consumption (dry matter intake) for each animal (per treatment group) during the dosing period.

²Average of the body weights of all cows per dose group (over study days -16/-15, -9/-8, -2/-1, 6/7, 13/14, 20/21, 24/25 and day 28)

³Calculated Average Dose (mg/kg bw/day) = mg pydiflumetofen consumed per day (mg/day)/average body weights (kg).

Milking was done twice per day (am and pm) throughout the acclimatisation and study period and milk samples were weighed. Samples from the pm milking were combined with the following am milk sample to give a 24-hr bulk sample. Milk collected on study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28 were kept for analysis of pydiflumetofen and relevant metabolites. After the appropriate subsamples and aliquots had been taken, the remaining bulk samples from study days 14 and 28 were retained to produce skimmed milk and cream.

All cattle were sacrificed after the 28 days of dosing, immediately after morning milking on day 29, within *ca* 22-24 h of the last morning dose. Edible tissues (loin and hind-leg muscle, liver, kidney, perirenal, mesenteric and subcutaneous fat) were removed postmortem and weighed. Once weighed, all samples were stored whole in a freezer set at -20°C. All tissue and milk samples taken were of an acceptable size in accordance with OECD 505.

Analysis/general procedures

Samples of tissue and milk were homogenised frozen with the aid of dry ice to form a fine powder and were returned to frozen storage.

For analysis of pydiflumetofen residues, samples of liver, muscle, kidney and milk were extracted with acetonitrile: water (80:20, v/v) whilst samples of fat were extracted with hexane and then partitioned into acetonitrile: water (80:20, v/v). Aliquots of muscle, liver, fat and milk samples were analysed by LC-MS/MS, whilst kidney sample extracts were cleaned up by SPE (solid phase extraction) before undergoing LC-MS/MS analysis. To prepare samples for the analysis of 2,4,6-Trichlorophenol, liver, muscle, kidney and milk were extracted with acetonitrile: water (80:20, v/v) whilst samples of fat were extracted with hexane and then partitioned into acetonitrile: water (80:20, v/v). For all samples, the organic solvent fraction was removed before overnight enzyme hydrolysis (deconjugation), the extracts were then cleaned up by SPE before analysis by LC-MS/MS. For the analysis of the

metabolites SYN548264 and SYN508272 in milk, the samples were extracted with acetonitrile and an aliquot was analysed by LC-MS/MS. The metabolites SYN548263 and SYN547897 were analysed in bovine liver and kidney. The samples were extracted with acetonitrile: water (80:20, v/v) and the organic solvent fraction was removed before overnight enzyme hydrolysis. Extracts were cleaned up by SPE before analysis by LC-MS/MS.

For all matrices, the samples from the high dose rate group were analysed first and if residues above LOQ (0.01 mg/kg) were found, samples from the mid dose rate dose group for that matrix were analysed. The same was done for the low dose group if the mid dose rate dose group showed residues >LOQ. All samples (liver, muscle, kidney, fat and milk) were analysed for pydiflumetofen and 2,4,6-TCP. Samples of milk were analysed for SYN548264 and SYN508272 and samples of liver and kidney for SYN548263 and SYN547897.

Samples of muscle, liver, kidney, fat and milk were analysed for pydiflumetofen using the procedure AP.224292.02 (based on the draft method GRM061.06A) and for 2,4,6-Trichlorophenol (free and conjugated) using procedure AP.287043A.02 (based on method GRM061.07A). The LOQ for these methods is 0.01 mg/kg (KCA1 4.1.2/29 and KCA1 4.1.2/26 respectively, please refer to Volume 3 CA, B.5.1.2). Milk was analysed for SYN548264 and SYN508272 content using the procedure AP.287043B.03 (based on the draft method GRM061.08A). The LOQ for this method is 0.01 mg/kg (KCA1 4.1.2/27, please refer to Volume 3 CA, B.5.1.2). SYN548263 and SYN547897 were measured in liver and kidney by the analytical procedure AP.287043C.02 (based on method GRM061.09A). This method has been validated to an LOQ of 0.01 mg/kg in relevant matrices (KCA1 4.1.2/28, please refer to Volume 3 CA, B.5.1.2). All methods analysed the samples using LC-MS/MS.

Procedural recoveries were provided for each compound being analysed for in each of its associated matrices. For pydiflumetofen and 2,4,6-TCP, both were analysed for in milk, cream, muscle, liver, kidney and fat. The mean recoveries were all acceptable at appropriate fortification levels (0.01 and 0.1 mg/kg). Mean recoveries were acceptable for both SYN508272 and SYN548264 in milk and cream (fortification levels 0.01 and 0.1 mg/kg). Mean procedural recoveries for SYN548263 and SYN547897 were acceptable in the matrices liver and kidney (fortification levels 0.01 and 0.1 mg/kg).

Storage stability of residues (considering the periods of storage of samples in the feeding study)

No storage stability investigations were conducted within the study report. The maximum storage period (at *ca* -20°C) from sampling to analysis was up to 306 days for milk, 317 days for skimmed milk and cream, 323 days for liver, 326 days for kidney, 258 days for muscle and 316 days for fat.

Storage stability studies conducted separately to the feeding study demonstrate the stability of pydiflumetofen in animal matrices for at least up to 24 months when stored at -20°C (██████████ (2016, final 2017)) and the stability of 2,4,6 Trichlorophenol for at least up to 12 months when stored at -18°C (██████████, ██████████. (2016)). Stability of metabolites SYN548264, SYN508272, SYN 547897 and SYN548263 were determined in different matrices in ██████████ (2016).

Residues of SYN508272 and SYN548264 were found to be stable in milk and SYN548263 stable in bovine kidneys for at least up to 12 months.

In the storage stability study, SYN548263 was not tested in liver, but was tested in that matrix in this feeding study. As SYN548263 was found to be stable in kidney for up to 12 months in the storage stability study, it can be assumed, subject to some uncertainty, it will be sufficiently stable in liver for the storage period in the feeding study (≤323 days). The metabolite was not present in liver tissue below the high dose rate dosing level. It is noted that another metabolite, SYN547897, had started in the freezer storage stability study (see below) to show some degradation, both bovine liver and bovine kidney towards the end of the 12 months study.

In the storage stability study (evaluated in section B.7.1.2) SYN547897 (██████████ (2016)) was considered to be sufficiently stable only for up to 9.5 months in bovine liver and only up to 11 months in bovine kidney. The tests were done for up to 12 months, a start of a decline in residues (degradation) of SYN547897 was observed towards the end of the study.

SYN547897 was only determined to be sufficiently stable for up to ~9.5 months in liver in the study by ██████████ (2016) whereas the liver samples being tested for SYN548263 and SYN547897 were stored for up to 10.5 months. SYN547897 was also determined in bovine kidney in this feeding study. SYN547897 was only

determined to be sufficiently stable for up to ~11 months in kidney in the study by [REDACTED] (2016); the kidney samples being tested for SYN547897 were stored for up to 10.5 months (just within the acceptable period).

There is some uncertainty therefore in the estimation of the residue levels of SYN547897 determined in liver in this particular ruminant feeding study. In the following feeding study on ruminants ([REDACTED] and [REDACTED], 2016), the samples are only stored up to a maximum of 9 days (frozen storage). The resultant residues found in liver and kidney samples are fairly similar between the two different feeding studies which use comparable dose rates; thus, it is unlikely that the storage period of 326 days has led to a significant decrease in the residue levels found in the samples.

Extract stability

Within this study, extracts of pydiflumetofen, 2,4,6-TCP, SYN508272, SYN548264, SYN548263 and SYN547897 were stored for a maximum of 6 days (the report did not state whether fridge or freezer) and are therefore covered by the stability of extracts given in reports no. GRM061.06A, GRM061.07A, GRM061.08A and GRM061.09A respectively (extracts stored refrigerated in the method validation work).

The stability of the analytes SYN548264, SYN508272, SYN548263, SYN547897 in final volume solutions was also proven by procedural recovery samples which were stored for the same period of time between extraction and LC-MS/MS analysis. See also section B.7.1.3.

Results and discussion

For all matrices, the samples from the high dose rate group (Group 4) were analysed first and if residues above LOQ (0.01 mg/kg) were found, samples from the mid dose rate group (Group 3) for that matrix were analysed. The same was done for the low dose rate group (Group 2) if the mid dose rate group showed residues >LOQ.

References to determination of 2,4,6-TCP (2,4,6-trichlorophenol) in this study relate to the determination as free and conjugated residues of 2,4,6-trichlorophenol. As noted in section B.5.1.2, the method determines 2,4,6-trichlorophenol after enzyme hydrolysis ‘deconjugating’ the residue, and the method is validated for both free and conjugated 2,4,6-trichlorophenol. However, we know from the metabolism data in ruminants (section B.7.2.3) that these residues of 2,4,6-TCP are expected to be exclusively in the (sulphate) conjugated form. The method of analysis releases the residues in the free form, prior to chromatographic analysis. This step is achieved by enzyme de-conjugation using β -glucuronidase to give de-conjugated 2,4,6-TCP; this same approach was used in the metabolism study to release 2,4,6-TCP from the conjugated form.

Residues in whole milk samples

No residues of pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP), SYN548264 or SYN508272 were found in non-treated control tissue samples at levels at or above the LOQ (0.01 mg/kg).

Magnitude of residues of pydiflumetofen, 2,4,6-TCP (trichlorophenol), SYN548264 and SYN508272 found in whole milk samples collected on days -1 to 28 are summarized in the following tables.

Table 7-88 Pydiflumetofen Residue Data for Bovine Milk

Dose Rate (mg pydiflumetofen /kg feed)		Animal	Study Day										
Target	Actual		-1	1	3	5	7	10	14	17	21	24	28
Control		1 or 11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
15	15.7 (4.1N Tier 1-10 year use; 2.4N Tier 2 long term use)	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
45	47.2	5	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	(11.1N 5.3N Tier 1 – 10 year use; 6.6N Tier 2 long term use)	6	ND	ND	ND	<0.01	<0.01	<0.01	ND	ND	<0.01	ND	<0.01
		7	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mean	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
150	152.5 (44.1N 21N Tier 1 – 10 year use; 26N Tier 2 long term use)	8	ND	<0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.02
		9	ND	<0.01	0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
		10	ND	<0.01	0.01	0.01	0.01	0.01	0.01	<0.01	0.01	0.02	0.01
		Mean	ND	<0.01	0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
		Max	ND	<0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-89 2,4,6-Trichlorophenol (TCP) Residue Data for Bovine Milk

Dose Rate (mg pydiflumetofen /kg feed)		Animal	Study Day										
Target	Actual		-1	1	3	5	7	10	14	17	21	24	28
Control		1 or 11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
15	15.7 (4.1N 1.9N Tier 1 – 10 year use; 2.4N Tier 2 long term use)	2	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		3	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		4	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Mean	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
45	47.2 (11.1N 5.3N Tier 1 – 10 year use; 6.6N Tier 2 long term use)	5	ND	<0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
		6	ND	<0.01	<0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.01
		7	ND	<0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		Mean	ND	<0.01	<0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.02	0.01
		Max	ND	<0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
150	152.5 (44.1N 21N Tier 1 – 10 year use; 26N Tier 2 long term use)	8	ND	0.06	0.09	0.10	0.10	0.09	0.09	0.08	0.08	0.08	0.08
		9	ND	0.06	0.09	0.06	0.10	0.08	0.08	0.09	0.10	0.10	0.08
		10	ND	0.06	0.09	0.08	0.07	0.09	0.08	0.07	0.08	0.08	0.06
		Mean	ND	0.06	0.09	0.08	0.09	0.09	0.08	0.08	0.09	0.09	0.07
		Max	ND	0.06	0.09	0.10	0.10	0.09	0.09	0.09	0.10	0.10	0.08

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-90 SYN548264 Residue Data for Bovine Milk

Dose Rate (mg pydiflumetofen /kg feed)		Animal	Study Day										
Target	Actual		-1	1	3	5	7	10	14	17	21	24	28
Control		1 or 11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
15	15.7 (4.1N Tier 1 -10 year use; 2.4N Tier 2 long term use)	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
45	47.2 (11.1N Tier 1 -10 year use; 6.6N Tier 2 long term use)	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
150	152.5 (44.1N Tier 1 -10 year use; 26N Tier 2 long term use)	8	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		9	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		10	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01
		Mean	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

ND = not detectable (<0.00250 mg/kg)

Table 7-91 SYN508272 Residue Data for Bovine Milk

Dose Rate (mg pydiflumetofen /kg feed)		Animal	Study Day										
Target	Actual		-1	1	3	5	7	10	14	17	21	24	28
Control		1 or 11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
15	15.7 (4.1N Tier 1 -10 year use; 2.4N Tier 2 long term use)	2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
45	47.2 (11.1N Tier 1 -10 year use; 6.6N Tier 2 long term use)	5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		7	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		Mean	-	-	-	-	-	-	-	-	-	-	-
		Max	-	-	-	-	-	-	-	-	-	-	-
150	152.5 (44.1N Tier 1 -10 year use; 26N Tier 2 long term use)	8	ND	ND	ND	ND	ND	ND	<0.01	ND	ND	ND	ND
		9	ND	ND	ND	<0.01	<0.01	<0.01	ND	<0.01	<0.01	ND	<0.01
		10	ND	ND	ND	<0.01	ND	<0.01	<0.01	ND	<0.01	<0.01	<0.01
		Mean	ND	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		Max	ND	ND	ND	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)
 ND = not detectable (<0.00250 mg/kg)

Mean residues of pydiflumetofen in whole milk at the high dose rate feeding level (Group 4) were <LOQ (0.01 mg/kg) on Study Days 1, 10, 14, 17 and 21. On Study Days 3, 5, 7, 24 and 28 the mean residues of pydiflumetofen were 0.01 mg/kg. On Study Days 21, 24 and 28 the maximum residues of pydiflumetofen in whole milk at the high dose rate feeding level were 0.02 mg/kg. At the mid dose rate (Group 3), no residues of pydiflumetofen at or above the LOQ were detected. Residues of pydiflumetofen reached a plateau concentration in whole milk at approximately 3 days after dosing began.

Positive residues of 2,4,6-TCP were detected in all samples of whole milk analysed from animals at the high dose rate dose level (Group 4). Mean residue levels were between 0.06 - 0.09 mg/kg and the maximum level detected in any one animal was 0.10 mg/kg. Mean residue levels in Group 3 (mid dose rate) ranged from <LOQ to 0.02 mg/kg, with a maximum level of 0.02 mg/kg in any individual sample. Study Day 1 was the only day where mean and individual samples of whole milk were <LOQ. All samples showed residues <LOQ for Group 2 animals (low dose rate). Residues of 2,4,6-trichlorophenol reached a plateau concentration in whole milk at approximately 3-5 days after dosing began.

No residues of SYN548264 at or above the LOQ were detected in samples from animals in the high dose rate dosing group (Group 4) with the exception of one sample on the 28th day of the study which showed results at the LOQ.

No residues of SYN508272 at or above the LOQ were detected in samples from animals in the high dose rate dosing group (Group 4).

Given the low residues (<LOQ) of SYN548264 and SYN508272, an estimation of the time to reach a plateau cannot be made.

Residues in skimmed milk samples

Magnitude of residues of pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP), SYN548264 and SYN508272 found in skimmed milk samples generated from milk collected on days 14 to 28 are summarized in the following tables.

Mean residues of pydiflumetofen in skimmed milk samples from the high dose rate dose group (Group 4) were <LOQ (0.01 mg/kg) on Study Day 14 and not detectable (<0.00250 mg/kg) on Study Day 28.

At the high dose rate dose group (Group 4) mean residues of 2,4,6-TCP in skimmed milk were 0.08 mg/kg on both Study days 14 and 28. For Group 3 (mid dose rate) and Group 2 (low dose rate) animals, skimmed milk samples on both study days (14 and 28) had mean residues of 0.01 mg/kg and <0.01 mg/kg respectively.

Mean residues of SYN548264 were below the LOQ (<0.01 mg/kg) on Study Days 14 and 28 for dose Groups 4 (high dose rate) and 3 (mid dose rate). Residues of SYN548264 were not detectable in skimmed milk samples from Group 2 animals on Study Days 14 or 28.

Mean residues of SYN508272 were <LOQ (0.01 mg/kg) on Study Day 14 and 28 for Group 4 (high dose rate).

Table 7-92 Residue Data for Bovine Skimmed Milk

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6- Trichlorophenol (mg/kg)			SYN548264 (mg/kg)			SYN508272 (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max
Control		14	1	ND	-	-	ND	-	-	NA	-	-	ND	-	-
		28	11	ND	-	-	ND	-	-	NA	-	-	ND	-	-
15		14	2	NA	-	-	<0.01			ND	ND	ND	NA	-	-

	15.7 (4.1N 1.9N Tier 1 10 year use; 2.4N Tier 2 long term use)	28	3	NA	-	-	<0.01	<0.0 1	<0.0 1	ND	ND	ND	NA	-	-
			4	NA			<0.01			ND			NA		
			2	NA			<0.01			ND			NA		
			3	NA			<0.01			ND			NA		
	47.2 (11.1N 5.3N Tier 1 10 year use; 6.6N Tier 2 long term use)	14	5	NA	-	-	0.01	0.01	0.01	<0.01	<0.0 1	<0.0 1	NA	-	-
			6	NA			0.01			<0.01			NA		
			7	NA			0.01			<0.01			NA		
			5	NA			0.01			<0.01			NA		
	152.5 (44.1N 21N Tier 1-10 year use; 26N Tier 2-long term use)	28	6	NA	-	-	0.01	0.01	0.01	<0.01	<0.0 1	<0.0 1	NA	-	-
			7	NA			0.01			ND			NA		
			8	ND			0.08			<0.01			<0.01		
			9	ND			0.08			<0.01			<0.01		
	150	14	10	<0.01	<0.0 1	<0.0 1	0.07	0.08	0.08	<0.01	<0.0 1	<0.0 1	<0.01	<0.0 1	<0.0 1
			8	ND			0.06			0.01			<0.01		
			9	ND			0.09			<0.01			<0.01		
			10	ND			0.08			0.01			<0.01		

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)
 ND = not detectable (<0.00250 mg/kg)

Residues in cream

Magnitude of residues of pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP), SYN548264 and SYN508272 found in cream samples generated from milk collected on days 14 to 28 are summarized in the following tables.

Mean residues of pydiflumetofen in cream samples were 0.13 mg/kg, 0.03 mg/kg and 0.01 mg/kg from the high dose rate (Group 4), mid dose rate (Group 3) and low dose rate groups (Group 2) respectively, on Study Day 14. Results from Study Day 28 were similar for all dose groups, with levels of 0.16 mg/kg, 0.04 mg/kg and 0.01 mg/kg for the high dose rate, mid dose rate and low dose rate groups, respectively.

Mean residues of 2,4,6-TCP in cream samples were 0.06 mg/kg, 0.02 mg/kg and <0.01 mg/kg from the high dose rate (Group 4), mid dose rate (Group 3) and low dose rate groups (Group 2) respectively, on Study Day 14. Mean residues of TCP in cream samples measured on Study Day 28 were 0.05 mg/kg, 0.02 mg/kg and <0.01 mg/kg for the high dose rate, mid dose rate and low dose rate groups, respectively.

Mean residues of SYN548264 were <LOQ (0.01 mg/kg) on Study Day 14 and 28 for Group 4 (high dose rate).

Mean residues of SYN508272 were not detectable (<0.00250 mg/kg) on Study Days 14 and 28 for Group 4 (high dose rate).

Table 7-93 Residue Data for Bovine Cream

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)			SYN548264 (mg/kg)			SYN508272 (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max
Control		14	1	ND	-	-	ND	-	-	ND	-	-	ND	-	-
		28	11	ND	-	-	ND	-	-	ND	-	-	ND	-	-
15	15.7 (4.1N 1.9N Tier 1 10-year use; 2.4N Tier 2 long term use)	14	2	0.01	0.01	0.01	<0.01	<0.01	0.02	NA	-	-	NA	-	-
			3	0.01			<0.01			NA			NA		
			4	0.01			0.02			NA			NA		
		28	2	0.01	0.01	0.01	<0.01	<0.01	0.01	NA	-	-	NA	-	-
			3	0.01			<0.01			NA			NA		
			4	0.01			0.01			NA			NA		
45	47.2 (11.1N 5.3N Tier 1 10-year use; 6.6N Tier 2 long term use)	14	5	0.04	0.03	0.04	0.02	0.02	0.02	NA	-	-	NA	-	-
			6	0.02			0.02			NA			NA		
			7	0.03			0.02			NA			NA		
		28	5	0.04	0.04	0.04	0.02	0.02	0.02	NA	-	-	NA	-	-
			6	0.03			0.02			NA			NA		
			7	0.04			0.02			NA			NA		
150	152.5 (44.1N 21N Tier 1 10-year use; 26N Tier 2 long term use)	14	8	0.14	0.13	0.14	0.06	0.06	0.06	<0.01	<0.01	<0.01	ND	ND	ND
			9	0.12			0.06			<0.01			ND		
			10	0.14			0.06			<0.01			ND		
		28	8	0.20	0.16	0.20	0.05	0.05	0.05	<0.01	<0.01	<0.01	ND	ND	ND
			9	0.14			0.05			<0.01			ND		
			10	0.13			0.05			<0.01			ND		

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)
 ND = not detectable (<0.00250 mg/kg)

Residues in muscle

Muscle samples (composite of loin and hind-leg) collected following 28 days of dosing were analysed for residues of pydiflumetofen and 2,4,6-trichlorophenol (2,4,6-TCP). These are summarized in Table 7-94.

Mean and maximum residues of pydiflumetofen found in muscle were <0.01 mg/kg in the high dose rate and mid dose rate groups. For 2,4,6-TCP, mean and maximum residues were <0.01 mg/kg in the high dose rate dose group.

Table 7-94 Residue Data for Bovine Muscle

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max

Control		28	11	ND	-	-	ND	-	-
15	15.7 (4.1N Tier 1 – 10 year use; 2.4N Tier 2 – long term use)	28	2	NA	-	-	NA	-	-
			3	NA			NA		
			4	NA			NA		
45	47.2 (11.1N Tier 1 – 10 year use; 6.6N Tier 2 – long term use)	28	5	<0.01	<0.01	<0.01	NA	-	-
			6	<0.01			NA		
			7	<0.01			NA		
150	152.5 (44.1N Tier 1 – 10 year use; 26N Tier 2 – long term use)	28	8	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
			9	<0.01			<0.01		
			10	<0.01			<0.01		

NA

= not analysed (<LOQ for the previous dose group)

ND

= not detectable (<0.00250 mg/kg)

<0.01

= <LOQ, lower limit of quantification (0.01 mg/kg)

Residues in liver

As noted above, there is some uncertainty in the determination of SYN547897 in this particular feeding study. In the below study (■■■■ and ■■■■, 2016), the samples were analysed quickly to provide valid results on the levels of residues, taking account of the need not to store samples with residues of SYN547897 for long periods in the freezer prior to analysis.

Liver samples collected following 28 days of dosing were analysed for residues of pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP), SYN547897 and SYN548263. These are summarized in Table 7-95.

Mean residues of pydiflumetofen in liver were 0.09 mg/kg in the high dose rate dose group, 0.04 mg/kg in the mid dose rate group and 0.01 mg/kg in the low dose rate group. The maximum residues were 0.12 mg/kg (high dose rate), 0.05 mg/kg (mid dose rate) and 0.02 mg/kg (low dose rate).

Mean residues of 2,4,6-trichlorophenol were 0.07 mg/kg for the high dose rate dose group, 0.03 mg/kg for the mid dose rate group and <LOQ (0.01 mg/kg) in the low dose rate group. The maximum residues were 0.08 mg/kg (high dose rate), 0.03 mg/kg (mid dose rate) and <LOQ (low dose rate).

Mean residues of SYN547897 were 0.56 mg/kg in the high dose rate dose group, 0.22 mg/kg in the mid dose rate group and 0.04 mg/kg in the low dose rate group. Maximum residues were 0.59 mg/kg (high dose rate), 0.36 mg/kg (mid dose rate) and 0.06 mg/kg (low dose rate).

At the high dose rate dose group, all residues of SYN548263 were <LOQ (0.01 mg/kg). Residues of SYN548263 were not detectable (<0.00250 mg/kg) in samples from the mid dose rate feeding group.

Table 7-95 Residue Data for Bovine Liver

Dose Rate	Study Day	Animal	pydiflumetofen (mg/kg)	2,4,6-Trichlorophenol (mg/kg)	SYN547897 (mg/kg)	SYN548263 (mg/kg)
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(mg pydiflumetofen /kg feed)															
Target	Actual			Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max
Control		28	11	ND	-	-	ND	-	-	ND	-	-	ND	-	-
15	15.7 (4.1N Tier 1-10 year use; 2.4N Tier 2 long term use)	28	2	0.01	0.01	0.02	<0.01	<0.01	<0.01	0.02	0.04	0.06	NA	-	-
			3	0.02			<0.01			0.04			NA		
			4	0.01			<0.01			0.06			NA		
45	47.2 (11.1N Tier 5.3N Tier 1-10 year use; 6.6N Tier 2 long term use)	28	5	0.05	0.04	0.05	0.03	0.03	0.03	0.36	0.19	0.10	ND	ND	ND
			6	0.04			0.02			0.19			ND		
			7	0.03			0.03			0.10			ND		
150	152.5 (44.1N Tier 21N Tier 1-10 year use; 26N Tier 2 long term use)	28	8	0.09	0.09	0.12	0.06	0.07	0.08	0.56	0.59	0.52	<0.01	<0.01	<0.01
			9	0.12			0.08			0.59			<0.01		
			10	0.07			0.08			0.52			<0.01		

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)
 ND = not detectable (<0.00250 mg/kg)

Residues in kidney

As noted above, there is some uncertainty in the determination of SYN547897 in this particular feeding study for liver. In the below study (■■■■ and ■■■■, 2016), the samples of both kidney and liver were analysed quickly to provide valid results on the levels of residues, taking account of the need not to store samples with residues of SYN547897 for long periods in the freezer prior to analysis.

Kidney samples collected following 28 days of dosing were analysed for residues of pydiflumetofen, 2,4,6-trichlorophenol (2,4,6-TCP), SYN547897 and SYN548263. These are summarized in Table 7-96.

Mean residues of pydiflumetofen in kidney were 0.02 mg/kg in the high dose rate dose group and <LOQ (0.01 mg/kg) in the mid dose rate group. Maximum residues were 0.03 mg/kg (high dose rate) and <LOQ (mid dose rate).

Mean residues of 2,4,6-trichlorophenol were 0.17 mg/kg for the high dose rate dose group, 0.05 mg/kg for the mid dose rate group and 0.01 mg/kg for the low dose rate group. Maximum residues were 0.21 mg/kg (high dose rate), 0.05 mg/kg (mid dose rate) and 0.01 mg/kg (low dose rate).

Mean residues of SYN547897 were 0.41 mg/kg in high dose rate dose group, 0.17 mg/kg in the mid dose rate dose group and 0.05 mg/kg in the low dose rate group. Maximum residues were 0.58 mg/kg (high dose rate), 0.24 mg/kg (mid dose rate) and 0.06 mg/kg (low dose rate).

Mean residues of SYN548263 were 0.08 mg/kg in the high dose rate dose group, 0.02 mg/kg in the mid dose rate group and <LOQ (0.01 mg/kg) in the low dose rate group. The maximum residues were 0.10 mg/kg (high dose rate), 0.02 mg/kg (mid dose rate) and <LOQ (low dose rate).

Table 7-96 Residue Data for Bovine Kidney

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)			SYN547897 (mg/kg)			SYN548263 (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max	Individual	Mean	Max
Control		28	11	ND	-	-	ND	-	-	ND	-	-	ND	-	-
15	15.7 (4.1N Tier 1 – 10 year use; 2.4N Tier 2 long term use)	28	2	NA	NA	NA	0.01	0.01	0.01	0.03	0.05	0.06	<0.01	<0.01	<0.01
			3	NA			0.01			0.06			<0.01		
			4	NA			0.01			0.06			<0.01		
45	47.2 (11.1N Tier 1 – 10 year use; 6.6N Tier 2 long term use)	28	5	<0.01	<0.01	<0.01	0.05	0.05	0.05	0.24	0.17	0.24	0.02	0.02	0.02
			6	<0.01			0.05			0.12			0.02		
			7	<0.01			0.04			0.15			0.02		
150	152.5 (44.1N Tier 1 – 10 year use; 26N Tier 2 long term use)	28	8	0.03	0.02	0.03	0.14	0.17	0.21	0.31	0.41	0.58	0.07	0.08	0.10
			9	0.02			0.21			0.58			0.10		
			10	0.01			0.17			0.36			0.07		

NA = not analysed (<LOQ for the previous dose group) <0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)
 ND = not detectable (<0.00250 mg/kg)

Residues in fat

Fat samples (subcutaneous, perirenal and mesenteric) collected following 28 days of dosing were analysed for residues of pydiflumetofen and 2,4,6-trichlorophenol (2,4,6-TCP). These are summarized in Table 7-97, Table 7-98 and Table 7-99.

Subcutaneous fat

Mean residues of pydiflumetofen in subcutaneous fat were <0.05 mg/kg in the high dose rate group, <0.03 mg/kg in the mid dose rate group and <LOQ (0.01 mg/kg) in the low dose rate group. Maximum residues of pydiflumetofen were 0.11 mg/kg (high dose rate), 0.04 mg/kg (mid dose rate) and 0.02 mg/kg (low dose rate).

Mean overall residues and maximum individual residues of 2,4,6-trichlorophenol (2,4,6-TCP) in subcutaneous fat were <LOQ (0.01 mg/kg) in the high dose rate group.

Table 7-97 Residue Data for Bovine Subcutaneous Fat

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	ND	-	-	ND	-	-
15	15.7 (4.1N Tier 1 – 10 year use; 2.4N Tier 2 long term use)	28	2	<0.01	<0.01	0.02	NA	NA	NA
			3	<0.01			NA		
			4	0.02			NA		

45	47.2 (11.1N 5.3N Tier 1 10 year use; 6.6N Tier 2 long term use)	28	5	<0.01	0.02	0.04	NA	NA	NA
			6	0.04			NA		
			7	0.03			NA		
150	152.5 (44.1N 24N Tier 1 10 year use; 26N Tier 2 long term use)	28	8	0.11	0.05	0.11	<0.01	<0.01	<0.01
			9	0.03			<0.01		
			10	<0.01			<0.01		

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Perirenal fat

Mean residues of pydiflumetofen in perirenal fat were 0.08 mg/kg in the high dose rate group, 0.05 mg/kg in the mid dose rate dose group and 0.01 mg/kg in the low dose rate group. Maximum residues were 0.11 mg/kg, 0.06 mg/kg and 0.01 mg/kg in the high dose rate, mid dose rate and low dose rate groups, respectively.

Mean residues of 2,4,6-trichlorophenol (TCP) in perirenal fat were <LOQ (0.01 mg/kg) in the high dose rate and mid dose rate groups and 0.01 mg/kg was the maximum residue for both dose groups. Residues of 2,4,6-TCP were not detectable (<0.00250 mg/kg) in the low dose rate group.

Table 7-98 Residue Data for Bovine Perirenal Fat

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	ND	-	-	ND	-	-
15	15.7 (4.1N 1.9N Tier 1 10 year use; 2.4N Tier 2 long term use)	28	2	0.01	0.01	0.01	ND	ND	ND
			3	0.01			ND		
			4	0.01			ND		
45	47.2 (11.1N 5.3N Tier 1 10 year use; 6.6N Tier 2 long term use)	28	5	0.06	0.05	0.06	<0.01	<0.01	0.01
			6	0.04			ND		
			7	0.04			0.01		
150	152.5 (44.1N 21N Tier 1 10 year use; 26N Tier 2 long term use)	28	8	0.11	0.08	0.11	<0.01	<0.01	0.01
			9	0.07			0.01		
			10	0.05			<0.01		

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Mesenterial fat

Mean residues of pydiflumetofen in mesenterial fat were 0.10 mg/kg in the high dose rate group, 0.05 mg/kg in the mid dose rate group and 0.01 mg/kg in the low dose rate group. Maximum residues were 0.17 mg/kg, 0.06 mg/kg and 0.02 mg/kg in the high dose rate, mid dose rate and low dose rate groups, respectively.

Mean residues of 2,4,6-trichlorophenol (2,4,6-TCP) were <LOQ (0.01 mg/kg) in the high dose rate feeding group. Within this dose group, one animal had 2,4,6-TCP levels <LOQ whilst residue levels were not detectable (<0.00250 mg/kg) in the remaining animals.

Table 7-99 Residue Data for Bovine Mesenterial Fat

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	pydiflumetofen (mg/kg)			2,4,6-Trichlorophenol (mg/kg)		
Target	Actual			Individual	Mean	Max	Individual	Mean	Max
Control		28	11	ND	-	-	ND	-	-
15	15.7 (4.1N Tier 1-10 year use; 2.4N Tier 2 long term use)	28	2	0.01	0.01	0.02	NA	-	-
			3	0.01			NA		
			4	0.02			NA		
45	47.2 (11.1N Tier 1-10 year use; 6.6N Tier 2 long term use)	28	5	0.06	0.05	0.06	NA	-	-
			6	0.04			NA		
			7	0.05			NA		
150	152.5 (44.1N Tier 1-10 year use; 26N Tier 2 long term use)	28	8	0.17	0.10	0.17	ND	<0.01	<0.01
			9	0.07			ND		
			10	0.05			<0.01		

NA = not analysed (<LOQ for the previous dose group)

ND = not detectable (<0.00250 mg/kg)

<0.01 = <LOQ, lower limit of quantification (0.01 mg/kg)

Conclusion

Mean and highest residues of pydiflumetofen found in bovine commodities from each dose group are summarised in Table 7-100 below.

Positive residues of (parent) pydiflumetofen were found in the commodities; whole milk, cream, liver, kidney and fat (subcutaneous, perirenal and mesenterial) at the highest dose level. Residues of pydiflumetofen were found at levels <LOQ (0.01 mg/kg) in skimmed milk and muscle at the high dose rate and in muscle, kidney and whole milk at the mid dose rate level. Residues ≥LOQ were found in cream, liver and subcutaneous, perirenal and mesenterial fat at the mid dose rate and low dose rate feeding levels.

Table 7-100 Mean and Highest Residues of pydiflumetofen in Bovine Commodities

Matrix	pydiflumetofen Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole milk (days -1 to 28)	NA	NA	<0.01	<0.01	0.01	0.02
Skimmed milk (days 14 and 21)	NA	NA	NA	NA	<0.01	<0.01
Cream (days 14 and 21)	0.01	0.01	0.04	0.04	0.15	0.20
Muscle	NA	NA	<0.01	<0.01	<0.01	<0.01
Liver	0.01	0.02	0.04	0.05	0.09	0.12
Kidney	NA	NA	<0.01	<0.01	0.02	0.03
Fat – subcutaneous	<0.01	0.02	0.02	0.04	0.05	0.11
Fat – perirenal	0.01	0.01	0.05	0.06	0.08	0.11
Fat – mesenterial	0.01	0.02	0.05	0.06	0.10	0.17

NA – not analysed

Mean and highest residues of 2,4,6-Trichlorophenol found in bovine commodities from each dose group are summarised in Table 7-101 below.

Positive residues of 2,4,6-Trichlorophenol were found in the commodities; whole milk, skimmed milk, cream, liver, kidney and perirenal fat at the highest dose level and the mid dose rate level. Residues of 2,4,6-Trichlorophenol were found at levels <LOQ (0.01 mg/kg) in muscle and subcutaneous and mesenterial fat at the high dose rate.

At the low dose rate feeding level, residues \geq LOQ (0.01 mg/kg) were found in cream and kidney. The remaining samples which were analysed (residues at higher feeding level \geq LOQ) were <LOQ.

Table 7-101 Mean and Highest Residues of 2,4,6-Trichlorophenol in Bovine Commodities

Matrix	2,4,6-Trichlorophenol Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole milk (days -1 to 28)	<0.01	<0.01	0.01	0.02	0.08	0.10
Skimmed milk (days 14 and 21)	<0.01	<0.01	0.01	0.01	0.08	0.09
Cream (days 14 and 21)	<0.01	0.02	0.02	0.02	0.06	0.06
Muscle	NA	NA	NA	NA	<0.01	<0.01
Liver	<0.01	<0.01	0.03	0.03	0.07	0.08
Kidney	0.01	0.01	0.05	0.05	0.17	0.21
Fat – subcutaneous	NA	NA	NA	NA	<0.01	<0.01
Fat – perirenal	ND	ND	<0.01	0.01	0.01	0.01
Fat – mesenterial	NA	NA	NA	NA	<0.01	<0.01

NA – not analysed

ND – not detected (< 0.00250 mg/kg)

Mean and highest residues of SYN548264 and SYN508272 found in whole milk, skimmed milk and cream from each dose group are summarised in Table 7-102 and Table 7-103 respectively.

Highest residues of SYN548264 were found at 0.01 mg/kg in whole milk and skimmed milk at the high dose rate. Whole milk and cream were not analysed at the mid dose rate and low dose rate levels. In skimmed milk, residues were <LOQ (<0.01 mg/kg) and not detectable (< 0.00250 mg/kg) at the mid dose rate and low dose rate levels respectively.

Table 7-102 Mean and Highest Residues of SYN548264 in Bovine Commodities

Matrix	SYN548264 Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole milk (days -1 to 28)	NA	NA	NA	NA	<0.01	0.01
Skimmed milk (days 14 and 28)	ND	ND	<0.01	<0.01	<0.01	0.01
Cream (days 14 and 28)	NA	NA	NA	NA	<0.01	<0.01

NA – not analysed

ND – not detected (< 0.00250 mg/kg)

At the high dose rate, residues of SYN508272 were found at <LOQ (<0.01 mg/kg) in whole milk and skimmed milk and were not detected (< 0.00250 mg/kg) in cream.

Table 7-103 Mean and Highest Residues of SYN508272 in Bovine Commodities

Matrix	SYN508272 Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Whole milk (days -1 to 28)	NA	NA	NA	NA	<0.01	<0.01
Skimmed milk (days 14 and 28)	NA	NA	NA	NA	<0.01	<0.01
Cream (days 14 and 28)	NA	NA	NA	NA	ND	ND

NA – not analysed

ND – not detected (< 0.00250 mg/kg)

As noted above, there is some uncertainty in the determination of SYN547897 in this particular feeding study for liver. In the below study (██████ and ██████, 2016), the samples of both kidney and liver were analysed quickly to provide valid results on the levels of residues, taking account of the need not to store samples with residues of SYN547897 for long periods in the freezer prior to analysis.

At the high dose rate, mid dose rate and low dose rate dose level, residues of SYN547897 were found at >LOQ (>0.01 mg/kg) in liver and kidney tissue samples. This is shown in Table 7-104 below.

Table 7-104 Mean and Highest Residues of SYN547897 in Bovine Commodities

Matrix	SYN547897 Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Liver	0.04	0.06	0.22	0.36	0.56	0.59
Kidney	0.05	0.06	0.17	0.24	0.41	0.58

At the high dose rate and mid dose rate level, residues of SYN548263 were found at >LOQ (>0.01 mg/kg) in kidney tissue samples. Residues were <LOQ in liver at high dose rate and in kidney at the low dose rate group. This is shown in Table 7-105 below.

Table 7-105 Mean and Highest Residues of SYN548263 in Bovine Commodities

Matrix	SYN548263 Residue (mg/kg)					
	Group 2 (low dose, 15.7 mg/kg)		Group 3 (mid dose, 47.2 mg/kg)		Group 4 (high dose, 152.5 mg/kg)	
	Mean	Highest	Mean	Highest	Mean	Highest
Liver	NA	NA	ND	ND	<0.01	<0.01
Kidney	<0.01	<0.01	0.02	0.02	0.08	0.10

Report:	K-CA 6.4.2/01, [REDACTED] [REDACTED] (2016)
Title:	SYN545974 – Magnitude of Residues in Tissues of Dairy Cows Following Multiple Oral Administrations of SYN545974
Report No:	Report No. 37460
Document No:	Document No. VV-465348 (Syngenta File No. SYN545974_10421)
Guidelines:	Regulation (EC) No 1107/2009 of the European Parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. OECD guideline 505 for testing of chemicals “Residues in Livestock”, 08-Jan-2007.
Guideline deviations:	None
GLP:	Yes

Materials and methods

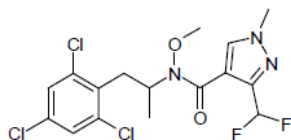
Materials

Pydiflumetofen

Description: SYN545974

Lot/Batch #: SMU2EP12007

Purity: 98.5% active substance in technical material



Methods

The magnitude of residues of the pydiflumetofen (SYN545974) metabolite SYN547897 in ruminant tissue (liver and kidney) was investigated following the administration of pydiflumetofen via gelatine capsules to ruminants for a period of 28 days. Non-pregnant, lactating, dairy cattle (breed *Ayrshire* and *Holstein/Friesian*) were fed gelatine capsules containing pydiflumetofen at three dosing levels of approximately 15 ppm, 45 ppm and 150 ppm (mg per kg) of dry matter (DM) feed.

A total of 11 dairy cows were selected for the study, of these, 9 were fed with pydiflumetofen via gelatine capsules whilst one control cow received gelatine dosing capsules containing no active substance. The 9 cows being given gelatine capsules containing pydiflumetofen were split into a total of 3 dosing groups: 15 mg pydiflumetofen /kg DM (Group 2, low dose rate), 45 mg pydiflumetofen /kg DM (Group 3, mid dose rate), and 150 mg pydiflumetofen /kg DM (Group 4, high dose rate). For each animal, the mean daily dose level achieved was within 2% of the target dose level. The mean daily dose levels per group, calculated on a body weight basis, was calculated as 0.40 (Group 2, low dose rate), 1.1 (Group 3, mid dose rate) and 3.4 (Group 4, high dose rate) mg pydiflumetofen /kg body weight/day. Details of the study outline are summarized in Table 7-106. The cattle were acclimatised for at least 5 days prior to dosing. The cattle were all fed commercially available concentrate twice daily and meadow hay and water were offered to the animals *ad libitum* per treatment group pen. Group hay and concentrate consumption were recorded daily during acclimatisation and dosing and the moisture content of each diet type (hay and concentrate) was determined once during the acclimatisation period.

Animals were assessed for general health and food consumption, and weights of the animals recorded over the course of the study. On one occasion, the initial dosing of the treated animal was not successful, but this was then managed as a follow up immediately post the initial attempt at dosing.

Table 7-106 Consumption of pydiflumetofen by lactating cattle

Dose (mg/kg dm)	Group	Number of cows	Animal	Achieved dose of pydiflumetofen (mg/kg dm) ¹	Average Body Weight (kg) ²	Average Dose pydiflumetofen (mg/kg body weight/day) ³	N rate (Tier 1 10 year use)	N rate (Tier 2 long term use)
0	1	1	1, 2	0	542	0	-	-
15	2 (low dose rate)	3	3, 4, 5	15.20	587	0.40	4.1N 1.9N	2.4N
45	3 (mid dose rate)	3	6, 7, 8	44.20	685	1.10	11.2N 5.3N	6.6N
150	4 (high dose rate)	3	9, 10, 11	149.90	737	3.40	34.7N 16N	20N

¹Calculation of actual dose levels - based on amount of test item dispensed into each capsule and actual mean food consumption (dry matter intake) for each animal (per treatment group) during the dosing period.

²Average of the body weights of all cows per dose group (over study days -7/-6, -1, 7/8, 14/15, 21/22 and day 28)

³Calculated Average Dose (mg/kg bw/day) = mg pydiflumetofen consumed per day (mg/day)/average body weights (kg).

Milking was done twice per day (am and pm) throughout the acclimatisation and study period and milk samples were weighed. Samples from the pm milking were combined with the following am milk sample to give a 24 hr bulk sample. Milk collected on study days -1, 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28 were kept in case analysis for residues of pydiflumetofen and metabolites was required. After the appropriate subsamples and aliquots had been taken, the remaining bulk samples from study days 14 and 28 were retained to produce skimmed milk and cream.

All cattle were euthanised after the 28 days of dosing, within *ca* 22-24 h of the last morning dose. Edible tissues (loin and hind-leg muscle, liver, kidney, perirenal, mesenteric and subcutaneous fat) were removed postmortem and weighed. Once weighed, all samples were stored whole in a freezer set at -20°C. All samples of liver and kidney taken were of an acceptable size in accordance with OECD 505.

Analysis/general procedures

Samples of liver and kidney were homogenised frozen with the aid of dry ice to form a fine powder and were returned to frozen storage.

For analysis of SYN547897 residues, samples of liver and kidney were extracted with acetonitrile: water (80:20, v/v) and the organic solvent fraction was removed before overnight enzyme hydrolysis (deconjugation). Extracts were then cleaned up by SPE before analysis by LC-MS/MS. Residues of SYN547897 were measured in the tissues by analytical procedure AP.287043C.02 (based on method GRM061.09A). This method has been validated to an LOQ of 0.01 mg/kg in relevant matrices (KCA1 4.1.2/28, please refer to Volume 3 CA, B.5.1.2).

Procedural recoveries were provided for SYN547897 in both liver and kidney; the mean recoveries were acceptable (84 and 89% for liver and kidney respectively) and were done for suitable fortification levels (0.01 and 0.1 mg/kg).

No storage stability investigations were conducted within the study report. The storage period (at *ca* -20°C) from sampling to analysis was up to 9 days in liver and kidney. In accordance with OECD 505, if samples are analysed within 30 days, demonstration of storage stability is not required. In addition, a separate storage stability study (██████████ (2016)), SYN547897 was determined to be sufficiently stable in liver for only up to ~9.5 months and in kidney for only up to 11 months, therefore the storage time for this feeding study samples is also supported by this freezer storage stability study. This study was performed (analysing the samples quickly) to obtain valid residue levels of SYN547897 taking account of the potential storage stability concerns. As noted below, the results for levels of SYN547897 in liver and kidney from this study were of a fairly similar level to those determined in the above study (██████████ ██████████ ██████████ (2017)).

The maximum time period between extraction and analysis of the samples was 5 days and is therefore covered by the stability of extracts given in report no. GRM061.09A. As discussed above, procedural recoveries were analysed alongside the study samples and were acceptable.

Results and discussion

Liver and kidney samples collected following 28 days of dosing were analysed for residues of SYN547897. These are summarized in Table 7-107 and Table 7-108 respectively.

Residues in liver

Residues in the control samples were <0.01 mg/kg.

Mean SYN547897 levels were 0.60 mg/kg in the highest dose group (high dose rate), 0.25 mg/kg in the mid dose rate group and 0.06 mg/kg in the lowest dose group (low dose rate). The maximum residues were 1.00 mg/kg in the high dose rate dose group, 0.33 mg/kg in the mid dose rate group and 0.07 mg/kg in the low dose rate dose group.

Table 7-107 Residues of SYN547897 in liver

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	SYN547897 (mg/kg)		
Target	Actual			Individual	Mean	Max
Control		28	1	<0.01		
15	15.2 (4.1N Tier 1-10 year use; 2.4N Tier 2 long term use)	28	3	0.04	0.06	0.07
			4	0.07		
			5	0.07		
45	44.2 (11.2N 5.3N Tier 1-10 year use; 6.6N Tier 2 long term use)	28	6	0.19	0.25	0.33
			7	0.23		
			8	0.33		
150	149.9 (34.7N 16N Tier 1-10 year use; 20N Tier 2 long term use)	28	9	0.30	0.60	1.0
			10	0.51		
			11	1.00		

Residues in kidney

Residues in the control samples were <0.01 mg/kg.

Mean residues of SYN547897 were 0.44 mg/kg in the highest dose group, 0.17 mg/kg in the mid dose rate group and 0.08 mg/kg in the lowest dose group. Maximum residues were 0.49 mg/kg in the high dose rate group, 0.19 mg/kg in the mid dose rate group and 0.09 mg/kg in the low dose rate dose group.

Table 7-108 Residues of SYN547897 in kidney

Dose Rate (mg pydiflumetofen /kg feed)		Study Day	Animal	SYN547897 (mg/kg)		
Target	Actual			Individual	Mean	Max
Control		28	1	<0.01		
15	15.2 (4.1N Tier 1-10 year use; 2.4N Tier 2 long term use)	28	3	0.09	0.08	0.09
			4	0.08		
			5	0.06		

	Tier 2 long term use)					
45	44.2 (11.2N 5.3N Tier 1 10 year use; 6.6N Tier 2 long term use)	28	6	0.19	0.17	0.19
			7	0.14		
			8	0.17		
150	149.9 (34.7N 46N Tier 1 10 year use; 20N Tier 2 long term use)	28	9	0.41	0.44	0.49
			10	0.49		
			11	0.41		

Conclusion

At the high dose rate, mid dose rate and low dose rate levels, residues of SYN547897 were found at >LOQ (>0.01 mg/kg) in all liver and kidney tissue samples.

The residues found in this study are of a fairly similar magnitude to those observed in the previous feeding study on ruminants.

B.7.4.3. Pigs

A feeding study on swine has not been submitted, but is not required due to the similar metabolic pathway demonstrated between rats and ruminants (see B.7.2.4).

B.7.4.4. Fish

Currently no test method or guidance document is available. It is considered that residues in fish do not need to be addressed in the current evaluation.

Please refer to section B.7.2.5 for further information relating to residues in fish. The requirement for fish feeding studies can only be considered once metabolism in fish has been addressed.

B.7.5. EFFECTS OF PROCESSING

B.7.5.1. Nature of the residue

The effect of processing on the nature of the residues of pydiflumetofen was investigated using the active substance radiolabelled in the Pyrazole-5-¹⁴C position. The labelling position is considered appropriate to provide sufficient information given that pydiflumetofen was demonstrated to be hydrolytically stable. The molecular structure and position of the ¹⁴C label in the test items used in the studies is shown in Table 7-109.

Report: KCA1 6.5.1; [REDACTED]; 2014
Title: [14 C] SYN545974 - Aqueous Hydrolysis at 90, 100 and 120°C
Report No.: 35072
Document No.: VV-410228, SYN545974_10110
Guidelines: OECD Guidelines for the Testing of Chemicals, Nature of the Pesticide Residues in Processed Commodities – High Temperature Hydrolysis, Guideline 507, 16th October 2007.
Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market.
Commission Regulation (EU) No. 283/2013 of 1 March 2013 setting out the 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.
Guideline deviations: A total of two deviations have been listed, these have all been considered to have no impact on the conduct or quality of the study
GLP/GEP: Yes

Summary

The aim of the study was to investigate the hydrolytic stability of [Pyrazole-5-¹⁴C]- pydiflumetofen under conditions representative of pasteurisation, baking, brewing, boiling and sterilisation, and to identify/characterise any major components produced. The conditions are summarised below:

Temperature (°C)	Time (min)	pH	Process represented
90	20	4	Pasteurisation
100	60	5	Baking, Brewing, Boiling
120	20	6	Sterilisation

Test solutions were prepared by adding an aliquot of the radiochemical stock solution to each buffer (citrate buffers were prepared at pH 4, 5 and 6) to give a final test concentration of 1.0 mg/L. Test solutions were dispensed into headspace vials (which were capable of withstanding the high temperature and pressures expected during incubation) and samples were incubated as per the conditions detailed above.

All reaction solutions were chromatographically profiled within 35 days of the initial experiment.

The radioactivity content of incubates sampled prior to and following incubation was determined by LSC analysis. Radioactivity content in incubated samples was compared to control samples.

The total recovery of applied radioactivity ranged from 100.37% to 107.73% for all hydrolysis experiments. Characterisation and identification of the radiolabelled components in treated solutions in this study was carried out by co-chromatography against reference standards using thin layer chromatography (TLC). Quantification of the degradates was conducted using the TLC data. In addition, the presence of parent [Pyrazole-5-¹⁴C]- pydiflumetofen in incubated and nonincubated (control) samples was confirmed using high performance liquid chromatography (HPLC).

The majority of the recovered radioactivity was present as ([Pyrazole-5-¹⁴C]- pydiflumetofen (95.5% – 97.5% chromatographed radioactivity). Between 1.3-1.9% of chromatographed radioactivity remained at the origin. Other degradation products (SYN545547 and SYN547891) were present at low levels accounting for ≤ 0.8% of the chromatographed radioactivity.

[Pyrazole-5-¹⁴C]- pydiflumetofen was found to be hydrolytically stable in buffer solutions at pH 4, 5 and 6 at temperatures simulating pasteurisation (90°C), baking/brewing/boiling (100°C) and sterilisation (120°C) respectively.

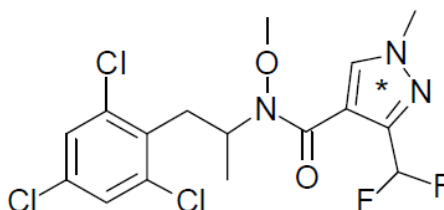
I. Materials and methods

A. Materials

In this study [Pyrazole-5-¹⁴C]- pydiflumetofen was used as test item with a specific activity of 5.06 MBq/mg. Radiochemical purity was > 98% (COA) and 96.9-97.8 determined at test facility. Chemical purity reported on the COA was >100% (101 %)

Table 7-109 Structural details of test item

Chemical structure



Radiolabel position	[pyrazole-5- ¹⁴ C] see * for ¹⁴ C-labeling position in chemical structure
Specific radioactivity	3.59 MBq/mg
Radiochemical purity	>98%
Chemical purity	>100%

B. Study Design

1. Preparation of radiolabelled Stock and Test Solutions:

The radio-labelled treatment solution was prepared by diluting 90 µL of the [Pyrazole-5-¹⁴C]-pydiflumetofen stock solution to 10 mL with acetonitrile. The treatment solution was analysed by counting portions (0.01 mL) by LSC confirming that it contained a test item concentration of 73.60 mg/L. The radiochemical purity of the stock solution was determined by TLC and HPLC.

Three [Pyrazole-5-¹⁴C]- pydiflumetofen radiolabelled test solutions were prepared by adding 349 µL aliquots of the Pyrazole-¹⁴C]- pydiflumetofen label radiochemical stock solution (equivalent to 25.7 µg) to three 25 mL volumetric flasks and drying under a stream of nitrogen. The radiolabelled test item was re-dissolved in 250 µL acetonitrile and the volumetric flasks made to volume with the appropriate buffer solution (i.e. pH 4, 5 or 6) to give a target rate of 1.03 mg/L.

The total radioactive content of each solution was determined by LSC for the pH 4, 5 and 6 buffer solutions, respectively, giving nominal concentrations of 1.0 mg/L.

2. Experimental conditions

Each flask of treatment solution was covered in aluminium foil to eliminate as far as possible any photolytic effects. The pH of each treatment solution was checked to ensure that the addition of the test substance had not caused the pH to change.

A total of 6 incubation vessels were prepared for incubation. Two vessels were set up at each pH and incubated at the required temperatures as detailed the table below. In addition, two vessels were set up at each pH and left to stand at ambient laboratory temperature for the incubation period (6 non-incubated solutions).

Table 7-110 Hydrolytic Conditions and Simulated Processes

Temperature (°C)	Time (min)	pH	Process represented
90±1	20	4±0.1	Pasteurisation
100±1	60	5±0.1	Baking, Brewing, Boiling
120±1	20	6±0.1	Sterilisation

In order to achieve the required temperature for each simulated process, the incubation vessels containing the pH 4 buffer were placed in a water bath, the incubation vessels containing the pH 5 buffer were placed in an oven and the incubation vessels containing the pH 6 buffer were placed in an autoclave for the appropriate incubation period.

Once the incubation time was reached, the samples were removed from their heat source and cooled to room temperature. After incubation and cooling, the radioactive content of each vessel was determined by LSC. The incubation vessels were emptied, rinsed with acetonitrile and the radioactive content in the rinse added to the determined radioactive content in the aqueous buffer solution to ensure complete recovery of radioactivity. Some minor deviations were noted. These are not considered to have a significant impact on the result of the study:

- pH values for test solutions (after addition of test item) are outside acceptance criteria of ± 0.1 pH units for some pH 4 and pH 6 samples.
- The temperature of the pH 6 test system was not fully monitored during the incubation period (no recordings at mid and endpoint of incubation period).

3. Sampling

Following incubation, the vessels were cooled to ambient temperature. The pH of all incubated and non-incubated solutions was measured. Aliquots of each incubated and nonincubated solution were analysed by LSC to determine the radioactive content. Subsamples of each incubated and non-incubated solution were taken for chromatographic analysis (TLC and HPLC). 10 mL of acetonitrile was added to each empty incubation vessel and all incubation vessels were ultra-sonicated for 5 minutes. The radioactive content of the acetonitrile washings was determined by liquid scintillation counting.

When not undergoing analysis, all samples were stored in a freezer set to maintain a temperature of -20°C. All hydrolysed samples were analysed by HPLC and TLC within 40 days of incubation.

C. Analytical procedures

The chromatographic analysis was used to determine the amount of test item ([Pyrazole-5-14C]- pydiflumetofen) and any degradation products that were present in the samples. Due to good recoveries (100.37% to 107.73%), results are based on the assumption that the chromatographed radioactivity represents 100% of the applied radioactivity.

HPLC was conducted on all samples generated in this study, in order to confirm qualitatively the presence of the test item ([Pyrazole-5-14C]- pydiflumetofen) and the absence of any significant degradation products.

II. Results and discussion

Material balance

The post-hydrolysis quantification results based on the actual amount of radioactivity applied to the solutions shows recoveries ranging from 100.37% to 107.73% confirming there was no significant loss of radioactivity during the reactions.

Detailed results for measured radioactivity balances are provided below.

Table 7-111 Recovery of Total Radioactivity From pH 4 Buffer. Treatment Solutions Incubated at 90°C

		Incubate pH 4 A				Incubate pH 4 B			
		Total dpm	Dpm/mL	Mg/L	% AR	Total dpm	Dpm/mL	Mg/L	% AR
[Pyrazole-5- ¹⁴ C]-pydiflumetofen	Treated Incubate	3058400	305840	1.007	105.52	3072000	307200	1.012	105.99
	Treated Apparatus Wash	53000	5300	0.017	1.83	50400	5040	0.017	1.74
	Total	3111400	311140	1.024	107.35	3122400	312240	1.029	107.73

% AR - % of applied radioactivity

Table 7-112 Recovery of Total Radioactivity From pH 5 Buffer. Treatment Solutions Incubated at 100°C

		Incubate pH 5 A				Incubate pH 5 B			
		Total dpm	Dpm/mL	Mg/L	% AR	Total dpm	Dpm/mL	Mg/L	% AR
[Pyrazole-5- ¹⁴ C]-pydiflumetofen	Treated Incubate	3040600	304060	1.002	104.47	3041600	304160	1.002	104.51
	Treated Apparatus Wash	54600	5460	0.018	1.88	50600	5060	0.017	1.74
	Total	3095200	309520	1.020	106.35	3092200	309220	1.019	106.25

% AR - % of applied radioactivity

Table 7-113 Recovery of Total Radioactivity From pH 6 Buffer. Treatment Solutions Incubated at 120°C

		Incubate pH 6 A				Incubate pH 6 B			
		Total dpm	Dpm/mL	Mg/L	% AR	Total dpm	Dpm/mL	Mg/L	% AR
[Pyrazole-5- ¹⁴ C]-pydiflumetofen	Treated Incubate	2914800	291480	0.960	99.95	2855400	285540	0.941	97.91
	Treated Apparatus Wash	115000	11500	0.038	3.94	71800	7180	0.024	2.46
	Total	3029800	302980	0.998	103.89	2927200	292720	0.965	100.37

% AR - % of applied radioactivity

The results from TLC analysis of the test incubates indicated that after elevated temperature hydrolysis no significant degradation had taken place. The majority of the recovered radioactivity was present as ([Pyrazole-5-¹⁴C]- pydiflumetofen (95.5% – 97.5% chromatographed radioactivity). Between 1.3 - 1.9% of chromatographed radioactivity remained at the origin. Other degradation products (SYN545547 and SYN547891) were present at low levels accounting for ≤ 0.8% of the chromatographed radioactivity and were identified by co-

chromatography against analytical reference standards. HPLC was conducted on all samples generated in this study, in order to confirm qualitatively the presence of the test item ([Pyrazole-5-¹⁴C]- pydiflumetofen) and the absence of any significant degradation products.

Table 7-114 Summary of Radioactive Residues in pH 4 Buffer Treatment Solutions incubated at 90°C for 20 Minutes (Results based on TLC data)

Label	incubate	pydiflumetofen		Origin		SYN545547		SYN547891		Unidentified		Total	
		%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L
[pyrazole-5- ¹⁴ C]	pH 4 A	97.47	0.998	1.29	0.0013	0.26	0.003	0.20	0.002	0.78	0.008	100	1.024
	pH 4 B	96.15	0.989	1.62	0.017	0.63	0.006	0.51	0.005	1.09	0.011	100	1.029

* % AR – Results are based on the assumption that the chromatographed radioactivity represents 100% of the applied radioactivity.

Table 7-115 Summary of Radioactive Residues in pH 5 Buffer Treatment Solutions incubated at 100°C for 60 Minutes (Results based on TLC data)

Label	incubate	pydiflumetofen		Origin		SYN545547		SYN547891		Unidentified		Total	
		%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L
[pyrazole-5- ¹⁴ C]	pH 5 A	96.48	0.982	1.69	0.017	0.35	0.004	0.51	0.005	0.97	0.01	100	1.018
	pH 5 B	95.97	0.978	1.71	0.017	0.62	0.006	0.37	0.004	1.33	0.014	100	1.019

* % AR – Results are based on the assumption that the chromatographed radioactivity represents 100% of the applied radioactivity.

Table 7-116 Summary of Radioactive Residues in pH 6 Buffer Treatment Solutions incubated at 120°C for 20 Minutes (Results based on TLC data)

Label	incubate	pydiflumetofen		Origin		SYN545547		SYN547891		Unidentified		Total	
		%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L	%AR*	mg/L
[pyrazole-5- ¹⁴ C]	pH 6 A	95.54	0.940	1.88	0.018	0.64	0.006	0.28	0.003	1.66	0.016	100	0.984
	pH 6 B	95.74	0.923	1.66	0.016	0.78	0.008	0.08	0.001	1.74	0.017	100	0.964

* % AR – Results are based on the assumption that the chromatographed radioactivity represents 100% of the applied radioactivity.

III. Conclusions

Following hydrolysis two minor degradation products (SYN545547 and SYN547891) were present at low levels accounting for ≤ 0.8% of the chromatographed radioactivity.

[Pyrazole-5-¹⁴C]- pydiflumetofen was found to be hydrolytically stable in buffer solutions, representing > 95 % of the TRR, at pH 4, 5 and 6 at temperatures simulating pasteurisation (90°C), baking/brewing/boiling (100°C) and sterilisation (120°C) respectively.

The use of a single radiolabel position for the high temperature hydrolysis study was considered acceptable as the minor degradation products (≤ 0.8%) are whole molecule related and unidentified material accounted for only ~3.5% of AR (sum of data at the TLC origin, plus ‘unidentified’ other material chromatographed (the latter is max 1.7%)), thus use of a second radiolabel position would not provide any additional information.

B.7.5.2. Distribution of the residue in peel and pulp

Not relevant for the representative uses on cereals and oilseed rape.

B.7.5.3. Magnitude of residues in processed commodities

Representative processing studies on barley, wheat and oilseed rape were conducted with field samples collected from supervised residue trials conducted in Europe.

B.7.5.3.1. Barley

Report:	KCA1 6.5.3; [REDACTED]; [REDACTED]; [REDACTED]; 2017
Title:	SYN545974 - Residue Study on Barley and Processed Specimens in Northern France, Germany and Poland in 2013
Report No.:	S13-02518
Document No.:	VV-463141 , A17573A_10004
Guidelines:	<p>Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document).</p> <p>Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.</p> <p>European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).</p> <p>The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9.</p> <p>Guidelines for the Generation of Data concerning Residues as provided in Annex II part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC concerning the placing of plant protection products on the market, EU 1607/VI/97 rev.2 (10 Jun 1999).</p> <p>OECD Test Guideline 508: Magnitude of the Pesticide Residues in Processed Commodities.</p> <p>Commission of the European Communities, Processing Studies; 7035/VI/95 (rev.5, working document).</p> <p>The national requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.</p>
Guideline deviations:	No significant deviations
GLP/GEP:	Yes

I. Materials and Methods

The study contained four field trials on barley conducted in northern Europe carried out in the 2013 growing season; samples from two of these trials were used for determinations of pydiflumetofen on barley (whole plant, grain, straw) and processed fractions.

Field part

In these four trials, one untreated control plot was used for the generation of the control samples. The study design included two plots for each trial and, in two of the trials (S13-02518-01 and S13-02518-02), an additional plot (P3) was included that was treated at a higher application rate. These additional plots (P3) were used to determine the magnitude of residues of pydiflumetofen (also known as SYN545974) on barley (whole plant, grain, straw) and processed fractions.

The following summaries cover only the control plot and the plots treated at higher application rates (Trial S13-02518-01 and S13-02518-02). Normal agricultural procedures were followed, and no unusual weather events were

recorded. Application data from the two higher application rate supervised residue trials are presented in the following Table 7-117.

Table 7-117 Planned application scenario for higher application rate trials

Trial No. / Location / EU zone / Year	Commodity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (BBCH)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL			
(a)	(b)					(c)	(d)	(f)
S13-02518-01 Rouvres Saint Jean, Loiret, France 45300	Barley – Sebastian	1, 6/3/2013 2, 18- 22/6/2013 3, 30/7/2013	376 378	200 201		19/6/2013 28/6/2013	59-61 75	Backpack / Broadcast foliar spray A17573A 100 EC No adjuvants used
S13-02518-02 Ascoux, Loiret, France, 45300	Barley – Chill	1, 5/3/2013 2, 12- 19/6/2013 3, 23/7/2013	376 381	205 203		12/6/2013 24/6/2013	57-61 75	Backpack / Broadcast foliar spray A17573A 100 EC No adjuvants used

To plot P3 (trials S13-02518-01 and -02 only), two applications (applied at growth stages BBCH 57-61 and 75), separated by a 9 - 12 day interval were made at a nominal application rate of 375 g ai/ha.

Treated samples for processing (2x~50kg) were collected from plot P3 at normal commercial harvest (NCH) (29-32 DALA).

Untreated samples (>1kg for RAC analysis) were collected at 0 days before the last application (DBLA) and at normal commercial harvest (NCH) (>1kg for RAC analysis and 81kg for processing).

Field samples for processing were shipped and stored at ambient temperature for Ca. 5 months, which was stated in the study to be representative of normal commercial practice. Positive residues were observed in both RAC and processed fractions following this storage, in line with the levels observed in the RAC frozen samples.

Relevant practices and standardised procedures were applied in this processing phase which simulated common industrial processes at a laboratory scale.

The processing of the barley specimens was performed at following processing locations:

Separation, sampling, cleaning, sieving,
storage, shipment, GLP-management

BioChem agrar
Labor für biologische und chemische Analytik
GmbH Kupferstraße 6
D-04827 Gerichshain
Germany

Processing to malt

VLB e.V. Seestraße 13
D-13353 Berlin

Processing to beer

Germany
fermtec GmbH Köpenicker Str. 325
D-12555 Berlin

Processing to pot and pearl barley

Germany
TU Berlin Seestraße 13

13353 Berlin
Germany

Each field specimen for processing was separated dependent on the processing destination and the respective final products for special processing parts as follows:

Table 7-118 Field specimen amount and processing destination

Specimen Code (field phase)	Specimen No. for processing Specimen weight		
	Pearl barley (A)	Pot barley (B)	Beer (C)
S13-02518-01-023*(Balance untreated) C1	S13-02518-01-023A 12.59 kg	S13-02518-01-023B 13.50 kg	S13-02518-01-023C 35.05 kg
S13-02518-01-030(Balance treated) P3	S13-02518-01-030A 10.00 kg	S13-02518-01-030B 10.00 kg	S13-02518-01-030C 30.00 kg
S13-02518-01-031(Follow-up treated) P3	S13-02518-01-031A 10.00 kg	S13-02518-01-031B 10.00 kg	S13-02518-01-031C 30.00 kg
S13-02518-02-023*(Balance untreated) C1	S13-02518-02-023A 13.28 kg	S13-02518-02-023B 13.74 kg	S13-02518-02-023C 35.60 kg
S13-02518-02-030(Balance treated) P3	S13-02518-02-030A 10.92 kg	S13-02518-02-030B 10.45 kg	S13-02518-02-030C 29.55 kg
S13-02518-02-031(Follow-up treated) P3	S13-02518-02-031A 10.08 kg	S13-02518-02-031B 10.00 kg	S13-02518-02-031C 30.27 kg

The specimens were stored immediately after processing at or below -18 °C and remained frozen during transportation to the test facility.

Processed fractions (including grain prior to processing) were stored frozen for a maximum period of 9 months from sampling to analysis for pydiflumetofen. These storage periods are covered by storage stability trials conducted summarised in section B.7.1, which shows stability in all matrices for 23 months

Extract solutions of field specimens were stored for a maximum of 32 days in the refrigerator before analysis of pydiflumetofen. Extract solutions of processed fractions were stored for a maximum of 10 days in the refrigerator before analysis of pydiflumetofen.

The stability of the analyte in the sample extracts was proven by the corresponding procedural recovery samples, which were stored under the same conditions together with the sample extracts.

At each independent trial site there were two trials on treated crops (described by the applicant as balance and follow up trials). Balance studies (untreated and treated) and follow-up studies (treated) were carried out on each process. Balance studies determined residues in more processing fractions to provide a greater understanding of where the residues resulted arising from the processing operation. The follow up studies were focussed on the processed commodities that are consumed. Flow diagrams showing the processing stages are provided in this section.

Processing summary

Pearl barley/Pot barley production

Before processing start the corresponding grain (immediately before processing after separation) was sampled. The grain specimens for this processing step were cleaned before the pearl barley/pot barley production was

started and cleaned grain (balance and follow up studies) and offal (impurities) (only balance studies) were sampled.

Conditioning

Before beginning of pearl barley/pot barley production, an optimal moisture content of barley grain of approx. 14 % should be achieved. The grain specimens for processing of trial S13-02518-01 had a moisture content of approx. 14 %, therefore conditioning was not necessary.

The grain specimens for processing of trial S13-02518-02 were damped to achieve the optimal moisture content of approx. 15 %.

The optimal moisture content for hulling can vary depending on the quality of the grain.

Hulling/Sieving

The corresponding samples were hulled using a mechanical vertical shelling machine (Vertikal-Schälmaschine). Each sample was hulled until the stipulated abrasion was reached (Ca. 2-3.5 mins); for pearl barley this was 30-35 %, for pot barley this was 20-25 %. The degree of abrasion (pearling dust/bran and flour) was determined by the proportion of pearl barley/pot barley with respect to the total portion of cleaned grain used for hulling process. Abrasion was sampled as abrasion dust (rub off), (only balance studies). The rest of the abrasion was sieved to bran and flour. Pearl barley/pot barley, bran and flour were sampled (balance and follow up studies).

Beer production

Malting

Directly before processing grain (immediately before processing after separation) was sampled. The grain was cleaned and sieved (sieve mesh 2.5 mm) before malting was started. Sieving was only done, if kernel sizes were great enough. Cleaned grains as well as offal were sampled (only balance studies).

Steeping

After sieving, a combined wet and dry steeping was conducted. Around 14.5 kg of barley per sample was used with an initial moisture content of 12-14 %.

Table 7-119 Steeping course

Specimen No.	S13-02518-01-023C	S13-02518-01-030C	S13-02518-01-031C	S13-02518-02-023C	S13-02518-02-030C	S13-02518-02-031C
1. Steeping	5h	5h	5h	5h	5h	5h
1. Dry steeping	22h	22h	22h	22h	22h	22h
2. Steeping	4h	4h	4h	4h	4h	4h
2. Dry steeping	18h	19h	19h	18h	19h	19h
1. Wet steeping	20 min	25 min	20 min	30 min	25 min	35 min
Casting	15 min	10 min	15 min	15 min	15 min	15 min
Steeping degree (%)	44.7	44.8	44.8	44.7	44.8	44.9

The exact steeping time was recorded.

Germination

After steeping, a germination procedure followed ("still" germination). Germination was conducted over 5 days (Ca. 118 – 120 hrs), with a mean temperature of 14 °C and > 90 % relative humidity. Material was turned continuously throughout.

Kiln drying

Kiln-drying was conducted in a dry chamber, using 3 runs of increasing temperature, over a period of 24 hrs (Run 1, 45-55 °C = 17hrs; Run 2, 60-70 °C = 2 hrs; Run 3, 80-90 °C = 5 hrs). Initial water content of the green malt was Ca. 45 – 46 %. Water content of the dried malt was Ca. 4 – 5 %.

After kiln-drying brewing malt was sampled as malt with sprouts (only balance studies). Subsequently the germs (malt sprouts) were removed mechanically by a trimmer. Malt sprouts and cleaned malt were sampled immediately after malting.

Until brewing (approx. 2 weeks malt rest) the malt was stored at room temperature at BioChem agrar GmbH.

Brewing

The specimens for processing were shipped by car at ambient temperature to the processing location. Directly before brewing, the sample denoted “directly before brewing” was taken.

The malt, processed as outlined above, was stored at ambient temperature conditions for approx. 2 weeks prior to brewing.

The malt was dry milled and mixed with brew water before being processed through each of the following steps: Mashing (Ca. 1 hr 50 mins); Lautering (Ca. 2hrs, 10 mins); Wort boiling (Ca. 1 hr 30 mins); rest (Ca. 20 mins). The processed fractions were then allowed to ferment for 6-10 days, warm mature for 2 days and cold mature for Ca. 1 month. More detail is provided in the subsequent paragraphs.

Mashing

Mashing was the homogeneous mixing of ground malt and water according to a definite temperature time regime (mash program). The main purpose of mashing was the dissolution and enzymatic conversion of ingredients. Mashing was started in a heatable tun at 46 °C and included the following steps:

Table 7-120 Mashing

Step	Time (mins)	Temperature (°C)
Heating	8-10	55
Rest (protein decomposition)	15	55
Heating	8-10	62
Rest (maltose formation)	30	62
Heating	14-15	72
Rest (sugar formation)	20	72
Heating	6-7	76
Mashing down	3	76

Lautering: Wort extraction and separation

After mash boiling, the wort was separated from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was extracted by washing with hot water (first filter runnings). The wort separation was done using a refining vat and took 2 - 3 hours (separated wort extract: Ca. 17 %; wort extract (before cooking):

Ca. 10 %)). Spent grain (brewers grain) (only balance studies) and dried brewers grain were sampled (Dry matter content of spent grain Ca. 96 %).

Wort boiling and conditioning

Before wort boiling the ‘wort before cooking’ was sampled (only balance studies).

After addition of hop pellets, the separated wort was boiled (90 mins). This deactivated the enzymes of the malt, sterilized the wort, extracted and isomerised the essential components of the hops, precipitated high molecular proteins (called „Bruch“) and expelled unwanted aromatic substances.

After boiling, the flocs were separated in a whirlpool causing the sludge to deposit on the bottom in the shape of a cone and were sampled as spent hops (flocs) (only balance studies).

Subsequently, wort was sampled as wort after cooking (only balance studies; Ca. 11 %). The pH of the spent wort was 5.7-5.9. For cooling and ventilating the wort, an intra-plant circulation was used (wort temperature after cooling: 12-13 °C). By adding oxygen (intra-plant circulation) the conditions for the start of the fermentation were prepared.

Fermentation and maturation

The pure culture yeast fermented sugar of the wort to alcohol and CO₂, but in the course of the yeast metabolism unwanted by-products were also formed (diacetyl, higher alcohols and others). In the pilot plant the classical primary fermentation (low fermentation) was carried out in bottom fermentation containers. The fermentation temperature was approx. 9 °C. Fermentation heat was dissipated by means of room ventilation. The duration of main fermentation depended on temperature, on starting extract concentration of the finished wort, on the ratio of non-fermentable sugars to the extract, on the final attenuation and on the yeast cell number (exact duration was recorded).

As soon as the extract content of the fermented young beer was 2 % higher than the final attenuation, the storing time started. Before maturation the young beer was cooled down. During the main fermentation (6-10 days) the yeast deposited on the tank bottom. After fermentation spent yeast (follow up and balance studies) and young beer (only balance studies) were sampled.

At the beginning of maturation, the young beer was stored at room temperature for warm maturation (2 days; mean temp 20 °C; 0-1.6 bar pressure) to break down the diacetyl in casks. Then the young beer was stored under pressure (0.7 - 1.0 bar) at approx. 0 - 2 °C (cold maturation) for about 4 weeks. In this time the remaining extract was fermented. Unwanted flavour and odorous substances were decomposed or expelled. Sludge particles and yeast settled at the bottom. The rack beer was filtered using a special filter combination (Seitz/Arauner). During filtration all organisms harming the beer (bacteria and yeast) were removed and sludge particles were separated. The final product beer was sampled.

Quality parameters beer

Table 7-121 Quality parameters beer

Specimen No.	S13-0251801-023C	S13-0251801-030C	S13-0251801-031C	S13-0251802-023C	S13-0251802-030C	S13-0251802-031C
Original wort (%)	12.2	11.6	11.6	11.9	11.6	11.7
Extract, apparent (g/100g)	1.73	1.83	1.72	1.95	2.28	2.14
Extract, real (g/100g)	3.73	3.68	3.60	3.84	4.05	3.96
Content Ethanol (Vol. %)	5.63	5.21	5.27	5.34	5.01	5.14
pH	4.55	4.45	4.47	4.44	4.44	4.49
Colour (EBC) Lovibond	8	7	7	8	7	7

Output fermentation degree, apparent (%)	85.8	84.2	85.1	83.6	80.4	81.7
Output fermentation degree, real (%)	71.1	69.7	70.4	69.3	66.7	67.8

Processing flowcharts

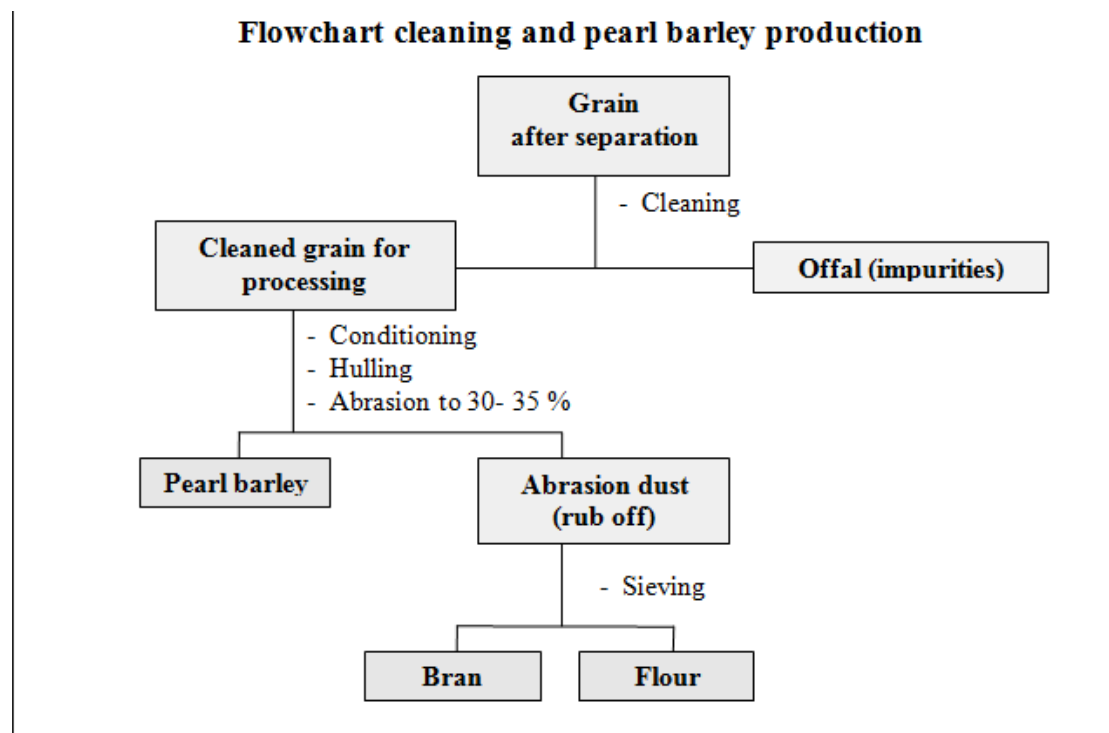
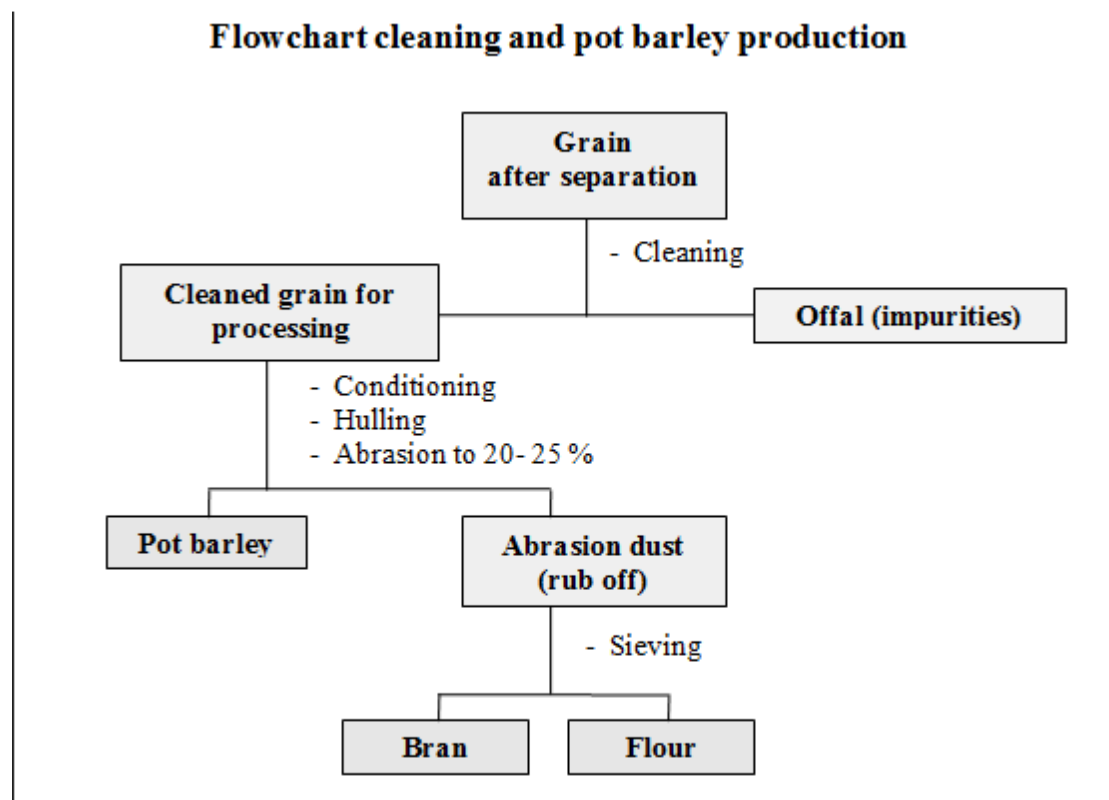
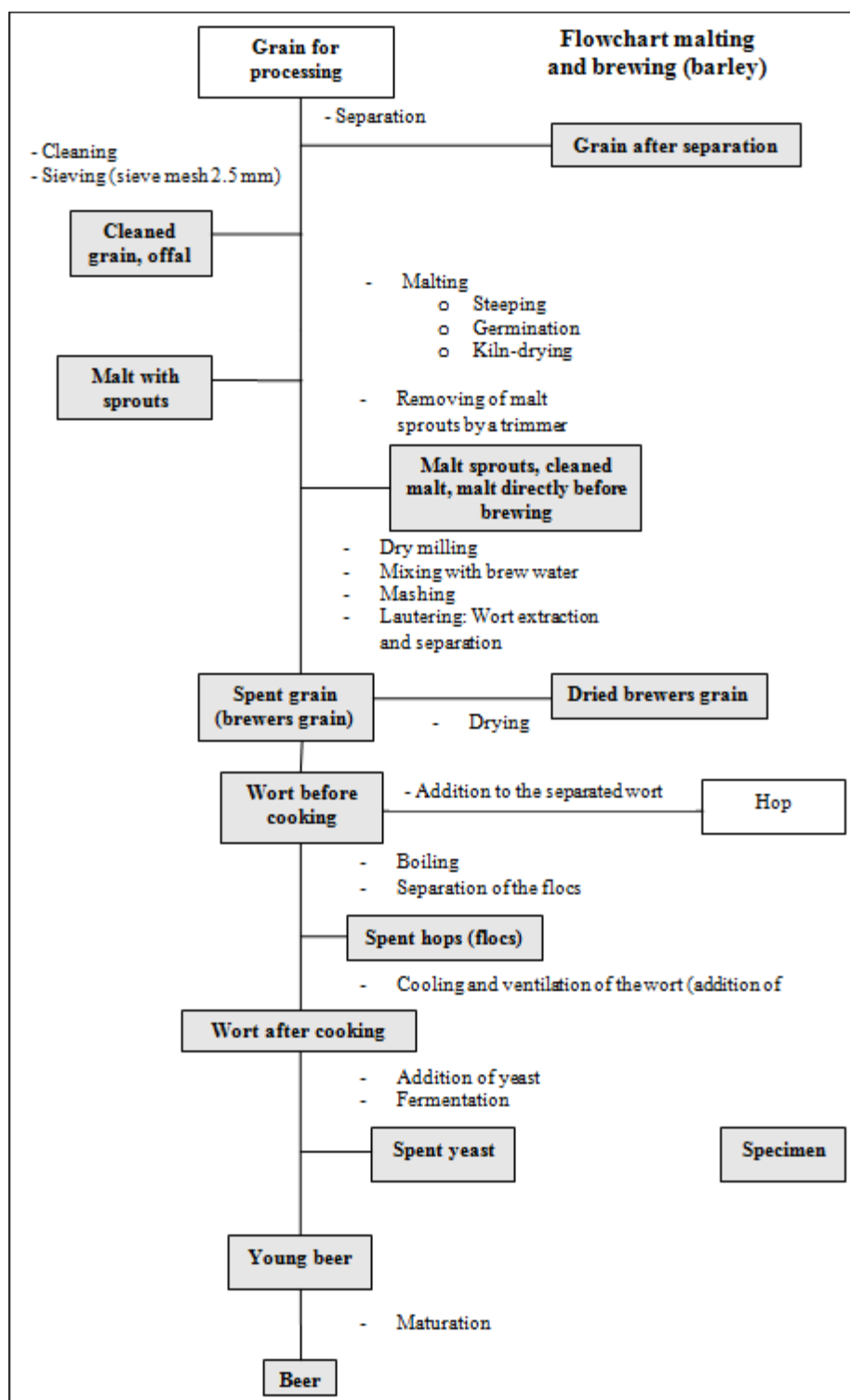
Figure 7-7 Cleaning and pearl barley productionFigure 7-8 Cleaning and pot barley production

Figure 7-9 Malting and brewing

II. Findings

Method performance for determining pydiflumetofen residues in/on barley was evaluated during method validation (method GRM061.03A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the

determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (cereal grain and potato), high protein (dry beans) and dry (cereal straw) commodities, with an LOQ of 0.01 mg/kg – see B 5.1.2.5) and by use of concurrent recovery by fortifying samples of barley and all processed fraction at 0.010 and 0.10 ppm (all analytes) and at higher levels (up to 12 mg/kg) for all substrates except grain prior to processing, cleaned grain, pearl barley, pot barley, wort before cooking, wort after cooking, young beer, beer and spent yeast. Note that for processed fractions, all samples should include a reduced validation, consisting of two fortifications and three repeats. This reduced data has not been supplied, however given the large number procedural recovery results and the large number of processed fractions, the data on the whole is considered sufficient.

Details on concurrent recovery data are shown in Table 7-122. The individual recoveries were generally within the acceptable range of mean recoveries of 70 – 110% (SANCO 3029-99 Rev.4) and all samples (except Abrasion rub off, 0.1 mg/kg fortification level and dried brewers yeast at 0.01 mg/kg fortification) are within 70-120% (for > 0.01 mg/kg < 0.1 mg/kg) or 60-120% (for > 1 µg/kg < 0.01 mg/kg) specified as range of mean recoveries in OECD guidance ENV/JM/MONO(2007)17. The RSD could not be calculated for most samples as there were not enough repeats. Residues above the LOQ were found in some of the control samples so alternate control samples were used for the fortification experiments.

Table 7-122 Recovery data for pydiflumetofen

Substrate (control)	Fortification level (mg/kg)	pydiflumetofen (%)
Whole plant	0.01	113
	0.1	99
	8.0	108
Grain	0.01	120
	0.10	90
	0.01	98
	4.0	110
Straw	0.01	105
	0.10	91
	8.0	98
	0.01	102
	0.10	85
Grain prior to processing	0.10	114 ^a (115)
	0.01	78
	0.10	100
	0.01	93
	0.10	76
Cleaned grain	0.01	62
	0.10	77
Pearl barley	0.01	86
Pot barley	0.10	83
Abrasion (rub-off)	0.01	74 ^b
	0.10	61 ^b
	0.01	88 ^b
	0.10	83 ^b
Offal	0.01	108 ^a (124)
	0.10	101 ^a (102)
	0.01	89 ^a (104)
	0.10	87 ^a (87)
	12	101
Bran	0.01	100 ^c
	0.10	82 ^c
	0.01	79 ^c
	0.10	78 ^c
Flour	0.10	90 ^a (97)
	0.10	72 ^a (77)
Malt with sprouts	0.01	76

	0.10	87
Cleaned malt	0.01	109
	0.10	108
	1.0	86
Malt before brewing	0.01	72
	0.10	71
Spent grain	0.01	104
	0.10	87
Dried brewers yeast	0.01	90 ^a (131)
	0.10	89 ^a (89)
	4.0	105
Wort before cooking	0.1	82
Wort after cooking	0.01	73
	0.01	77
	0.1	118
Young beer	0.01	82
	0.1	78
Beer	-	-
Spent hops	0.01	99
	0.1	92
	0.5	86
Spent yeast	0.01	74
	0.1	79
Malt sprouts	0.01	83
	0.1	99
	2.0	94

a The peak area of the fortification experiment was corrected for the peak area of the control (untreated) sample. Uncorrected values are given in brackets

b Due to unacceptable levels of pydiflumetofen contamination being found in the control samples collected from this study, the control sample S12-01355-CMN-12-13300-122 (pydiflumetofen residue < 0.003 mg/kg) from study S12-01355 has been used for the fortification experiments. This use of alternative control samples is considered acceptable.

c Due to unacceptable levels of pydiflumetofen contamination being found in the control samples collected from this study, the control sample S12-01355-01-CMN-12-13300-255 (pydiflumetofen residue < 0.003 mg/kg) from study S12-01355 has been used for the fortification experiments. This use of alternative control samples is considered acceptable.

Residues

The levels of residues of pydiflumetofen in the treated samples are summarised in Table 7-123 to Table 7-124.

Residues of pydiflumetofen at or above LOQ (0.01 mg/kg) were found in the untreated control plot in S13-02518-01 for straw at 41 DALA with residues of 0.02 mg/kg.

Note that the applicant contended that contamination may have occurred through spray drift from earlier applications.

Residues in untreated processed specimens were below LOQ (0.01 mg/kg) with the exception of the samples described below and in Table 7-125:

- Residues of pydiflumetofen in the untreated offal samples S13-02518-01-023A-B1-A-003 and S13-02518-01-023C-B1-C-015 were 0.01 mg/kg.
- Residues of pydiflumetofen in the untreated abrasion dust (rub off) samples S13-02518-01-023B-B1-B-009, S13-02518-02-023A-B3-A-081, S13-02518-02-023B-B3-B-086 were in the range of 0.01 to 0.17 mg/kg (mean of two determinations).
- Residues of pydiflumetofen in the untreated bran samples S13-02518-02-023A-B3-A-083 and S13-02518-02-023B-B3-B-088 were 0.11 and 0.05 mg/kg (mean of two determinations).

- Residues of pydiflumetofen in the untreated flour samples S13-02518-02-023A-B3-A-084 and S13-02518-02-023B-B3-B-089 were 0.14 and 0.06 mg/kg (mean of two determinations).

Note that the applicant contended that potential residues in untreated processed specimens may have been from treated grain being processed prior to untreated grain.

Residues of pydiflumetofen in the duplicate treated barley (grain) samples prior to processing from trial S13-02518-01 were in the range of 0.83 to 1.2 mg/kg (mean values) and from trial S13-02518-02 were in the range of 1.5 to 2.3 mg/kg (mean values).

The pydiflumetofen residues in pearl barley were significantly lower than in the barley (grain) samples prior to processing. A total of 71 % and 83 % of the pydiflumetofen on the barley (grain) were accounted for in the pearl barley and the processing by-products.

The pydiflumetofen residues in pot barley were significantly lower than in the barley (grain) samples prior to processing. A total of 62 % and 73 % of the pydiflumetofen on the barley (grain) were accounted for in the pearl barley and the processing by-products.

The pydiflumetofen residues in beer were significantly lower than in the barley (grain) samples prior to processing. A total of 58 % and 74 % of the pydiflumetofen on the barley (grain) were accounted for in the beer and the processing by-products.

Some processing factors shows a large difference (>50%) between trials (based on both the individual values from the balance and follow up trials and their mean values) so in principle a third trial should be performed, and the median value reported. This was not considered necessary due to the data supplied from the follow up trials giving a total of four separate values from two independent trial sites.

Note that the values given below for the overall transfer factors (Table 7-128) show data from two independent test sites (and some commodities have results for both balance and follow up studies). As the follow up studies have independently gone through the same processing as the balance trials and as the difference between the trials are not irreconcilable (10 fold different according to OECD 508), the median transfer factor was considered most appropriate and has been calculated.

Field specimens (RAC for the trial that were analysed separately to the processed samples) were analysed following frozen storage, within an acceptable timeframe that was covered by storage stability studies (see section B.7.1).

The maximum time field samples for processing were stored at ambient temperature prior to the start of processing was < 6 months, this is considered acceptable as sufficient levels of detectable incurred residues were present in the samples prior to processing.

Processed fractions (including grain prior to processing) were stored frozen for a maximum period of 9 months from sampling to analysis for pydiflumetofen. These storage periods are covered by storage stability trials summarised in section 7.1 which shows stability in all matrices for 23 months

III. Conclusions

The study contained four field trials on barley (two for processing trials) conducted in northern Europe and was carried out to generate results on the magnitude of residues and to generate samples for processing. Determinations of pydiflumetofen on barley (whole plant, grain, straw) and processed fractions were undertaken to evaluate the magnitude of residues.

The plots designated for the processing study, P3 in trials S13-02518-01 and S13-02518-02 had an application rate of nominally 375 g ai/ha, applied at growth stages BBCH 57-61 and 75, treatment interval was 9-12 days.

The study was conducted to GLP.

The results indicate that residues of pydiflumetofen do not concentrate in the processing of barley into pearl barley, pot barley or beer (processing factor <0.01 to 0.11) but do concentrate in the processing of barley into flour (in

both pearl and pot barley – processing factor 2.78-2.93), abrasion dust (pf 3.15-3.74), offal (pf 4.98) and dried brewers grain (pf 1.94).

Table 7-123 Field samples

Trial number	Sample No. S13-02518- 01/02-	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop Part	pydiflumetofen Residue (mg/kg)
S13-02518- 01	Treated Plot (P3)				
	028	2 x 375	NCH (32 DALA)	Grain	1.3
	Control Plot (C1)				
	001	Control	0 DBLA	Whole plant	< 0.01
	019	Control	NCH (41 DALA)	Grain	< 0.01
	021	Control	NCH (41 DALA)	Straw	0.02 [#] (0.02, 0.02, 0.02, 0.02)
S13-02518- 02	Treated Plot (P3)				
	029	2 x 375	NCH (29 DALA)	Grain	2.1
	Control Plot (C1)				
	001	Control	0 DBLA	Whole plant	< 0.01
	019	Control	NCH (41 DALA)	Grain	< 0.01
	021	Control	NCH (41 DALA)	Straw	< 0.01

Plot C1: Untreated, Plot P3: Treated with formulation A17573A DBLA – Days before last application; DALA – Days after last application; NCH – Normal commercial harvest.

mean value of four determinations (in brackets: individual results)

No correction of results for either control residues or recovery values has been performed.

Table 7-124 Pydiflumetofen residue results for processing samples

Event	Crop Part	pydiflumetofen Residue (mg/kg)
Field Specimen S13-02518-01-030 (Balance Trial)		
balance / pearl barley and flour		
prior to processing	grain	1.1
prior to processing	grain	1.0
after cleaning	cleaned grain	0.90
after cleaning	offal (impurities)	3.6
after hulling	abrasion dust (rub off)	2.9
after hulling	pearl barley	0.13

after sieving	bran	0.14
after sieving	flour	2.0
balance / pot barley and flour		
prior to processing	grain	1.1
prior to processing	grain	1.0
after hulling	abrasion dust (rub off)	3.9
after hulling	pot barley	0.13
after sieving	bran	0.18
after sieving	flour	3.1
balance / beer		
prior to processing	grain	1.1
prior to processing	grain	1.2
after cleaning	cleaned grain	0.87
after cleaning	offal	3.7
after kiln-drying	malt with sprouts	0.27
after kiln-drying	malt sprouts	0.67
after kiln-drying	cleaned malt	0.36
directly before brewing	malt directly before brewing	0.26
after lautering	spent grain (brewing grain)	0.30
after lautering	dried brewers grain	1.8
before boiling	wort before cooking	< 0.01 [#] (<0.01, <0.01)
after boiling	wort after cooking	< 0.01 [#] (<0.01, <0.01)
after boiling	spent hops (flocs)	0.28
after fermentation	spent yeast	0.02
after fermentation	young beer	< 0.01
after maturation	beer	< 0.01
follow up / pearl barley and flour		
prior to processing	grain	0.96 [#] (0.62, 1.3)
prior to processing	grain	0.89 [#] (0.77, 1.0)
after cleaning	cleaned grain	0.75
after hulling	pearl barley	0.13
after sieving	bran	0.15
after sieving	flour	2.2
follow up / pot barley and flour		
prior to processing	grain	1.0 [#] (0.71, 1.3)
prior to processing	grain	0.78 [#] (0.45, 1.1)
after hulling	abrasion dust (rub off)	4.5
after hulling	pot barley	0.17
after sieving	bran	0.19
after sieving	flour	2.7
follow up / beer		

prior to processing	grain	0.94 [#] (0.58, 1.3)
prior to processing	grain	0.72 [#] (0.44, 1.0)
after kiln-drying	malt sprouts	0.70
after kiln-drying	cleaned malt	0.45
directly before brewing	malt directly before brewing	0.35
after lautering	dried brewers grain	2.3
after fermentation	spent yeast	0.02
after maturation	beer	< 0.01
Field Specimen S13-02518-02-030 (Balance Trial)		
balance / pearl barley and flour		
prior to processing	grain	1.8
prior to processing	grain	1.5
after cleaning	cleaned grain	1.3
after cleaning	offal (impurities)	7.0
after hulling	abrasion dust (rub off)	6.2
after hulling	pearl barley	0.11
after sieving	bran	0.23
after sieving	flour	5.3
balance / pot barley and flour		
prior to processing	grain	2.2
prior to processing	grain	2.3
after hulling	abrasion dust (rub off)	8.6
after hulling	pot barley	0.13
after sieving	bran	0.34
after sieving	flour	6.0
balance / beer		
prior to processing	grain	1.3
prior to processing	grain	1.8
after cleaning	cleaned grain	1.4
after cleaning	offal	11
after kiln-drying	malt with sprouts	0.44
after kiln-drying	malt sprouts	1.1
after kiln-drying	cleaned malt	0.53
directly before brewing	malt directly before brewing	0.48
after lautering	spent grain (brewing grain)	0.44
after lautering	dried brewers grain	3.2
before boiling	wort before cooking	0.01 [#] (0.01, 0.01)
after boiling	wort after cooking	< 0.01 [#] (< 0.01, 0.01)
after boiling	spent hops (flocs)	0.43
after fermentation	spent yeast	0.04
after fermentation	young beer	< 0.01
after maturation	beer	< 0.01

follow up / pearl barley and flour		
prior to processing	grain	1.5
prior to processing	grain	1.4
after cleaning	cleaned grain	1.1
after hulling	pearl barley	0.08
after sieving	bran	0.27
after sieving	flour	4.8
follow up / pot barley and flour		
prior to processing	grain	2.2
prior to processing	grain	1.6
after hulling	abrasion dust (rub off)	7.1
after hulling	pot barley	0.12
after sieving	bran	0.36
after sieving	flour	6.3
follow up / beer		
prior to processing	grain	1.4
prior to processing	grain	1.5
after kiln-drying	malt sprouts	1.1
after kiln-drying	cleaned malt	0.47
directly before brewing	malt directly before brewing	0.42
after lautering	dried brewers grain	2.8
after fermentation	spent yeast	0.03
after maturation	beer	< 0.01

mean value of two determinations (in brackets: individual results)

No correction of results for either control residues or recovery values has been performed.

No residues of pydiflumetofen at or above the LOQ (< 0.01 mg/kg) were found in any of the untreated processing specimens except for following processing specimens:

Table 7-125 Untreated processing samples giving residues at or above LOQ

Event	Crop Part	pydiflumetofen Residue (mg/kg)
Field Specimen S13-02518-01-023 (Balance Trial)		
balance / pearl barley and flour		
after cleaning	offal (impurities)	0.01 [#] (0.01, 0.01)
balance / pot barley and flour		
after hulling	abrasion dust (rub off)	0.01 [#] (0.01, 0.01)
mass balance / beer		
after cleaning	offal	0.01 [#] (0.01, 0.01)

Field Specimen S13-02518-02-023 (Balance Trial)		
balance / pearl barley and flour		
after hulling	abrasion dust (rub off)	0.17 [#] (0.15, 0.18)
after sieving	bran	0.11 [#] (0.09, 0.12)
after sieving	flour	0.14 [#] (0.15, 0.12)
balance / pot barley and flour		
after hulling	abrasion dust (rub off)	0.07 [#] (0.06, 0.08)
after sieving	bran	0.05 [#] (0.04, 0.05)
after sieving	flour	0.06 [#] (0.06, 0.06)

[#] mean value of two determinations (in brackets: individual results)

No correction of results for either control residues or recovery values has been performed.

Table 7-126 Processing factors for pydiflumetofen – Field trial S13-02518-01

Processing product	pydiflumetofen residue (mg/kg)		Processing Factor (PF)
	RD-Mo	RD-RA	
Field Specimen S13-02518-01-030 (Balance)			
Pearl barley production			
RAC – Barley grain	1.1 ^{a,b}	1.1 ^{a,b}	-
Cleaned grain	0.9	0.9	0.82
Offal (impurities)	3.6	3.6	3.27
Abrasion dust (rub off)	2.9	2.9	2.64
Pearl barley	0.13	0.13	0.12
Bran	0.14	0.14	0.13
Flour	2.0	2.0	1.82
Pot barley production			
RAC – Barley grain	1.1 ^{a,b}	1.1 ^{a,b}	-
Abrasion dust (rub off)	3.9	3.9	3.55
Pot barley	0.13	0.13	0.12
Bran	0.18	0.18	0.16
Flour	3.1	3.1	2.82
Beer production			
RAC – Barley grain	1.2 ^c	1.2 ^c	-
Cleaned grain	0.87	0.87	0.73

Offal	3.7	3.7	3.08
Malt with sprouts	0.27	0.27	0.23
Malt sprouts	0.67	0.67	0.56
Cleaned malt	0.36	0.36	0.30
Malt directly before brewing	0.26	0.26	0.22
Spent grain (brewing grain)	0.3	0.3	0.25
Dried brewers grain	1.8	1.8	1.5
Wort before cooking	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	<0.01
Wort after cooking	<0.01 (<0.01, <0.01)	<0.01 (<0.01, <0.01)	<0.01
Spent hops (flocs)	0.28	0.28	0.23
Spent yeast	0.02	0.02	0.02
Young beer	<0.01	<0.01	<0.01
Beer	< 0.01	< 0.01	< 0.01
Field Specimen S13-02518-01-031 (Follow up)			
Pearl barley production			
RAC – Barley grain	0.92 ^d	0.92 ^d	-
Cleaned grain	0.75	0.75	0.82
Pearl barley	0.13	0.13	0.14
Bran	0.15	0.15	0.16
Flour	2.2	2.2	2.39
Pot barley production			
RAC – Barley grain	0.89 ^e	0.89 ^e	-
Abrasion dust (rub off)	4.5	4.5	5.06
Pot barley	0.17	0.17	0.19
Bran	0.19	0.19	0.21
Flour	2.7	2.7	3.03
Beer production			
RAC – Barley grain	0.83 ^f	0.83 ^f	-
Malt sprouts	0.7	0.7	0.84
Cleaned malt	0.45	0.45	0.54
Malt directly before brewing	0.35	0.35	0.42
Dried brewers grain	2.3	2.3	2.77
Spent yeast	0.02	0.02	0.02
Beer	< 0.01	< 0.01	< 0.01

The pydiflumetofen residues before processing were calculated as mean of the individual residues found for the grain samples prior to processing:

^a

sample No.: S13-02518-01-030A-B2-A-028 and -029

^b

sample No.: S13-02518-01-030B-B2-B-036 and -037

^c

sample No.: S13-02518-01-030C-B2-C-042 and -043

^d

sample No.: S13-02518-01-031A-F1-A-058 and -059

^e

sample No.: S13-02518-01-031B-F1-B-064 and -065

^f

sample No.: S13-02518-01-031A-F1-C-070 and -071

Table 7-127 Processing factors for pydiflumetofen – Field trial S13-02518-02

Processing product	pydiflumetofen residue (mg/kg)		Processing Factor
	RD-Mo	RD-RA	
Field Specimen S13-02516-02-030 (Balance)			
Pearl barley production			
RAC – Barley grain	1.7 ^g	1.7 ^g	-
Cleaned grain	1.3	1.3	0.76
Offal (impurities)	7	7	4.12
Abrasion dust (rub off)	6.2	6.2	3.65
Pearl barley	0.11	0.11	0.06
Bran	0.23	0.23	0.14
Flour	5.3	5.3	3.12
Pot barley production			
RAC – Barley grain	2.3 ^h	2.3 ^h	-
Abrasion dust (rub off)	8.6	8.6	3.74
Pot barley	0.13	0.13	0.06
Bran	0.34	0.34	0.15
Flour	6.0	6.0	2.61
Beer production			
RAC – Barley grain	1.6 ⁱ	1.6 ⁱ	-
Cleaned grain	1.4	1.4	0.88
Offal	11	11	6.88
Malt with sprouts	0.44	0.44	0.28
Malt sprouts	1.1	1.1	0.69
Cleaned malt	0.53	0.53	0.33
Malt directly before brewing	0.48	0.48	0.30
Spent grain (brewing grain)	0.44	0.44	0.28
Dried brewers grain	3.2	3.2	2

Wort before cooking	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	<0.01
Wort after cooking	<0.01 (<0.01, 0.01)	<0.01 (<0.01, 0.01)	<0.01
Spent hops (flocs)	0.43	0.43	0.27
Spent yeast	0.04	0.04	0.03
Young beer	<0.01	<0.01	<0.01
Beer	< 0.01	< 0.01	< 0.01
Field Specimen S13-02516-02-031 (Follow up)			
Pearl barley production			
RAC – Barley grain	1.5 ^j	1.5 ^j	-
Cleaned grain	1.1	1.1	0.73
Pearl barley	0.08	0.08	0.05
Bran	0.27	0.27	0.18
Flour	4.8	4.8	3.20
Pot barley production			
RAC – Barley grain	1.9 ^k	1.9 ^k	-
Abrasion dust (rub off)	7.1	7.1	3.74
Pot barley	0.12	0.12	0.06
Bran	0.36	0.36	0.19
Flour	6.3	6.3	3.32
Beer production			
RAC – Barley grain	1.5 ^l	1.5 ^l	-
Malt sprouts	1.1	1.1	0.73
Cleaned malt	0.47	0.47	0.31
Malt directly before brewing	0.42	0.42	0.28
Dried brewers grain	2.8	2.8	1.87
Spent yeast	0.03	0.03	0.02
Beer	< 0.01	< 0.01	< 0.01

The pydiflumetofen residues before processing were calculated as mean of the individual residues found for the grain samples prior to processing:

^g sample No.: S13-02518-02-030A-B4-A-105 and -106

^h sample No.: S13-02518-02-030B-B4-B-113 and -114

ⁱ sample No.: S13-02518-02-030C-B4-C-119 and -120

^j sample No.: S13-02518-02-031A-F2-A-135 and -136

^k sample No.: S13-02518-02-031B-F2-B-141 and -142

^l sample No.: S13-02518-02-031C-F2-C-147 and -148

Table 7-128 Overall processing factors

Crop (RAC)/Processed product	Number of studies ^(a)	Processing Factor (PF)	
		Individual values	Median PF
Barley/Cleaned grain	2 (4)	0.73, 0.76, 0.82, 0.82	0.79
Barley/Pearl barley offal (impurities)	2	3.27, 4.12	3.71
Barley/Pearl barley abrasion dust (rub off)	2	2.64, 3.65	3.15
Barley/Pearl barley	2 (4)	0.05, 0.06, 0.12, 0.14	0.09
Barley/ Pearl barley bran	2 (4)	0.13, 0.14, 0.16, 0.18	0.15
Barley/Pearl barley flour	2 (4)	1.82, 2.39, 3.12, 3.20	2.76 2.78
Barley/Pot barley abrasion dust (rub off)	2 (4)	3.55, 3.74, 3.74, 5.06	3.74
Barley/Pot barley	2 (4)	0.06, 0.06, 0.12, 0.19	0.09
Barley/Pot barley bran	2 (4)	0.15, 0.16, 0.19, 0.21	0.18
Barley/Pot barley flour	2 (4)	2.32, 2.61, 2.82, 3.03, 3.32	2.93
Barley/Cleaned grain for beer	2	0.73, 0.88	0.81
Barley/Offal for beer	2	3.08, 6.88	4.98
Barley/Malt with sprouts	2	0.23, 0.28	0.26
Barley/Malt sprouts	2 (4)	0.54, 0.56, 0.69, 0.73	0.63
Barley/Cleaned malt	2 (4)	0.30, 0.31, 0.33, 0.54	0.32
Barley/Malt directly before brewing	2 (4)	0.22, 0.28, 0.30, 0.42	0.29
Barley/Spent grain (brewing grain)	2	0.25, 0.28	0.27
Barely/Dried brewers grain	2 (4)	1.5, 1.87, 2, 2.77	1.94
Barley/Wort before cooking	2	<0.01, <0.01	<0.01
Barley/Wort after cooking	2	<0.01, <0.01	<0.01
Barley/Spent hops (flocs)	2	0.23, 0.27	0.25
Barley/Spent yeast	2 (4)	0.02, 0.02, 0.02, 0.03	0.02
Barley Young beer	2	<0.01, <0.01	<0.01
Barley/Beer	2 (4)	< 0.01, < 0.01, < 0.01, < 0.01	< 0.01

^a Number of studies signifies number of independent trials. Figure in brackets gives the number of individual processed fractions assessed (includes both main trial and follow ups from each independent trial)

B.7.5.3.2. Wheat

Report:	KCA1 6.5.3; [REDACTED]; [REDACTED]; [REDACTED]; [REDACTED]; 2017
Title:	SYN545974 - Residue Study on Wheat in Northern France and the United Kingdom in 2013
Report No.:	S13-02516
Document No.:	VV-467692, A17573A_10005
Guidelines:	<p>Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document).</p> <p>Guidelines and Criteria for the Preparation and Presentation of Complete Dossiers and of Summary Dossiers for the Inclusion of Active Substances in Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009.</p> <p>European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000).</p> <p>OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009</p> <p>OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)</p> <p>EU 1999: 1607/VI/97</p> <p>OECD Test Guideline 508: Magnitude of the Pesticide Residues in Processed Commodities.</p> <p>OECD (2008). Guidance document on magnitude of pesticide residues in processed commodities. Environment, Health and Safety Publications. Series on Testing and Assessment No. 96.</p> <p>Commission of the European Communities, Processing Studies; 7035/VI/95 (rev.5, working document).</p> <p>The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO (2002) 9.</p> <p>The national requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.</p>
Guideline deviations:	None
GLP/GEP:	Yes

I. Materials and Methods

The study contained four field trials on wheat conducted in northern Europe, carried out in the 2012-2013 growing season; samples from two of these trials were used for determinations of pydiflumetofen on wheat (whole plant, grain, straw) and processed fractions.

Field part

In these four trials, one untreated control plot was used for the generation of the control samples.

The study design included three treated plots for trials S13-02516-01 and S13-02516-03 and two treated plots for S13-02516-02 and S13-02516-04.

To plot 2 (all trials) application rate was 125 g ai/ha and 150 g ai/ha at BBCH 43-65 and 69 respectively, treatment interval was 14-19 days.

To plot 3 (all trials) application rate was 200 g ai/ha at BBCH 69.

To plot 4, trials S13-02516-01 and S13-02516-03 only, application rate was 375 g ai/ha and 450 g ai/ha at BBCH 69 and 77-79 respectively, treatment interval was 14-21 days.

Trials on Plot 4 were used to determine the magnitude of residues of pydiflumetofen (also known as SYN545974) on wheat (whole plant, grain, straw) and processed fractions.

The following summaries cover only the control plot and the plots treated at higher application rates. Application data from the two higher application rate supervised residue trials are presented in the following table (Table 7-129).

Table 7-129 Planned application scenario for higher application rate trials

Trial No. / Location / EU zone / Year	Commo dity / Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting	Application rate per treatment			Dates of treatment / Application interval	Growth stage at last treatment (BBCH)	Details on trial
			g a.s./ha	Water (L/ha)	g a.s./hL			
(a)	(b)					(c)	(d)	(f)
S13-02516-01 Mespuits, Ile de France, France, 91150	Wheat - Hystar	1, 7/11/2012	369	197		14/6/2013	69	Backpack / Broadcast Foliar Spray A17573A 100 EC No adjuvants used
		2, 7-14/6/2013 3, 6/8/2013	451	200		5/7/2013	77	
S13-02516-03 Church Lawford, Warwickshire, UK, CV23 9HD	Wheat - Granary	1, Not recorded	380	203		15/7/2012	69	Backpack / Broadcast Foliar Spray A17573A 100 EC No adjuvants used
		2, Jun – Jul 2013 3, 4/9/2013	449	199		29/7/2012	79	

Treated samples of grain for processing were collected from plot P4 at NCH (31-37 DALA) from trials S13-02516-01 and -03 (2 x 51 kg from trial S13-02516-01 and ~30 kg and 27 kg from trial S13-02516-03). One sample for balance study and one sample for follow-up study were collected from plot P4 for each trial.

For all trials, untreated samples of whole plant (>1 kg) were collected from the control plot C1 immediately before last application (0 DBLA) and untreated samples of grain (>1 kg) and straw (>0.5 kg) were collected at NCH (42-52 DALA). For trials S13-02516-01 and -03, untreated samples of grain (81 kg and 72 kg respectively) were also collected at NCH from the control plot C1 at 51-52 DALA which coincided with the sampling day of the equivalent treated samples for processing from plot P4.

Field samples for processing were shipped and stored at ambient temperature for Ca. 3-4 months, which was stated in the study as being representative of normal commercial practice. Positive residues were observed in both RAC and processed fractions following this storage, in line with the levels observed in the RAC frozen samples.

Relevant practices and standardised procedures were applied in this processing phase which simulated the common industrial processes at a laboratory scale.

The processing of the wheat specimens was performed at the test site and in a specialised pilot plant at the following processing location:

BioChem agrar GmbH
Kupferstraße 6
04827 Gerichshain, Germany

Each field specimen for processing was separated dependent on the processing destination. The respective final products for special processing parts are shown below:

Table 7-130 Field specimen amount and processing destination

Specimen Code (field phase)	Specimen weight for division	Specimen No. for processing Specimen weight (approx.)			
		Flour (type 550)	Whole-meal flour and whole-meal bread	Wheat germ	Production of starch and gluten
S13-02516-01-035 (Balance untreated) C1	80.45 kg	S13-02516-01-035-A 13.03 kg	S13-02516-01-035-B 14.77 kg	S13-02516-01-035-C 21.62 kg	S13-02516-01-035-D 12.34 kg
S13-02516-01-046 (Balance treated) P4	51.08 kg	S13-02516-01-046-A 10.06 kg	S13-02516-01-046-B 10.18 kg	S13-02516-01-046-C 20.41 kg	S13-02516-01-046-D 10.24 kg
S13-02516-01-047 (Follow up treated) P4	52.55 kg	S13-02516-01-047-A 10.45 kg	S13-02516-01-047-B 10.69 kg	S13-02516-01-047-C 20.95 kg	S13-02516-01-047-D 10.44 kg
S13-02516-03-035 (Balance untreated) C1	71.50 kg	S13-02516-03-035-A 12.41 kg	S13-02516-03-035-B 13.89 kg	S13-02516-03-035-C 22.60 kg	S13-02516-03-035-D 12.65 kg
S13-02516-03-046 (Balance treated) P4	29.50 kg	S13-02516-03-046-A 6.83 kg	S13-02516-03-046-B 6.68 kg	S13-02516-03-046-C 10.51 kg	S13-02516-03-046-D 5.40 kg
S13-02516-03-047 (Follow up treated) P4	27.01 kg	S13-02516-03-047-A 6.00 kg	S13-02516-03-047-B 6.00 kg	S13-02516-03-047-C 10.00 kg	S13-02516-03-047-D 5.00 kg

C1 = untreated; P4 = treated with formulation A17573A

The specimens were stored immediately after processing at or below -18 °C until shipment to the specimen consignee. The specimens were dispatched deep-frozen and arrived deep-frozen on the same day.

Processed specimens (including grain prior to processing) were stored frozen for a maximum period of 10 months from sampling to analysis for pydiflumetofen. These storage periods are covered by storage stability trials summarised in section B.7.1 which shows stability in all matrices for 23 months

Extract solutions of field specimens were stored for a maximum of 17 days in the refrigerator before analysis of pydiflumetofen. Extract solutions of processed specimens were stored for a maximum of 14 days in the refrigerator before analysis of pydiflumetofen. The stability of the analytes in the sample extracts was proven by the corresponding procedural recovery samples, which were stored under the same conditions together with the sample extracts.

At each independent trial site there were two trials on treated crops (described by the applicant as balance and follow up trials). Balance studies (untreated and treated) and follow-up studies (treated) were carried out on each process. Balance studies determined residues in more processing fractions to provide a greater understanding of where the residues resulted arising from the processing operation. The follow up studies were focussed on the processed commodities that are consumed. Flow diagrams showing the processing stages are provided in this section.

Processing summary

Each of the separated field sample for processing was cleaned by air cleaning. Samples of aspirated grain (cleaned grain) and aspirated grain fraction (offal) were taken (balance and follow-up (flour type 550) studies). The sampled offal consisted of dust, parts of straw and husks. Afterwards the grain was shipped by car at ambient temperature from BioChem agrar to the processing location.

Conditioning

In order to obtain acceptable milling results, grain with a moisture content of approximately 15-16 % was necessary. Therefore, the cleaned grain samples for processing of trial 01 (moisture 13.0-13.7%) were moistened (addition of tap water) until the optimal moisture content was reached (15.1% - 15.9%). Cleaned grain samples for trial 03 were acceptable (15.1-15.3%). Samples of grain after conditioning were taken (only balance studies).

Milling to white flour (type 550)

In a closed system with different pairs of smooth rollers and sifter passages of the “Bühler Mahlautomat” the grain was milled to straight flour, bran (coarse bran) and middlings (fine bran). Samples of bran (coarse bran), middlings (fine bran) and straight flour were taken (only balance studies).

In a further processing step bran (coarse bran) and middlings (fine bran) were mixed together (mixed bran was sampled; only balance studies) and low grade meal was separated using a centrifuge/scouring machine. This process resulted in shorts (total bran) and low grade meal (toppings). Samples of shorts (total bran), (balance and follow-up studies) and low grade meal (toppings) (only balance studies) were taken.

After determination of the mineral content of straight flour and low grade meal, both fractions were mixed to the final product white flour (type 550) until a mineral content of 510-630 g/100 kg flour was reached. Samples of white flour were taken (balance and follow-up studies)

Milling to whole-meal flour

For the generation of whole-meal flour and whole-meal bread the same milling procedure as for the production of flour type 550 was used.

In a closed system with different pairs of smooth rollers and sifter passages of the “Bühler Mahlautomat” the grain was milled to straight flour, bran (coarse bran) and middlings (fine bran). Samples of bran (coarse bran), middlings (fine bran) and straight flour were taken (only balance studies).

In a further processing step bran (coarse bran) and middlings (fine bran) were mixed together (mixed bran was sampled; only balance studies) and low grade meal was separated using a centrifuge/scouring machine. This process resulted in shorts (total bran) and low grade meal (toppings). A sample of shorts (total bran), (only balance studies) was taken.

After milling the shorts (total bran) were cracked with an impact mill to smaller pieces. All milling products of the process were completely used for the whole-meal and mixed homogeneously. A sample of the whole-meal flour was collected (balance and follow-up studies).

Baking of whole-meal bread

For baking a 1.0 kg whole-meal bread, whole-meal (1.3 kg), yeast (52 g), salt (26 g) and water (0.91 L) were mixed. Subsequently, the resulting dough was kneaded for 7 min. After kneading the dough fermented for about 20 min. It was then moved for 10 min and a second rest for fermentation followed (40 min in a baking tin). A sample of dough was taken (only balance studies). The baking process conducted at 210°C for 50 min. A sample of whole-meal bread was taken (balance and follow-up studies).

Production of wheat germs

In the first step in the production of wheat germs the grain was broken to bruised grain in a special mill (roller mill with 0.5 mm roller distance). The fraction 400-1000 µm was collected; the fraction above 1000 µm was broken once more (0.3 mm roller distance). This milling/sieving process was performed in total three times, with a final roller distance of 0.2 mm. The fractions obtained below 400 µm and the last fraction above 1000 µm were excluded completely from further processing. Samples of the fractions of bruised grain (> 1000 µm, 400 – 1000 µm and < 400 µm) were taken (only balance studies) as specimens.

The fraction 400-1000 µm, a mixture of bran, middlings and germs, was fed to a special separator (Leichtgewichtsausleser). Due to the different specific weights of the bran, middlings and germs, this allowed separation of the middlings/germ mixture from most parts of the bran. Samples of middlings/germ mixture and bran were collected (only balance studies).

Subsequently, in a mill with a pair of smooth rollers the middlings/germ mixture was milled to flour, bran and small wheat germ discs. This product was then sieved to allow separation of the various fractions. The first sieving

step resulted in flour and a bran/germ fraction, from which samples were taken (only balance studies). The bran/germ fraction was sieved once again to fine bran/germ fraction and coarse bran/germ fraction, from which samples were collected (only balance studies).

From the separated germ discs small parts of bran were removed manually.

Samples of wheat germs (balance and follow up studies) and remaining bran (only balance studies) were taken.

Production of starch and gluten

The first step of the production of starch and gluten was milling the grain to straight flour, bran (coarse bran) and middlings (fine bran). Samples of straight flour, bran (coarse bran) and middlings (fine bran) were taken (only balance studies).

1000 g of straight flour and 1200 g water were mixed to obtain a hydrated dough. The dough was separated by centrifugation into wet starch, process water I and gluten (containing starch). A sample of process water I was taken (only balance studies).

Subsequently, the starch was washed out (washing I) with 1500 g water and was separated by centrifugation into starch A, process water II and gluten. This process was repeated. The starch was washed out (washing II) with 1500 g water and was separated by centrifugation into starch A, process water III and gluten. A sample of wet starch A was taken (only balance studies).

The gluten (containing starch) of the process was washed out with the process water II and was resulting in gluten after washing and process water IV (containing starch B and fibre). This process was repeated. The gluten (containing starch) of the process was washed out with the process water III and was resulting in gluten after washing and process water V (containing starch B and fibre). A sample of wet gluten was taken (only balance studies).

Remaining process water IV + V were separated by centrifugation into starch B, fibre and process water VI. Subsequently fibre was washed out with process water VI into wet starch B, fibre and process water VII. Samples of wet starch B and process water VII were taken (only balance studies). Afterwards fibre was dried at 60 °C and milled. A sample of dried milled fibre was taken (only balance studies). Wet starch (A and B) was dried at approx. 60 °C and wet gluten was dried by freeze drying.

After the drying process the dried products were milled. Starch A, starch B and gluten were collected as sample (balance and follow-up studies). The dried and milled fraction fibre and milled starch B and gluten were mixed, in the ratio in which they were generated after drying / milling, to gluten feed meal. Samples of gluten feed meal were taken (balance and follow-up studies).

Processing flowcharts

Figure 7-10 Grain processing (Part I)

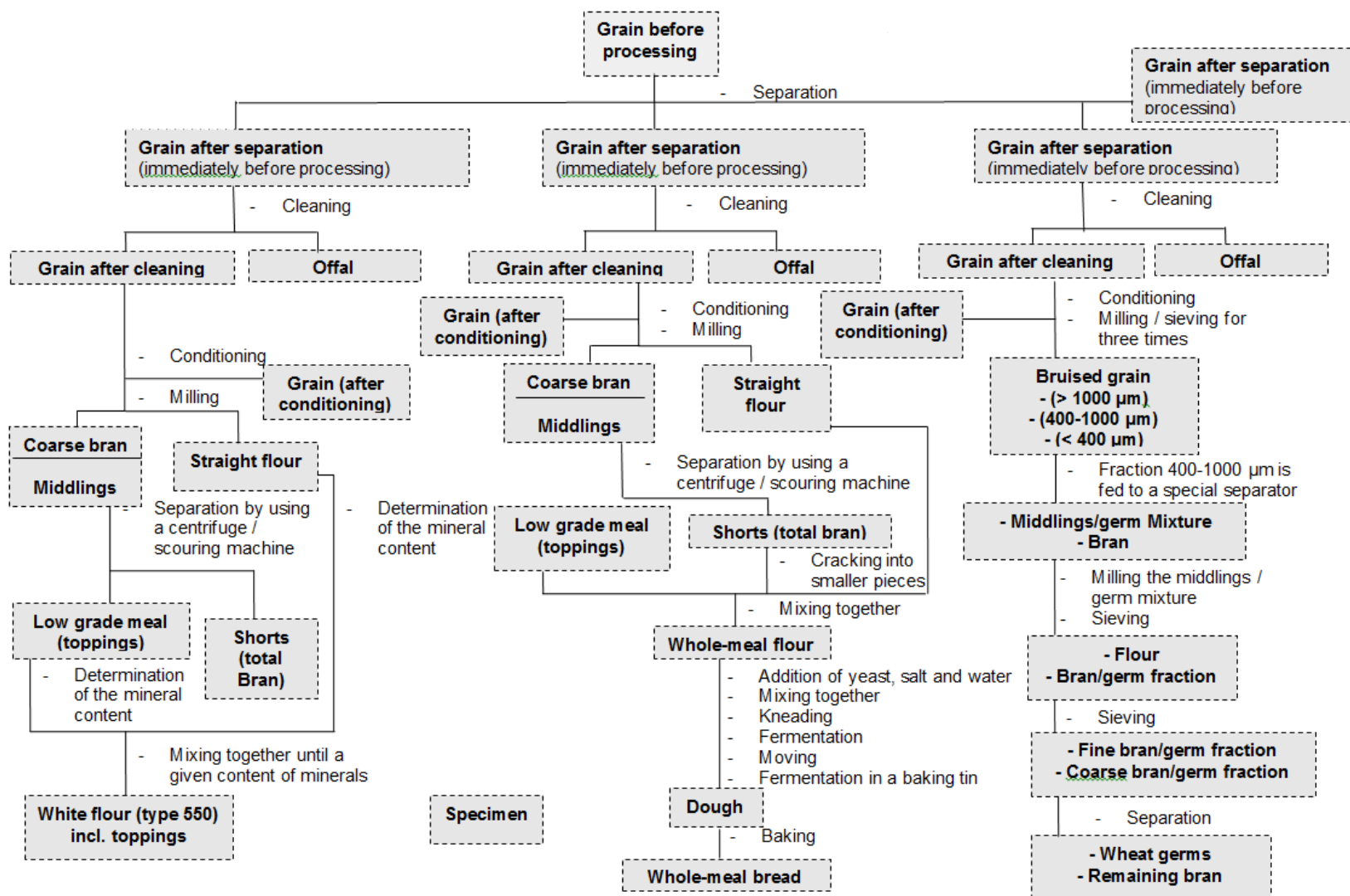


Figure 7-11 Grain processing (Part II)

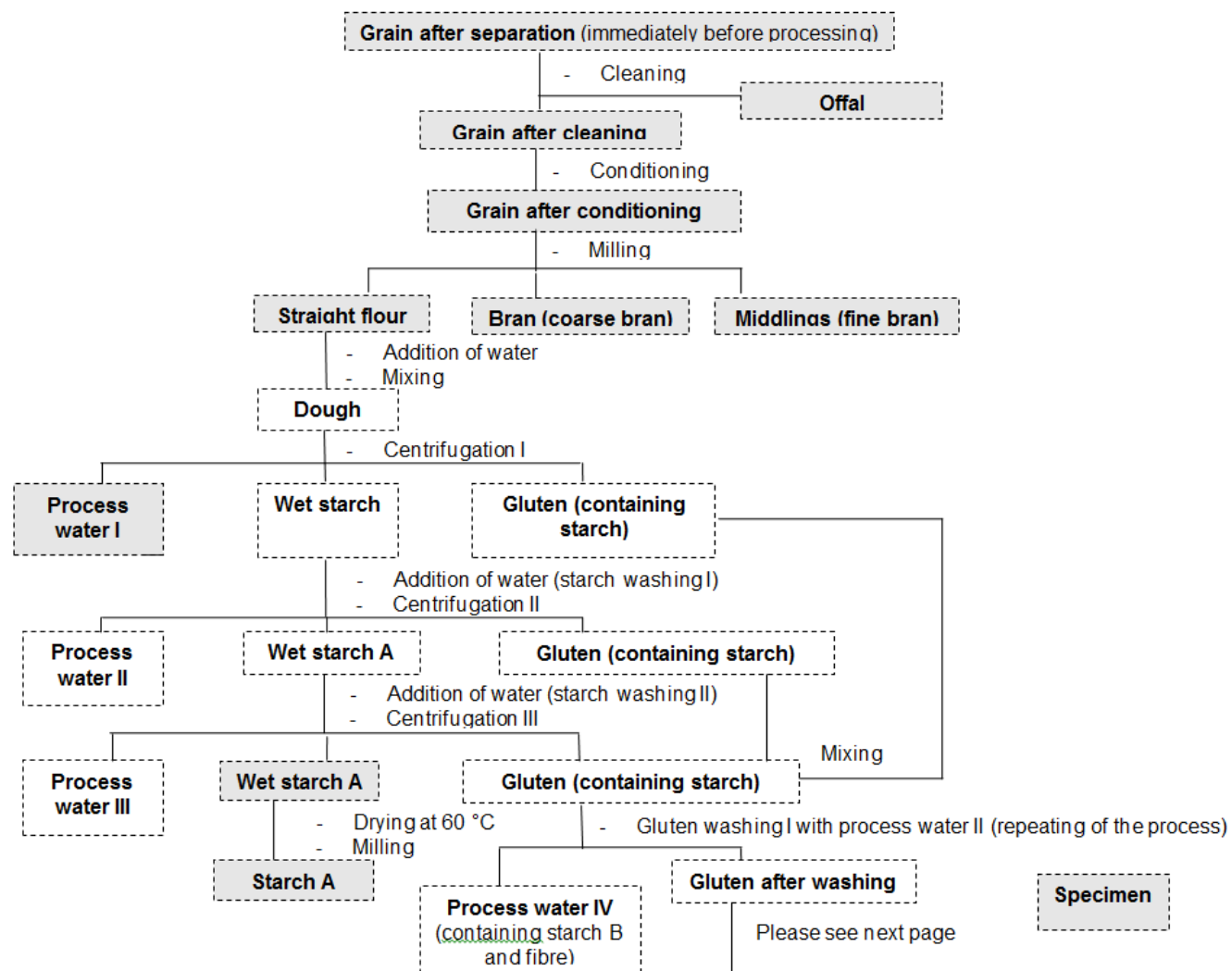
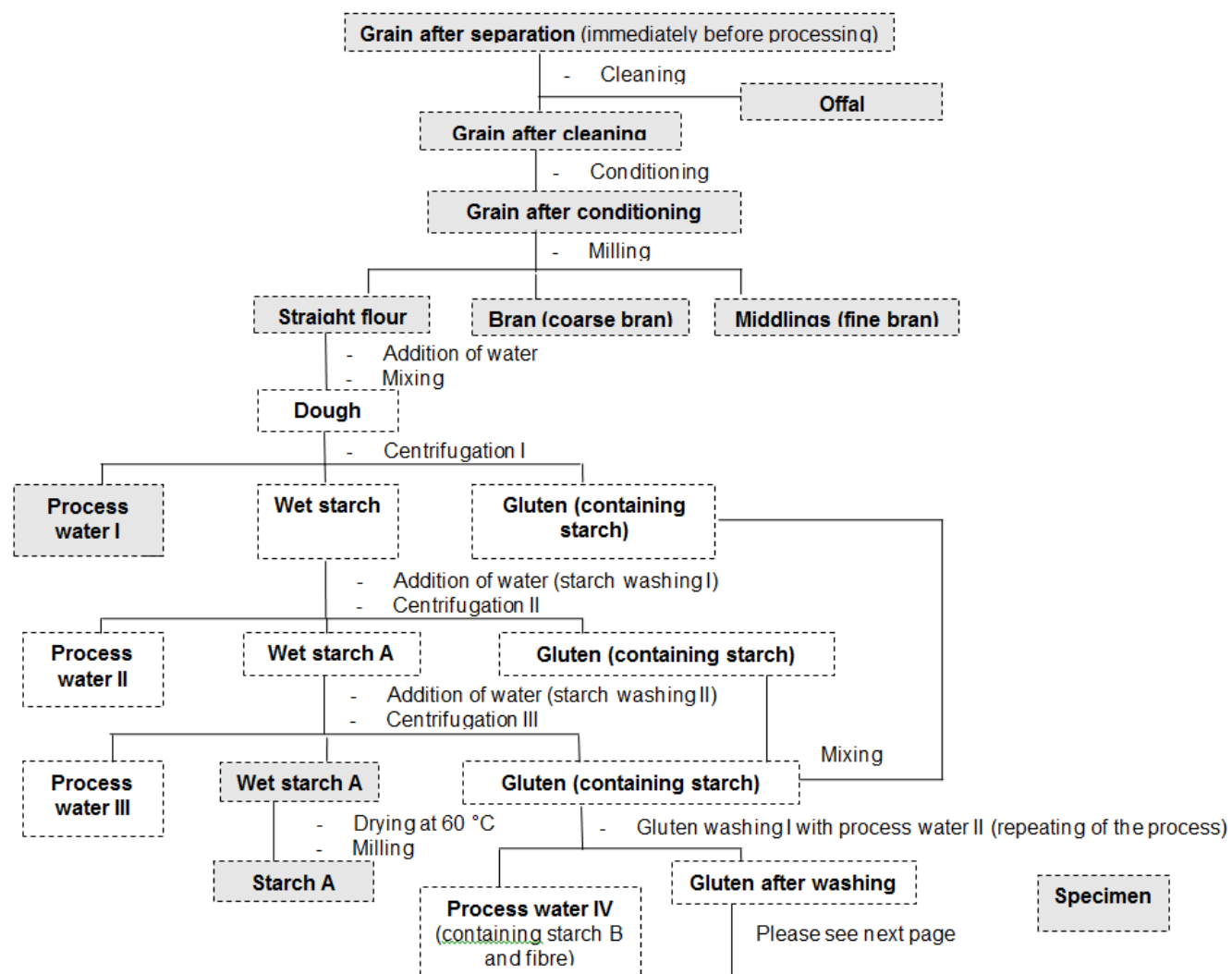


Figure 7-12 Grain processing (Part III)



II. Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Method GRM061.03A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch/dry (cereal grain and potato), high protein (dry beans) and dry (cereal straw) commodities, with an LOQ of 0.01 mg/kg – see B 5.1.2.5. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. Note that for processed fractions, all samples should include a reduced validation, consisting of two fortifications and three repeats. This reduced data has not been supplied, however given the large number procedural recovery results and the large number of processed fractions, the data on the whole is considered sufficient.

Details on concurrent recovery data are shown in Table 7-131. Average recoveries were only available for a limited number of substrates, these were all within the acceptable range of mean recoveries of 70 – 110% (SANCO 3029-99 Rev.4) 70 – 110% (except grain after separation, immediately before processing, fortified at 0.01 mg/kg), as were the single values which were not used in calculations of average values (with the exception of low grade meal, fortified at 0.1 mg/kg). RSD could not be calculated due to the limited number of samples.

Table 7-131 Recovery data for pydiflumetofen

Substrate (control)	Fortification level (mg/kg)	pydiflumetofen (%)
Whole plant	0.01	77, 97; Mean 87
	0.1	76, 85; Mean 81
	5	92
Straw	0.01	86
	0.1	102
	8	92
Wheat grain	0.01	96
	0.10	91
	0.5	69, 80; Mean 75
Grain after separation (immediately before processing)	0.01	130, 106; Mean 118
	0.10	74, 88; Mean 81
Aspirated grain (cleaned grain)	0.01	88
	0.10	83
Grain (after conditioning)	0.01	101
	0.10	84
Aspirated grain fraction (offal)	0.01	91
	0.10	91
	2.0	94
Middlings (fine bran)	0.10	90
Bran (coarse bran)	0.01	91
Shorts (total bran)	0.01	77
	0.10	87
	2.0	103
Bruised grain (400-1000 µm)	0.01	104
	0.1	73
Mixed bran	0.01	95
	0.1	74
Bran/germ fraction	0.01	97
	0.1	83
Fine bran/germ fraction	0.01	88
Coarse bran/germ fraction	0.10	81
Germs	0.01	101
	0.10	98
	0.50	89
	0.01	104

Low grade meal (toppings)	0.1	120
	0.5	90
Flour	0.01	110
	0.10	78
White flour (type 550) incl. toppings	0.01	89
	0.10	83
Dough	0.01	71
	0.10	83
Wholemeal bread	0.01	107
	0.10	93
Starch A	0.01	80
Starch B	0.10	83
Dried fibre	0.01	71
	0.10	75
Gluten	0.01	90
Gluten feed meal	0.10	109
Gluten meal	0.01	100
	0.5	98
Wet starch A	0.01	93
Wet starch B	0.10	89
Process Water I	0.01	73
Process water VII	0.10	83

Residues

No residues of pydiflumetofen at or above the LOQ (0.01 mg/kg) were found in any of the untreated wheat samples taken at 0 DBLA (whole plant), at 42-46 DALA (grain and straw) and at NCH (grain and straw), with the exception of the wheat grain and straw samples S13-02516-01-019 and -021 at 42 DALA where residues of 0.01 mg/kg were found for pydiflumetofen in both samples. Because of the very low level of residues found in the control samples compared to the treated samples and the fact that no residues were found at NCH in neither grain or straw control samples, this contamination has no impact on the level of residues found in the treated samples and consequently no impact on the study.

Note that the applicant contended that contamination may have occurred through spray drift from earlier applications.

The residues of pydiflumetofen in the treated wheat (grain) samples taken from plot P4 from trials S13-02516-01 and -03 at 31-37 days after the last application (DALA), normal commercial harvest (NCH) were in the range of 0.22 to 0.26 mg/kg.

No residues of pydiflumetofen at or above the limit of quantification (LOQ, 0.01 mg/kg) were found in any of the untreated processing samples.

Residues of pydiflumetofen in the duplicate treated wheat (grain) samples taken after separation before processing were in the range of 0.04 to 0.40 mg/kg.

Residues of pydiflumetofen in the processing end product white flour (type 550) samples were in the range of 0.01 to 0.04 mg/kg, residues in whole-meal flour samples were in the range of 0.03 to 0.15 mg/kg, residues in whole meal bread samples were in the range of 0.05 to 0.17 mg/kg, residues in wheat germs samples were in the range of 0.05 to 0.24 mg/kg, residues in starch A samples were < 0.01 mg/kg and the residues in gluten samples were in the range of 0.06 to 0.22 mg/kg.

Some processing factors shows a large difference (>50%) between trials (based on both the individual values from the balance and follow up trials and their mean values) so in principle a third trial should be performed, and the median value reported. This was not considered necessary due to the data supplied from the follow up trials giving a total of four separate values from two independent trial sites.

Note that the values given below for the overall transfer factors (Table 7-136) show data from two independent test sites (and some commodities have results for both balance and follow up studies). As the follow up studies

have independently gone through the same processing as the balance trials and as the difference between the trials are not irreconcilable (10 fold different according to OECD 508) the median transfer factor was considered most appropriate and has been calculated.

The processing factors are summarised in Table 7-135.

Field specimens (RAC for the trial that were analysed separately to the processed samples) were analysed following frozen storage, within an acceptable timeframe that was covered by storage stability studies (see section B.7.1).

The maximum time field samples for processing were stored at ambient temperature prior to the start of processing was < Ca.3-4 months, this is considered acceptable as sufficient levels of detectable incurred residues were present in the samples prior to processing.

Processed specimens (including grain prior to processing) were stored frozen for a maximum period of 10 months from sampling to analysis for pydiflumetofen. These storage periods are covered by storage stability trials summarised in section B.7.1, which shows stability in all matrices for 23 months.

III. Conclusions

The study contained four field trials on wheat (two for processing studies) conducted in northern Europe (France and UK) and was carried out to generate results on the magnitude of residues and to generate samples for processing and subsequent residue analysis.

Determinations of pydiflumetofen on wheat (whole plant, grain, straw) and processed fractions were undertaken to evaluate the magnitude of residues following application and allow calculation of mass balances and residue transfer factors.

The plots designated for the processing study, plot 4 in trials S13-02516-01 and S13-02516-03, had an application rate of 375 g ai/ha and 450 g ai/ha at BBCH 69 and 77-79 respectively, treatment interval was 14-21 days.

The study was conducted according to GLP.

The results of the study indicate that residues of pydiflumetofen do not concentrate in the processing of wheat into grain after conditioning, straight flour, Flour (type 550), aspirated grain, whole meal flour, dough and whole meal bread, wheat germs, starch or gluten (processing factors between 0.11 and 0.95) but does concentrate in offal, fine bran, coarse bran, mixed bran, total bran and toppings (pf between 1.01 and 13.16)

Table 7-132 Field samples

Trial number	Sample number	Number and nominal rate of application (g ai/ha)	Sampling interval (days)	Crop part	pydiflumetofen residue (mg/kg)
S13-02516-01	Treated plot (P4)				
	044	1 x 375; 1 x 450	NCH (31 DALA)	Grain	0.22
	Control plot (C1)				
	001	Control	0 DBLA	Whole plant	<0.01
	021	Control**	42 DALA	Straw	0.01
	019	Control**	42 DALA	Grain	0.01# (0.01, 0.01)
	033	Control	NCH (52 DALA)	Straw	<0.01
	031	Control	NCH (52 DALA)	Grain	<0.01

S13-02516-03	Treated plot (P4)				
	045	1 x 375; 1 x 450	NCH (37 DALA)	Grain	0.26
	Control plot (C1)				
	001	Control	0 DBLA	Whole plant	<0.01
	021	Control	46 DALA	Straw	<0.01
	019	Control	46 DALA	Grain	<0.01
	033	Control	NCH (51 DALA)	Straw	<0.01
	031	Control	NCH (51 DALA)	Grain	<0.01

Plot C1: Untreated, Plot P2-P4: Treated with formulation A17573A

DBLA – Days before last application; DALA – Days after last application; NCH – Normal commercial harvest

No correction of results for either control residues or recovery values has been performed.

Mean of two results (duplicate analyses from single sample)

** Because of the very low level of pydiflumetofen residues found in the control grain and straw samples at 42 DALA and the fact that no residues were found at NCH in neither grain or straw samples, this contamination had no impact on the level of residues found in the treated samples and consequently no impact on the study.

Table 7-133 Treated processing samples

Trial number	Event	Crop part	pydiflumetofen residue (mg/kg)
13-02516-01	Field specimen S13-02516-01-046 (Balance Trial)		
	Mass balance / flour (type 550)		
	Separation	Grain after separation *	0.10
	Separation	Grain after separation *	0.08
	Conditioning	Aspirated grain fraction (offal)	0.22
	Conditioning	Aspirated grain (cleaned grain)	0.07
	Conditioning	Grain (after conditioning)	0.07
	Milling	Straight flour	0.01
	Milling	Middlings (fine bran)	0.18
	Milling	Bran (coarse bran)	0.25
	Milling	Mixed bran	0.32
	Separation	Shorts (total bran)	0.38
	Determination of minerals	Low grade meal (toppings)	0.04
	Mixing of given minerals	White flour (type 550) incl. toppings	0.01
	Mass balance / whole meal flour and whole meal bread		
	Separation	Grain after separation *	0.09
	Separation	Grain after separation *	0.09
	Cleaning	Aspirated grain fraction (offal)	0.37
	Cleaning	Aspirated grain (cleaned grain)	0.07
	Conditioning	Grain (after conditioning)	0.10
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	0.14
	Milling	Bran (coarse bran)	0.26
	Milling	Mixed bran	0.38
	Separation	Shorts (total bran)	0.29
	Cracking	Low grade meal (toppings)	0.23
	Mixing	Whole meal flour	0.03
	Baking	Dough	0.03
	Baking	Whole meal bread	0.05
	Mass balance / Wheat germs		
	Separation	Grain after separation *	0.09

	Separation	Grain after separation *	0.06
	Cleaning	Aspirated grain fraction (offal)	1.6
	Cleaning	Aspirated grain (cleaned grain)	0.08
	Conditioning	Grain (after conditioning)	0.08
	Conditioning	Bruised grain (400-1000 µm)	0.05
	Conditioning	Bruised grain (>1000 µm)	0.17
	Conditioning	Bruised grain (<400 µm)	0.03
	Separation	Middlings/germ mixture	0.07
	Milling and sieving	Bran	0.13
	Milling and sieving	Flour	0.03
	Sieving	Bran/germ fraction	0.10
	Sieving	Fine bran/germ fraction	0.11
	Sieving	Coarse bran/germ fraction	0.08
	Separation	Remaining bran	0.10
	Separation	Germ	0.05
	Mass balance / Starch and gluten		
	Separation	Grain after separation *	0.05
	Separation	Grain after separation *	0.04
	Cleaning	Aspirated grain fraction (offal)	1.1
	Cleaning	Aspirated grain (cleaned grain)	0.08
	Conditioning	Grain (after conditioning)	0.07
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	0.24
	Milling	Bran (coarse bran)	0.27
	Centrifugation	Wet starch A	<0.01
	Drying and milling	Starch A	<0.01
	Washing	Wet starch B	<0.01
	Drying and milling	Starch B	0.01
	Drying and milling	Dried fibre	0.01
	Washing	Wet gluten	0.02
	Freeze-drying and milling	Gluten	0.06
	Mixing	Gluten feed meal	0.01
	Centrifugation	Process water I	<0.01
	Washing	Process water VII	<0.01
	Field Specimen S13-02516-01-047 (Follow up Trial)		
	Follow up / Flour (type 550)		
	Separation	Grain after separation *	0.12
	Separation	Grain after separation *	0.14
	Cleaning	Aspirated grain fraction (offal)	0.27
	Cleaning	Aspirated grain (cleaned grain)	0.05
	Separation	Shorts (total bran)	0.49
	Mixing of given minerals	White flour (type 550) incl. toppings	0.02
	Follow up / Whole meal flour and whole meal bread		
	Separation	Grain after separation *	0.12
	Separation	Grain after separation *	0.12
	Mixing	Whole meal flour	0.05
	Baking	Whole meal bread	0.06
	Follow up / Wheat germs		
	Separation	Grain after separation *	0.04
	Separation	Grain after separation *	0.09
	Separation	Germ	0.07
	Follow up / Starch and gluten		

	Separation	Grain after separation *	0.08
	Separation	Grain after separation *	0.10
	Drying and milling	Starch A	<0.01
	Drying and milling	Starch B	<0.01
	Freeze-drying and milling	Gluten	0.11
	Mixing	Gluten feed meal	0.02
13-02516-03	Field specimen S13-02516-03-046 (Balance Trial)		
	Mass balance / Flour (type 550)		
	Separation	Grain after separation *	0.20
	Separation	Grain after separation *	0.25
	Conditioning	Aspirated grain fraction (offal)	0.65
	Conditioning	Aspirated grain (cleaned grain)	0.2
	Conditioning	Grain (after conditioning)	0.14
	Milling	Straight flour	0.03
	Milling	Middlings (fine bran)	0.65
	Milling	Bran (coarse bran)	0.69
	Milling	Mixed bran	0.71
	Separation	Shorts (total bran)	0.72
	Determination of minerals	Low grade meal (toppings)	0.40
	Mixing of given minerals	White flour (type 550) incl. toppings	0.04
	Mass balance / Whole meal flour and whole meal bread		
	Separation	Grain after separation *	0.32
	Separation	Grain after separation *	0.31
	Cleaning	Aspirated grain fraction (offal)	0.72
	Cleaning	Aspirated grain (cleaned grain)	0.11
	Conditioning	Grain (after conditioning)	0.25
	Milling	Straight flour	0.03
	Milling	Middlings (fine bran)	0.71
	Milling	Bran (coarse bran)	0.64
	Milling	Mixed bran	1.6
	Separation	Shorts (total bran)	1.2
	Cracking	Low grade meal (toppings)	0.53
	Mixing	Whole meal flour	0.15
	Baking	Dough	0.12
	Baking	Whole meal bread	0.17
	Mass balance / Wheat germs		
	Separation	Grain after separation *	0.22
	Separation	Grain after separation *	0.27
	Cleaning	Aspirated grain fraction (offal)	0.58
	Cleaning	Aspirated grain (cleaned grain)	0.12
	Conditioning	Grain (after conditioning)	0.10
	Conditioning	Bruised grain (400-1000 µm)	0.08
	Conditioning	Bruised grain (>1000 µm)	0.40
	Conditioning	Bruised grain (<400 µm)	0.02
	Separation	Middlings/germ mixture	0.08
	Milling and sieving	Bran	0.16
	Milling and sieving	Flour	0.05
	Sieving	Bran/germ fraction	0.19
	Sieving	Fine bran/germ fraction	0.22
	Sieving	Coarse bran/germ fraction	0.26
	Separation	Remaining bran	0.21
	Separation	Germes	0.23
	Mass balance / Starch and gluten		
	Separation	Grain after separation *	0.40

	Separation	Grain after separation *	0.34
	Cleaning	Aspirated grain fraction (offal)	1.6
	Cleaning	Aspirated grain (cleaned grain)	0.17
	Conditioning	Grain (after conditioning)	0.11
	Milling	Straight flour	0.05
	Milling	Middlings (fine bran)	0.72
	Milling	Bran (coarse bran)	1.0
	Centrifugation	Wet starch A	<0.01
	Drying and milling	Starch A	<0.01
	Washing	Wet starch B	<0.01
	Drying and milling	Starch B	<0.01
	Drying and milling	Dried fibre	0.02
	Washing	Wet gluten	0.07
	Freeze-drying and milling	Gluten	0.22
	Mixing	Gluten feed meal	0.08
	Centrifugation	Process water I	<0.01
	Washing	Process water VII	<0.01
	Field Specimen S13-02516-03-046 (Follow up Trial)		
	Follow up / Flour (type 550)		
	Separation	Grain after separation *	0.17
	Separation	Grain after separation *	0.16
	Cleaning	Aspirated grain fraction (offal)	0.55
	Cleaning	Aspirated grain (cleaned grain)	0.17
	Separation	Shorts (total bran)	0.49
	Mixing of given minerals	White flour (type 550) incl. toppings	0.04
	Follow up / Whole meal flour and whole meal bread		
	Separation	Grain after separation *	0.23
	Separation	Grain after separation *	0.22
	Mixing	Whole meal flour	0.09
	Baking	Whole meal bread	0.11
	Follow up / Wheat germs		
	Separation	Grain after separation *	0.34
	Separation	Grain after separation *	0.31
	Separation	Germes	0.24
	Follow up / Starch and gluten		
	Separation	Grain after separation *	0.30
	Separation	Grain after separation *	0.31
	Drying and milling	Starch A	<0.01
	Drying and milling	Starch B	<0.01
	Freeze-drying and milling	Gluten	0.15
	Mixing	Gluten feed meal	0.05

No correction of results for either control residues or recovery values has been performed.

* grain immediately before processing and after separation (the mean value of the two residues values for each process is used for calculating the transfer factors and the residue mass balances)

Table 7-134 Untreated processing samples

Trial number	Event	Crop part	pydiflumetofen residue (mg/kg)
S13-02516-01	Field Specimen S13-02516-01-035 (Balance Trial)		
	Mass balance / Flour (type 550)		
	Separation	Grain after separation *	<0.01
	Conditioning	Aspirated grain fraction (offal)	<0.01
	Conditioning	Aspirated grain (cleaned grain)	<0.01

	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Milling	Mixed bran	<0.01
	Separation	Shorts (total bran)	<0.01
	Determination of minerals	Low grade meal (toppings)	<0.01
	Mixing of given materials	White flour (type 550) incl. toppings	<0.01
	Mass balance / Whole meal flour and whole meal bread		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Milling	Mixed bran	<0.01
	Separation	Shorts (total bran)	<0.01
	Cracking	Low grade meal (toppings)	<0.01
	Mixing	Whole meal flower	<0.01
	Baking	Dough	<0.01
	Baking	Whole meal bread	<0.01
	Mass balance / Wheat germs		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Conditioning	Bruised grain (400-1000 µm)	<0.01
	Conditioning	Bruised grain (>1000 µm)	<0.01
	Conditioning	Bruised grain (<400 µm)	<0.01
	Separation	Middlings / germ mixture	<0.01
	Milling and sieving	Bran	<0.01
	Milling and sieving	Flour	<0.01
	Sieving	Bran / germ fraction	<0.01
	Sieving	Fine bran / germ fraction	<0.01
	Sieving	Coarse bran / germ fraction	<0.01
	Separation	Remaining bran	<0.01
	Separation	Germs	<0.01
	Mass balance / Starch and gluten		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Centrifugation	Wet starch A	<0.01
	Drying and milling	Starch A	<0.01
	Washing	Wet starch B	<0.01
	Drying and milling	Starch B	<0.01
	Drying and milling	Dried fibre	<0.01
	Washing	Wet gluten	<0.01
	Freeze-drying and milling	Gluten	<0.01
	Mixing	Gluten feed meal	<0.01

	Centrifugation	Process water I	<0.01
	Washing	Process water VII	<0.01
S13-02516-03	Field Specimen S13-02516-03-035 (Balance Trial)		
	Mass balance / Flour (type 550)		
	Separation	Grain after separation *	<0.01
	Conditioning	Aspirated grain fraction (offal)	<0.01
	Conditioning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Milling	Mixed bran	<0.01
	Separation	Shorts (total bran)	<0.01
	Determination of minerals	Low grade meal (toppings)	<0.01
	Mixing of given materials	White flour (type 550) incl. toppings	<0.01
	Mass balance / Whole meal flour and whole meal bread		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Milling	Mixed bran	<0.01
	Separation	Shorts (total bran)	<0.01
	Cracking	Low grade meal (toppings)	<0.01
	Mixing	Whole meal flower	<0.01
	Baking	Dough	<0.01
	Baking	Whole meal bread	<0.01
	Mass balance / Wheat germs		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Conditioning	Bruised grain (400-1000 µm)	<0.01
	Conditioning	Bruised grain (>1000 µm)	<0.01
	Conditioning	Bruised grain (<400 µm)	<0.01
	Separation	Middlings / germ mixture	<0.01
	Milling and sieving	Bran	<0.01
	Milling and sieving	Flour	<0.01
	Sieving	Bran / germ fraction	<0.01
	Sieving	Fine bran / germ fraction	<0.01
	Sieving	Coarse bran / germ fraction	<0.01
	Separation	Remaining bran	<0.01
	Separation	Germs	<0.01
	Mass balance / Starch and gluten		
	Separation	Grain after separation *	<0.01
	Cleaning	Aspirated grain fraction (offal)	<0.01
	Cleaning	Aspirated grain (cleaned grain)	<0.01
	Conditioning	Grain (after conditioning)	<0.01
	Milling	Straight flour	<0.01
	Milling	Middlings (fine bran)	<0.01
	Milling	Bran (coarse bran)	<0.01
	Centrifugation	Wet starch A	<0.01
	Drying and milling	Starch A	<0.01

	Washing	Wet starch B	<0.01
	Drying and milling	Starch B	<0.01
	Drying and milling	Dried fibre	<0.01
	Washing	Wet gluten	<0.01
	Freeze-drying and milling	Gluten	<0.01
	Mixing	Gluten feed meal	<0.01
	Centrifugation	Process water I	<0.01
	Washing	Process water VII	<0.01

No correction of results for either control residues or recovery values has been performed.

* grain immediately before processing and after separation

Table 7-135 Processing factors for pydiflumetofen

Processing product	pydiflumetofen residue (mg/kg)		Processing Factor (PF)
	RD-Mo	RD-RA	
Field Specimen S13-02516-01-046 (Balance)			
Flour (type 550) production			
RAC – Wheat grain	0.09	0.09	-
Aspirated grain fraction (offal)	0.22	0.22	2.44
Aspirated grain (cleaned grain)	0.07	0.07	0.78
Grain (after conditioning)	0.07	0.07	0.78
Straight flour	0.01	0.01	0.11
Middlings (fine bran)	0.18	0.18	2.00
Bran (coarse bran)	0.25	0.25	2.78
Mixed bran	0.32	0.32	3.26
Shorts (total bran)	0.38	0.38	4.22
Low grade meal (toppings)	0.04	0.04	0.44
Flour (type 550)	0.01	0.01	0.11
Wholemeal flour / wholemeal bread production			
RAC – Wheat grain	0.09	0.09	-
Aspirated grain fraction (offal)	0.37	0.37	4.11
Aspirated grain (cleaned grain)	0.07	0.07	0.78
Grain (after conditioning)	0.10	0.10	1.11
Straight flour	<0.01	<0.01	<0.11
Middlings (fine bran)	0.14	0.14	1.56
Bran (coarse bran)	0.26	0.26	2.89
Mixed bran	0.38	0.38	4.22
Shorts (total bran)	0.29	0.29	3.22
Low grade meal (toppings)	0.23	0.23	2.56
Whole meal flour	0.03	0.03	0.33
Dough	0.03	0.03	0.33

Whole meal bread	0.05	0.05	0.56
Wheat germ production			
RAC – Wheat grain	0.08	0.08	-
Aspirated grain fraction (offal)	1.6	1.6	20.00
Aspirated grain (cleaned grain)	0.08	0.08	1.00
Grain (after conditioning)	0.08	0.08	1.00
Bruised grain (400-1000 µm)	0.05	0.05	0.63
Bruised grain (>1000 µm)	0.17	0.17	2.13
Bruised grain (<400 µm)	0.03	0.03	0.38
Middlings/germ mixture	0.07	0.07	0.88
Bran	0.13	0.13	1.63
Flour	0.03	0.03	0.38
Bran/germ fraction	0.10	0.10	1.25
Fine bran/germ fraction	0.11	0.11	1.38
Coarse bran/germ fraction	0.08	0.08	1.00
Remaining bran	0.10	0.10	1.25
Wheat germs	0.05	0.05	0.63
Starch and gluten production			
RAC – Wheat grain	0.05	0.05	-
Aspirated grain fraction (offal)	1.1	1.1	22.00
Aspirated grain (cleaned grain)	0.08	0.08	1.60
Grain (after conditioning)	0.07	0.07	1.40
Straight flour	<0.01	<0.01	0.2
Middlings (fine bran)	0.24	0.24	4.80
Bran (coarse bran)	0.27	0.27	5.40
Wet starch A	<0.01	<0.01	<0.2
Starch A	<0.01	<0.01	<0.2
Wet starch B	<0.01	<0.01	<0.2
Starch B	0.01	0.01	0.2
Starch	< 0.01 [#]	< 0.01 [#]	<0.20
Dried fibre	0.01	0.01	0.2
Wet gluten	0.02	0.02	0.4
Gluten	0.06	0.06	1.20
Gluten feed meal	0.01	0.01	0.2
Field Specimen S13-02516-01-047 (Follow up)			
Flour (type 550) production			

RAC – Wheat grain	0.13	0.13	-
Aspirated grain fraction (offal)	0.27	0.27	2.08
Aspirated grain (cleaned grain)	0.05	0.05	0.38
Shorts (total bran)	0.49	0.49	3.77
Flour (type 550)	0.02	0.02	0.15
Wholemeal flour / wholemeal bread production			
RAC – Wheat grain	0.07	0.07	-
Whole meal flour	0.05	0.05	0.42
Whole meal bread	0.06	0.06	0.50
Wheat germ production			
RAC – Wheat grain	0.07	0.07	-
Wheat germs	0.07	0.07	1.00
Starch and gluten production			
RAC – Wheat grain	0.09	0.09	-
Starch A	<0.01	<0.01	<0.11
Starch B	<0.01	<0.01	<0.11
Starch	< 0.01 [#]	< 0.01 [#]	<0.11
Gluten	0.11	0.11	1.22
Gluten feed meal	0.02	0.02	0.22
Field Specimen S13-02516-03-046 (Balance)			
Flour (type 550) production			
RAC – Wheat grain	0.23	0.23	-
Aspirated grain fraction (offal)	0.65	0.65	2.83
Aspirated grain (cleaned grain)	0.2	0.2	0.87
Grain (after conditioning)	0.14	0.14	0.61
Straight flour	0.03	0.03	0.13
Middlings (fine bran)	0.65	0.65	2.83
Bran (coarse bran)	0.69	0.69	3.00
Mixed bran	0.71	0.71	3.09
Shorts (total bran)	0.72	0.72	3.13
Low grade meal (toppings)	0.40	0.40	1.74
Flour (type 550)	0.04	0.04	0.17
Wholemeal flour / wholemeal bread production			
RAC – Wheat grain	0.32	0.32	-
Aspirated grain fraction (offal)	0.72	0.72	2.25
Aspirated grain (cleaned grain)	0.11	0.11	0.34
Grain (after conditioning)	0.25	0.25	0.78
Straight flour	0.03	0.03	0.09

Middlings (fine bran)	0.71	0.71	2.22
Bran (coarse bran)	0.64	0.64	2.00
Mixed bran	1.6	1.6	5.00
Shorts (total bran)	1.2	1.2	3.75
Low grade meal (toppings)	0.53	0.53	1.66
Whole meal flour	0.15	0.15	0.47
Dough	0.12	0.12	0.38
Whole meal bread	0.17	0.17	0.53
Wheat germ production			
RAC – Wheat grain	0.25	0.25	-
Aspirated grain fraction (offal)	0.58	0.58	2.32
Aspirated grain (cleaned grain)	0.12	0.12	0.48
Grain (after conditioning)	0.10	0.10	0.40
Bruised grain (400-1000 µm)	0.08	0.08	0.32
Bruised grain (>1000 µm)	0.40	0.40	1.60
Bruised grain (<400 µm)	0.02	0.02	0.08
Middlings/germ mixture	0.08	0.08	0.32
Bran	0.16	0.16	0.64
Flour	0.05	0.05	0.20
Bran/germ fraction	0.19	0.19	0.76
Fine bran/germ fraction	0.22	0.22	0.88
Coarse bran/germ fraction	0.26	0.26	1.04
Remaining bran	0.21	0.21	0.84
Wheat germs	0.23	0.23	0.92
Starch and gluten production			
RAC – Wheat grain	0.37	0.37	-
Aspirated grain fraction (offal)	1.6	1.6	4.32
Aspirated grain (cleaned grain)	0.17	0.17	0.46
Grain (after conditioning)	0.11	0.11	0.3
Straight flour	0.05	0.05	0.14
Middlings (fine bran)	0.72	0.72	1.95
Bran (coarse bran)	1.0	1.0	2.70
Wet starch A	<0.01	<0.01	<0.03
Starch A	<0.01	<0.01	<0.03
Wet starch B	<0.01	<0.01	<0.03
Starch B	<0.01	<0.01	<0.03

Starch	< 0.01 [#]	< 0.01 [#]	<0.03
Dried fibre	0.02	0.02	0.05
Wet gluten	0.07	0.07	0.19
Gluten	0.22	0.22	0.59
Gluten feed meal	0.08	0.08	0.22
Field Specimen S13-02516-03-047 (Follow up)			
Flour (type 550) production			
RAC – Wheat grain	0.17	0.17	-
Aspirated grain fraction (offal)	0.55	0.55	3.24
Aspirated grain (cleaned grain)	0.17	0.17	1.00
Shorts (total bran)	0.49	0.49	2.88
Flour (type 550)	0.04	0.04	0.24
Wholemeal flour / wholemeal bread production			
RAC – Wheat grain	0.23	0.23	-
Whole meal flour	0.09	0.09	0.39
Whole meal bread	0.11	0.11	0.48
Wheat germ production			
RAC – Wheat grain	0.33	0.33	-
Wheat germs	0.24	0.24	0.73
Starch and gluten production			
RAC – Wheat grain	0.31	0.31	-
Starch A	<0.01	<0.01	<0.03
Starch B	<0.01	<0.01	<0.03
Starch	< 0.01 [#]	< 0.01 [#]	<0.03
Gluten	0.15	0.15	0.48
Gluten feed meal	0.05	0.05	0.16

* Residues in the grain before processing are mean values of analysis of the two grain samples taken immediately before processing after separation.

[#] Mean of pydiflumetofen residues found in starch A and starch B.

Table 7-136 Overall Median Processing Factors for pydiflumetofen

Crop (RAC)/Processed product	Number of studies ^(a)	Processing Factor (PF)	
		Individual values	Median PF
Flour (type 550)			
Wheat/Aspirated grain fraction (offal)	2 (4)	2.08, 2.44, 2.83, 3.24	2.64
Wheat/Aspirated grain (cleaned grain)	2 (4)	0.38, 0.78, 0.87, 1.00	0.83
Wheat/Grain (after conditioning)	2	0.61, 0.78	0.70

Wheat/Straight flour	2	<0.11, 0.13	0.12 (best estimate)
Wheat/Middlings (fine bran)	2	2.00, 2.83	2.42
Wheat/Bran (coarse bran)	2	2.78, 3.00	2.89
Wheat/Mixed bran	2	3.09, 3.26	3.18
Wheat/Shorts (total bran)	2 (4)	2.88, 3.13, 3.77, 4.22	3.45
Wheat/Low grade meal (toppings)	2	0.44, 1.74	1.09
Wheat/Flour (type 550)	2 (4)	0.11, 0.15, 0.17, 0.24	0.16
Whole meal flour and wholemeal bread			
Wheat/Aspirated grain fraction (offal)	2	2.25, 4.11	3.18
Wheat/Aspirated grain (cleaned grain)	2	0.34, 0.78	0.56
Wheat/Grain (after conditioning)	2	0.78, 1.11	0.95
Wheat/Straight flour	2	0.09, 0.11	0.10
Wheat/Middlings (fine bran)	2	1.56, 2.22	1.89
Wheat/Bran (coarse bran)	2	2.00, 2.89	2.45
Wheat/Mixed bran	2	4.22, 5.00	4.61
Wheat/Shorts (total bran)	2	3.22, 3.75	3.49
Wheat/Low grade meal (toppings)	2	1.66, 2.56	2.11
Wheat/Whole meal flour	2 (4)	0.33, 0.39, 0.42, 0.47	0.41
Wheat/Dough	2	0.33, 0.38	0.36
Wheat/Whole meal bread	2 (4)	0.48, 0.50, 0.53, 0.56	0.52
Wheat germs			
Wheat/Aspirated grain fraction (offal)	2	2.32, 20.00	11.16
Wheat/Aspirated grain (cleaned grain)	2	0.48, 1.00	0.74
Wheat/Grain (after conditioning)	2	0.40, 1.00	0.70
Wheat/Bruised grain (400-1000 µm)	2	0.32, 0.63	0.47
Wheat/Bruised grain (>1000 µm)	2	1.60, 2.13	1.87
Wheat/Bruised grain (<400 µm)	2	0.08, 0.38	0.23
Wheat/Middlings/germ mixture	2	0.32, 0.88	0.6
Wheat/Bran	2	0.64, 1.63	1.14
Wheat/Flour	2	0.20, 0.38	0.29
Wheat/Bran/germ fraction	2	0.76, 1.25	1.01
Wheat/Fine bran/germ fraction	2	0.88, 1.38	1.13
Wheat/Coarse bran/germ fraction	2	1.00, 1.04	1.02
Wheat/Remaining bran	2	0.84, 1.25	1.05

Wheat/Wheat germs	2 (4)	0.63, 0.73, 0.92, 1.00	0.83
Starch and gluten			
Wheat/Aspirated grain fraction (offal)	2	4.32, 22.00	13.16
Wheat/Aspirated grain (cleaned grain)	2	0.46, 1.60	1.03
Wheat/Grain (after conditioning)	2	0.3, 1.40	0.85
Wheat/Straight flour	2	0.14, 0.2	0.17
Wheat/Middlings (fine bran)	2	1.95, 4.80	3.38
Wheat/Bran (coarse bran)	2	2.70, 5.40	4.05
Wheat/Wet starch A	2	<0.03, <0.2	<0.2 best estimate
Wheat/Starch A	2 (4)	<0.03, <0.03, <0.11, <0.2	<0.11 best estimate
Wheat/Wet starch B	2	<0.03, <0.2	<0.2 best estimate
Wheat/Starch B	2 (4)	<0.03, <0.03, <0.11, 0.2	<0.11 best estimate
Wheat/Starch**	2 (4)	<0.03, <0.03, <0.11, <0.2	<0.11 best estimate
Wheat/Dried fibre	2	0.05, 0.2	1.03
Wheat/Wet gluten	2	0.19, 0.4	0.30
Wheat/Gluten	2 (4)	0.48, 0.59, 1.2, 1.22	0.90
Wheat/Gluten feed meal	2 (4)	0.16, 0.2, 0.22, 0.22	0.21

** Mean of PF for starch A and starch B.

^a Number of studies signifies number of independent trials. Figure in brackets gives the number of individual processed fractions assessed (includes both main trial and follow ups from each independent trial)

B.7.5.3.3. Oilseed rape

Report:	KCA1 6.3.13; [REDACTED], [REDACTED]; 2017
Title:	SYN545974 - Residue Study on Oilseed Rape and Processed Products in Northern France, Germany and United Kingdom in 2014
Report No.:	CEMR-6531
Document No.:	VV-468119, A19649B_10334
Guidelines:	Commission of the European Communities, General Recommendations for the Design, Preparation and Realization of Residue Trials; 7029/VI/95 (rev. 5, working document). Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC. European Commission Guidance for Generating and Reporting Methods of Analysis in Support of Pre-registration Requirements for Annex II (Part A, Section 4) of Directive 91/414, SANCO/3029/99 revision 4 (11 Jul 2000). OECD Guidelines for the Testing of Chemicals – Crop Field Trial, No. 509, OECD, Paris 2009 OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66) Commission of the European Communities, Processing Studies; 7035/VI/95 (rev.5, working document). OECD Test Guideline 508 for the testing of chemicals concerning the Magnitude of the Pesticide Residues in Processed Commodities (03 Oct 2008). The Application of the OECD Principles of GLP to the Organisation and Management of Multi-Site Studies, ENV/JM/MONO(2002) 9. The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHW, MAFF and METI) on the basis of intergovernmental agreements.
Guideline deviations:	None
GLP/GEP:	Yes

I. Materials and Methods

The study contained four trials on oilseed rape conducted in northern Europe (Northern France, Germany and the United Kingdom), carried out in the 2013-2014 growing season. Samples from two of these trials were used for determinations of pydiflumetofen on oilseed rape (seed, whole plant and processed fractions).

Field part

In these four trials, one untreated control plot was used for the generation of the control samples. The study design included two treated plots for trial GBU-14-18587 FR01 and GBU-14-18587 FR02 and one treated plot for the other two trials.

Pydiflumetofen was applied to oilseed rape as A19649B, a suspension concentrate (SC) formulation containing 200 g of pydiflumetofen per litre.

To treated plot P2, one application applied at or just before growth stage BBCH 69 was made at a nominal rate of 200 g ai/ha for pydiflumetofen.

To treated plot P3 (processing trials only (GBU-14-18587 FR01 and GBU-14-18587 FR02), one application applied at growth stage BBCH 73-75 was made at an exaggerated nominal rate of 400 g ai/ha for pydiflumetofen. Normal agricultural procedures were followed, and no unusual weather events were recorded.

Application data from the two higher application rate supervised residue trials are presented in the following table (Table 7-137).

Table 7-137 Planned application scenario for higher application rate trials

Trial No. / Location / EU zone / Year	Commodity / Variety (a)	Date of 1. Sowing or planting 2. Flowering 3. Harvest 4. Transplanting (b)	Application rate per treatment			Dates of treatment / Application interval (c)	Growth stage at last treatment (BBCH) (d)	Details on trial (f)
			g a.s./ha	Water (L/ha)	g a.s./hL			
GBU-14-18587 FR01 Perthes, Champagne- Ardennes, 08300	Oilseed rape - DK Explicit	1, 23/8/2013 2, 9-30/4/2014 3, July/2014	391	222		19/5/2014	73-75	Backpack / Broadcast Foliar Spray A19649B 200 SC No adjuvants used
GBU-14-18587 FR02 Dame Marie les Bois, Centre, 37110	Oilseed rape – PR44W2 9	1, 6/9/2013 2, April /2014 3, July/2014	400	150		14/5/2014	73	Backpack / Broadcast Foliar Spray A19649B 200 SC No adjuvants used

The following summaries cover only the control plot and plot P3 for the trials. The trials for plot P2 are summarised in section 7.3

Treated oilseed rape (whole plant) samples (> 1 kg) were collected at 0 and 7 days after application (DAA).
Untreated oilseed rape (whole plant) samples (> 1 kg) were collected at 0 DAA.

Treated and untreated oilseed rape (seed) samples (>0.5 kg field samples, > 30 kg for processing samples)) were collected at normal commercial harvest (NCH) for the field and processing samples (63-78 DAA on plot P2 and 49-58 DAA on plot P3).

Field samples of oilseed rape for processing were shipped and stored at ambient temperature for ~30 days, this is representative of normal commercial practice. Samples were processed into pressed crude oil, pressed presscake, refined pressed oil, extracted crude oil, extracted presscake and refined extracted oil. Positive residues were observed in both RAC and processed fractions following this storage, in line with the levels observed in the RAC frozen samples.

Relevant practices and standardised procedures were applied in this processing phase which simulated common industrial processes at a laboratory scale.

The processing of the oilseed rape specimens was performed at the test site, in a specialised pilot plant, at the following location:

Staphyt Processing,
La Paluzette,
Route des Mas,
F-34590 Marsillargues,

France.

The processed fractions were stored immediately after processing, at or below -18 °C until shipment to the analytical facility where they were analysed for pydiflumetofen.

Processed specimens (including seed prior to processing) were stored frozen for a maximum period of 183 days from sampling to analysis of pydiflumetofen. These storage periods are covered by storage stability trials summarised in B.7.1, which shows stability in all matrices for 23 months

Extract solutions of field specimens were stored for a maximum of 2 days in a refrigerator in the dark before analysis of pydiflumetofen. Extract solutions of processed specimens were stored for a maximum of 5 days in the refrigerator in the dark before analysis of pydiflumetofen.

Stability of the analytes in the specimen extracts was proven by the corresponding procedural recovery specimens, which were stored under the same conditions together with the sample extracts.

At each independent trial site there were two trials on treated crops, each trial having independently gone through the same processing procedures. Flow diagrams showing the processing stages are provided in this section.

Processing Summary

Pressed seed oil processing

Before oilseed pressing, oilseed rape seeds were cooked in an oven set at 60°C for at least 12 hours. Cooked rapeseeds were introduced into the press.

Presscake was collected in a plastic container placed at the press head exit and oil was collected in a plastic bucket placed under the press head.

A part of obtained crude oil was filtered to produce crude oil. Two >400 g pressed crude oil sub-specimens were collected in plastic bottles, labelled and frozen (below -18°C).

Soda (concentration: 115 g/L) was added to the other part of crude oil. The mixing was maintained for a minimum of 30 minutes in an oven at a temperature of about 80-90 °C.

After a settling period of minimum 30 minutes, waste (soap) was removed from saponified oil, weighed and discarded. Saponified oil was stored in a freezer until the start of deodorization.

For deodorization, oil was defrosted and after weighing, poured into a reactor equipment, heated to approximately 240 °C and kept up at this temperature for about 1 hour.

After cooling, refined oil was filtered. Two 400 g refined oil sub-specimens were collected in plastic bottle, labelled and then frozen (below -18°C).

Extracted seed oil processing

For each specimen, rape seeds were crushed. Crushed rape seeds were used to produce crude oil by extraction. Some oil (1) obtained was kept at ambient temperature.

The separation to oil from crushed seeds was produced in an extractor with the solvent hexane. Hexane was injected through the presscake seven times (8 L for the first washing and 5 L for six other washings, during 10 min for each washing). For all specimens two batches of presscake were used except for U1 specimen a single batch.

Presscake obtained after extraction was stored at ambient temperature in an aired part of the laboratory (except for T2B specimen which were frozen below -18°C) to permit the evaporation of residual hexane. The obtained oil/solvent mixture (miscella) was heated to distil off the solvent directly. This oil (2) obtained was weighed and mixed with oil (1) obtained during the crushing of rape seeds. Extracted crude oil was filtered. Pressed crude oil collected into glass demijohns was out of the freezer the day before the refining.

Soda (concentration: 115 g/L) was added to the remaining extracted crude oil. The mixing was maintained for 30 minutes in an oven at a temperature of about 80-90 °C. After a settling period of minimum 30 minutes, waste (soap) was removed from saponified oil and discarded.

For deodorization, oil was, poured into a reactor equipment, heated to approximately 240 °C and kept up at this temperature for about 1 hour.

After cooling, refined oil was filtered. Two ≥400 g refined oil sub-specimens were collected in plastic bottle, labelled and then frozen (below -18°C).

Processing was carried out at Staphyt Processing, La Paluzette, Route des Mas, F-34590 Marsillargues, France

Processing flowcharts

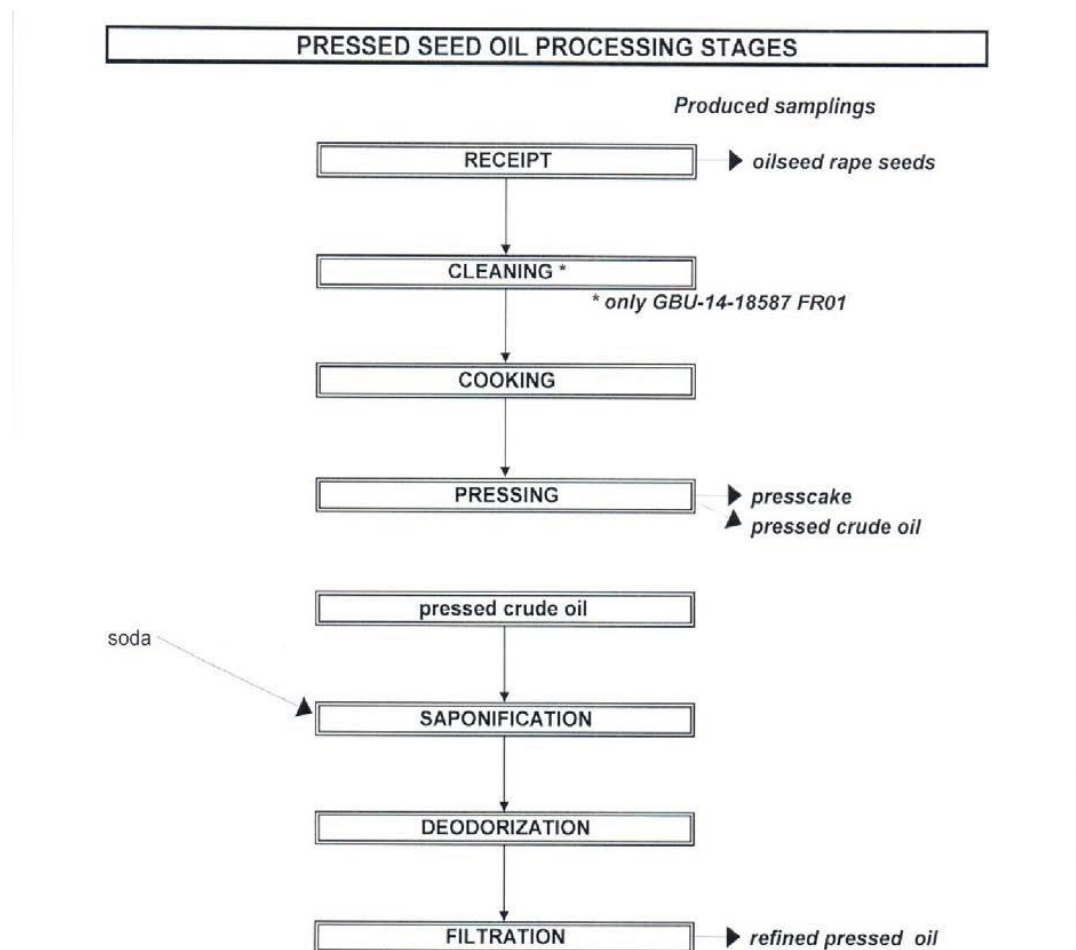
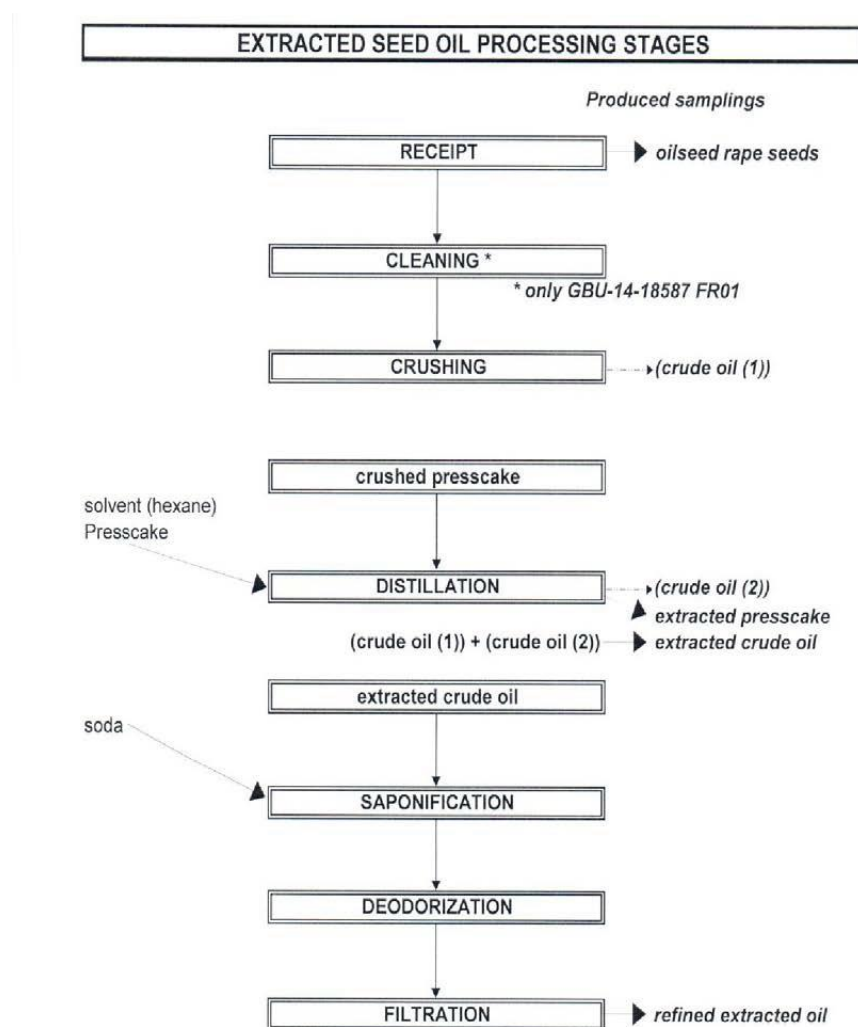
Figure 7-13 Pressed seed oil processing stages

Figure 7-14 Extracted seed oil processing stages

II. Findings

In order to check the performance of the method, recovery determinations were included in each set of analyses. Control samples from the study were fortified to be used as the recovery samples. All the recovery determinations were performed concurrently to the analyses of control and treated samples from the study. Method GRM061.03A is acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (cereal grain and potato), high protein (dry beans) and dry (cereal straw) commodities, with an LOQ of 0.01 mg/kg – see B 5.1.2.5.

Details on concurrent recovery data are shown in Table 7-138.

Average recoveries were available for most substrates, the majority of which were within the acceptable range for mean recovery of 70 – 110% (SANCO/3029/99 rev. 4) the remainder of the samples remained within the criteria laid down in OECD guidance ENV/JM/MONO(2007)17 for % mean recovery. RSD was within an acceptable level (<20%)

Table 7-138 Procedural recovery data

Substrate (control)	Fortification level (mg/kg)	pydiflumetofen Recovery (%)
Oilseed Rape (seed)	0.01	87
	0.1	110
	1.0	114
Oilseed Rape (whole plant)	0.01	113
	0.1	116
	5.0	96
Oilseed Rape (Seed before processing)	0.01	109, 111, 80
	Mean	100
	RSD (%)	17.3
	1.0	109, 106, 108
	Mean	108
	RSD (%)	1.4
Pressed presscake	0.01	95, 105, 100
	Mean	100
	RSD (%)	5
	1.0	98, 96, 93
	Mean	96
	RSD (%)	2.6
Extracted presscake	0.01	107, 114, 124
	Mean	115
	RSD (%)	7.4
	1.0	119, 113, 113
	Mean	115
	RSD (%)	3
Pressed crude oil	0.01	79, 78, 86
	Mean	81
	RSD (%)	5.4
	1.0	96, 92, 96
	Mean	95
	RSD (%)	2.4
Extracted crude oil	0.01	97, 94, 107
	Mean	99
	RSD (%)	6.9
	1.0	93, 91, 93
	Mean	92
	RSD (%)	1.3
Refined pressed oil	0.01	92, 103, 112
	Mean	102
	RSD (%)	9.8
	1.0	99, 103, 97

	Mean	100
	RSD (%)	3.1
Refined extracted oil	0.01	98, 91, 91
	Mean	93
	RSD (%)	4.3
	1.0	87, 87, 88
	Mean	87
	RSD (%)	0.7

The lowest fortification level is at the limit of quantification.

Mean and RSD (%) are calculated using rounded figures.

Residues

No residues of pydiflumetofen were found at or above the limit of quantification (0.01 mg/kg) in any of the untreated oilseed rape (whole plant) samples taken at either 0 or 7 DAA, or in the untreated oilseed rape (seed) samples taken at NCH.

Following foliar application (P3 in GBU-14-18587 FR01 and GBU-14-18587 FR02) residues of pydiflumetofen in treated oilseed rape (seed) samples were between 0.05 mg/kg and 0.13 mg/kg at NCH.

No residues of pydiflumetofen were found at or above the limit of quantification (0.01 mg/kg) in any of the untreated seed before processing samples or processed fractions.

For the processed fractions, residues of pydiflumetofen at NCH in treated oilseed rape (seed before processing) samples were between 0.02 mg/kg and 0.15 mg/kg, in pressed crude oil (after filtration); between 0.04 mg/kg and 0.11 mg/kg, in pressed presscake (after pressing); between <0.01 mg/kg and 0.02 mg/kg, in refined pressed oil (after filtration); between 0.05 mg/kg and 0.11 mg/kg, in extracted crude oil (after filtration); between 0.05 mg/kg and 0.12 mg/kg and in refined extracted oil (after filtration); between 0.06 mg/kg and 0.12 mg/kg. No residues of pydiflumetofen were found at or above the limit of quantification (0.01 mg/kg) in the extracted presscake (after solvent extraction).

Some processing factors shows a large difference (>50%) between trials (based on both the individual values from the two processing studies and the mean of the values from each independent site) so in principle a third trial should be performed, and the median value reported. This was not considered necessary due to the data supplied from the two processing trials per site, giving a total of four separate values.

Note that the values given below for the overall transfer factors (Table 7-142) show data from two independent test sites and with each independent test site having two samples that have independently gone through the same processing procedures. As the difference between the trials are not irreconcilable (10 fold different according to OECD 508) the median transfer factor was considered most appropriate and has been calculated.

The processing factors are summarised in Table 7-141.

Field specimens (RAC for the trial that were analysed separately to the processed samples) were analysed following frozen storage, within an acceptable timeframe that was covered by storage stability studies (see section B.7.1).

The maximum time field samples for processing were stored at ambient temperature prior to the start of processing was ~30 days, this is considered acceptable as sufficient levels of detectable incurred residues were present in the samples prior to processing

Processed specimens (including seed prior to processing) were stored frozen for a maximum period of 183 days from sampling to analysis of pydiflumetofen. These storage periods are covered by storage stability trials summarised in B.7.1, which shows stability in all matrices for 23 months.

III. Conclusions

The study contained four trials (two for processing studies) on field oilseed rape conducted in northern Europe (Northern France, Germany and the United Kingdom), and were carried out to generate results on the magnitude of residues and to generate samples for processing and subsequent residue analysis in order to support the registration or market value of plant protection products.

Determination of pydiflumetofen on oilseed rape (seed, whole plant and processed fractions) was undertaken to evaluate the magnitude of residues and to allow the calculation of residue transfer factors.

The plots designated for the processing study, P3 (processing trials only (GBU-14-18587 FR01 and GBU-14-18587 FR02), one application applied at growth stage BBCH 73-75 was made at an exaggerated nominal rate of 400 g ai/ha for pydiflumetofen.

The study was conducted according to GLP.

The results of the study indicate that residues of pydiflumetofen do not concentrate in the processing of oilseed rape into pressed presscake and extracted presscake (processing factors of 0.26 and 0.22 respectively). Residues of pydiflumetofen do concentrate in the processing of oilseed rape to pressed crude oil, pressed oil, extracted crude oil and refined extracted oil (processing factors of 1.40, 1.54, 1.50 and 1.69 respectively).

Table 7-139 Field samples

Trial number	Sample No.	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Crop Part	pydiflumetofen Residue (mg/kg)
					Uncorrected
GBU-14-18587 FR01	011	1 × 400	58 DAA (NCH)	Seed	0.13
	001	Control	0 DBA	Whole Plant	<0.01
	007	Control	78 DAA (NCH)	Seed	<0.01
GBU-14-18587 FR02	011	1 × 400	49 DAA (NCH)	Seed	0.05
	001	Control	0 DAA	Whole Plant	<0.01
	007	Control	63 DAA (NCH)	Seed	<0.01

DAA: Days after application, NCH: Normal Commercial Harvest, DBA: Days before application. No correction of results for either control residues or recovery values has been performed. Note: in error, control sample for GBU-14-18587 FR01 at 0 DAA was collected at 0 DBA.

Table 7-140 Pydiflumetofen residue results for processed samples

Trial number	Number and Nominal Rate of Application (g ai/ha)	Sampling Interval (days)	Processed Fraction	pydiflumetofen Residue (mg/kg)
				Uncorrected
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.09
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.09
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.11
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.15

GBU-14-18587 FR01	1 × 400	NCH (58 DAA)	Pressed crude oil (after filtration)	0.11
	1 × 400	NCH (58 DAA)	Pressed crude oil (after filtration)	0.08
	1 × 400	NCH (58 DAA)	Pressed presscake (after pressing)	0.02
	1 × 400	NCH (58 DAA)	Pressed presscake (after pressing)	0.01
	1 × 400	NCH (58 DAA)	Refined pressed oil (after filtration)	0.11
	1 × 400	NCH (58 DAA)	Refined pressed oil (after filtration)	0.09
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.09
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.09
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.10
	1 × 400	NCH (58 DAA)	Seed (before processing)	0.12
	1 × 400	NCH (58 DAA)	Extracted crude oil (after filtration)	0.11
	1 × 400	NCH (58 DAA)	Extracted crude oil (after filtration)	0.12
	1 × 400	NCH (58 DAA)	Extracted presscake (after solvent extraction)	<0.01
	1 × 400	NCH (58 DAA)	Extracted presscake (after solvent extraction)	<0.01
	1 × 400	NCH (58 DAA)	Refined extracted oil (after filtration)	0.12
	1 × 400	NCH (58 DAA)	Refined extracted oil (after filtration)	0.12
	Control	NCH (58 DAA)	Seed (before processing)	<0.01
	Control	NCH (58 DAA)	Pressed crude oil (after filtration)	<0.01
	Control	NCH (58 DAA)	Pressed presscake (after pressing)	<0.01
	Control	NCH (58 DAA)	Refined pressed oil (after filtration)	<0.01
	Control	NCH (58 DAA)	Seed (before processing)	<0.01
	Control	NCH (58 DAA)	Extracted crude oil (after filtration)	<0.01
	Control	NCH (58 DAA)	Extracted presscake (after solvent extraction)	<0.01
	Control	NCH (58 DAA)	Refined extracted oil (after filtration)	<0.01
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.02
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.02
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.04
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.04

GBU-14-18587 FR02	1 × 400	NCH (49 DAA)	Pressed crude oil (after filtration)	0.04
	1 × 400	NCH (49 DAA)	Pressed crude oil (after filtration)	0.07
	1 × 400	NCH (49 DAA)	Pressed presscake (after pressing)	<0.01
	1 × 400	NCH (49 DAA)	Pressed presscake (after pressing)	0.01
	1 × 400	NCH (49 DAA)	Refined pressed oil (after filtration)	0.05
	1 × 400	NCH (49 DAA)	Refined pressed oil (after filtration)	0.07
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.03
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.02
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.03
	1 × 400	NCH (49 DAA)	Seed (before processing)	0.03
	1 × 400	NCH (49 DAA)	Extracted crude oil (after filtration)	0.05
	1 × 400	NCH (49 DAA)	Extracted crude oil (after filtration)	0.06
	1 × 400	NCH (49 DAA)	Extracted presscake (after solvent extraction)	<0.01
	1 × 400	NCH (49 DAA)	Extracted presscake (after solvent extraction)	<0.01
	1 × 400	NCH (49 DAA)	Refined extracted oil (after filtration)	0.06
	1 × 400	NCH (49 DAA)	Refined extracted oil (after filtration)	0.07
	Control	NCH (49 DAA)	Seed (before processing)	<0.01
	Control	NCH (49 DAA)	Pressed crude oil (after filtration)	<0.01
	Control	NCH (49 DAA)	Pressed presscake (after pressing)	<0.01
	Control	NCH (49 DAA)	Refined pressed oil (after filtration)	<0.01
	Control	NCH (49 DAA)	Seed (before processing)	<0.01
	Control	NCH (49 DAA)	Extracted crude oil (after filtration)	<0.01
	Control	NCH (49 DAA)	Extracted presscake (after solvent extraction)	<0.01
	Control	NCH (49 DAA)	Refined extracted oil (after filtration)	<0.01

Table 7-141 Processing factors for pydiflumetofen

Trial number	Number and Nominal Rate of Application	Plot	Commodity	pydiflumetofen Residue (mg/kg)	pydiflumetofen Mean Residue (mg/kg)	Processing Factor (PF)
	(g ai/ha)			Uncorrected	Uncorrected	

Pre-processed and processing fractions from Sample GBU-14-18587 FR01-014					
1 × 400	P3	Seed (before processing)	0.09	0.09	N/A
1 × 400	P3		0.09		
1 × 400	P3	Pressed crude oil (after filtration)	0.11	N/A	1.22
1 × 400	P3	Pressed presscake (after pressing)	0.02	N/A	0.22
1 × 400	P3	Refined pressed oil (after filtration)	0.11	N/A	1.22
1 × 400	P3	Seed (before processing)	0.09	0.09	N/A
1 × 400	P3		0.09		
1 × 400	P3	Extracted crude oil (after filtration)	0.11	N/A	1.22
1 × 400	P3	Extracted presscake (after solvent extraction)	<0.01	N/A	<0.11 [#]
1 × 400	P3	Refined extracted oil (after filtration)	0.12	N/A	1.33
Pre-processed and processing fractions from Sample GBU-14-18587 FR01-015					
1 × 400	P3	Seed (before processing)	0.11	0.13	N/A
1 × 400	P3		0.15		
1 × 400	P3	Pressed crude oil (after filtration)	0.08	N/A	0.62
1 × 400	P3	Pressed presscake (after pressing)	0.01	N/A	0.08
1 × 400	P3	Refined pressed oil (after filtration)	0.09	N/A	0.69
1 × 400	P3	Seed (before processing)	0.10	0.11	N/A
1 × 400	P3		0.12		
1 × 400	P3	Extracted crude oil (after filtration)	0.12	N/A	1.09
1 × 400	P3	Extracted presscake (after solvent extraction)	<0.01	N/A	<0.09 [#]
1 × 400	P3	Refined extracted oil (after filtration)	0.12	N/A	1.09
Pre-processed and processing fractions from Sample GBU-14-18587 FR02-014					
1 × 400	P3	Seed (before processing)	0.02	0.02	N/A
1 × 400	P3		0.02		
1 × 400	P3	Pressed crude oil (after filtration)	0.04	N/A	2.00
1 × 400	P3	Pressed presscake (after pressing)	<0.01	N/A	<0.50 [#]

1 × 400	P3	Refined pressed oil (after filtration)	0.05	N/A	2.50
1 × 400	P3	Seed (before processing)	0.03	0.03	N/A
1 × 400	P3		0.02		
1 × 400	P3	Extracted crude oil (after filtration)	0.05	N/A	1.67
1 × 400	P3	Extracted presscake (after solvent extraction)	<0.01	N/A	<0.33 [#]
1 × 400	P3	Refined extracted oil (after filtration)	0.06	N/A	2.00
Pre-processed and processing fractions from Sample GBU-14-18587 FR02-015					
1 × 400	P3	Seed (before processing)	0.04	0.04	N/A
1 × 400	P3		0.04		
1 × 400	P3	Pressed crude oil (after filtration)	0.07	N/A	1.75
1 × 400	P3	Pressed presscake (after pressing)	0.01	N/A	0.25
1 × 400	P3	Refined pressed oil (after filtration)	0.07	N/A	1.75
1 × 400	P3	Seed (before processing)	0.03	0.03	N/A
1 × 400	P3		0.03		
1 × 400	P3	Extracted crude oil (after filtration)	0.06	N/A	2.00
1 × 400	P3	Extracted presscake (after solvent extraction)	<0.01	N/A	<0.33 [#]
1 × 400	P3	Refined extracted oil (after filtration)	0.07	N/A	2.33

[#] calculated by taking the value of the residue in the processed commodity (mg/kg) as being equal to the LOQ (0.01 mg/kg)

Table 7-142 Median processing factor results

Crop (RAC)/Processed product	Number of studies ^(a)	Processing Factor (PF)	
		Individual values	Median PF
Oilseed rape/ Pressed crude oil	2 (4)	0.62, 1.22, 1.75, 2.00	1.49
Oilseed rape/ Pressed presscake	2 (4)	0.08, 0.22, 0.25, <0.50	0.24
Oilseed rape/ Refined pressed oil	2 (4)	0.69, 1.22, 1.75, 2.50	1.49
Oilseed rape/ Extracted crude oil	2 (4)	1.09, 1.22, 1.67, 2.00	1.45
Oilseed rape/ Extracted presscake	2 (4)	<0.09, <0.11, <0.33, <0.33	<0.22 (best estimate)

Oilseed rape/ Refined extracted oil	2 (4)	1.09, 1.33, 2.00, 2.33	1.67
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Calculation performed using unrounded values.

^a Number of studies signifies number of independent trials. Figure in brackets gives the number of individual processed fractions assessed across both trials

B.7.6. RESIDUES IN SUCCEEDING OR ROTATIONAL CROPS

Metabolism of pydiflumetofen in soil has been investigated (see section B.8). DT90 values of pydiflumetofen are significantly above 100 days, therefore further investigation of residues in rotational crops is required. The fate and behaviour evaluation considering residues in soil concluded (see section Vol 1 2.8.1) that levels of metabolite formation were low; HSE consider on the basis of the results that no metabolites formed in soil trigger inclusion in risk assessment, and no soil metabolites are significant when considering accumulation potential. Therefore, the below studies were evaluated in the context of expected soil exposures of parent pydiflumetofen only. See Figure 7-1, in section 7.2 for representative label positions.

B.7.6.1. Metabolism in rotational crops

Report:	K-CA 6.6.1/01. [REDACTED] and [REDACTED] (2015)
Title:	SYN545974 –Uptake and Metabolism of [14C]-SYN545974 in Confined Rotational Crops
Report No:	Report Number 34316, (Syngenta Report No. TK0061717).
Document No:	VV-412848 (Syngenta File No. SYN545974_10227)
Guidelines:	OECD Guideline for the Testing of Chemicals. Metabolism in Rotational Crops. Guideline 502, January 2007. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 64. Series on Pesticides No. 32. Guidance Document on Overview of Residue Chemistry Studies ENV/JM/MONO (2009)31 (July 2009). Confined Accumulation in Rotational Crops; US Environmental Protection Agency; Residue Test Guidelines OPPTS 860.1850 (August 1996). Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market. Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the 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. Japanese Ministry of Agriculture, Forestry and Fisheries; Guideline on the Application for Agricultural Chemicals Registration; 12 Nohsan No. 8147; November 2000.
Guideline deviations:	None
GLP:	Yes

All expressions of the results in the rotational crop metabolism study are given in mg/kg parent equivalents.

Table 7-143 Summary of the study design for the metabolism study on rotational crops

Crop group	Crop	Label position	Application and sampling details			
			Method, F or G ^a	Target rate (kg a.s./ha)	Sowing intervals (days)	Harvest time
Leafy vegetables	Lettuce	[phenyl-U-14C] & [pyrazole-5-14C]-pydiflumetofen	Bare soil, F(a)	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					BBCH 49
Cereals	Wheat					Forage (BBCH 15-30), hay (BBCH 49-60) and maturity (BBCH 89) growth stage

a: Field or Glasshouse (whilst the application was made to the soil outside; the soil was in containers and the containers were moved into the glasshouse part way through the growing period, see the further explanation below).

Materials and methods

Materials

1. C-label pydiflumetofen

Description: Phenyl-U-14C – SYN545974 (spec. activity of a.s. 4.11 MBq/mg)

Lot/Batch #: DAD-XIII-38-4

Radiochemical Purity: 98.7 %

2. C-label pydiflumetofen

Description: Pyrazole-5-14C – SYN545974 (spec. activity of a.s. 4.01 MBq/mg)

Lot/Batch #: 5222GAR001-1

Radiochemical Purity: 97.7 %

Methods

Summarised application parameters are presented in Table 7-143; full details are provided below.

The metabolism and distribution of pydiflumetofen (radiolabelled on the phenyl or the pyrazole ring, as shown in Figure 7-1) was investigated in succeeding crops following a single soil treatment at a nominal rate of 400 g a.s./ha. The test solution was applied using a handheld sprayer. The actual application rates were 387.8 and 408.6 g a.s./ha for the phenyl and pyrazole labelled test substances, respectively. The radiochemicals were formulated as a suspension concentrate (SC200), using the blank SC formulation 'A20048A'. The crops tested are as follows:

- Lettuce (*var. 'toniva'*) – Leafy vegetables
- Turnip (*var. 'Tokyo cross'*) – Root and tuber vegetables
- Spring wheat (*var. 'Tybalt'*) – Cereals

The study was conducted in 2012-2015 at Charles River Laboratories in Edinburgh, Scotland. Plant back intervals of 30, 120 and 270 days were studied. 18 test containers were prepared (9 for each radiolabel); the containers had a spraying surface of approx. 0.42 m² and a volume of approx. 0.147 m³. The containers were filled with a small layer of gravel, followed by sandy loam soil. The containers were kept outdoors for 28 days following treatment;

they were then taken into separate greenhouses, where they remained for the rest of the study. Whilst outdoors, the containers were protected by netting for birds and animals; the containers were also fully covered when rain was falling or forecast.

Multiple crops were sown in each container at the various time points investigated, as shown in Table 7-144. Before each planting, the soil was mixed and any remaining root mass from the previous rotation was removed. Fertiliser and maintenance sprays were applied to ensure healthy crops.

Table 7-144 Containers and rotational intervals

Container No.	Crop Grown	Rotational Interval (DALA)	Harvest Stage
1	Lettuce	30	Immature
	Wheat	120	Straw and Grain
	Wheat	270	Straw and Grain
2	Lettuce	30	Mature
	Wheat	120	Straw and Grain
	Wheat	270	Straw and Grain
3	Turnip	30	Mature
	Wheat	120	Hay
	Lettuce	270	Immature
4	Wheat	30	Forage
	Turnip	120	Mature
	Wheat	270	Hay
5	Wheat	30	Hay
	Wheat	120	Forage
	Lettuce	270	Mature
6	Wheat	30	Straw and Grain
7	Wheat	30	Straw and Grain
8	Wheat	30	Forage
	Lettuce	120	Mature
	Turnip	270	Mature
9	Wheat	30	Hay
	Lettuce	120	Immature
	Wheat	270	Forage

Analysis/general procedures

Samples were collected according to the study design. Samples were pooled according to PBI, crop fraction and radiolabel. The samples were transferred to the freezer, set to maintain -20 °C as soon as practicable. Samples were homogenized with dry ice, shortly after freezing; samples were then refrozen, and the dry ice was allowed to sublime. Soil samples were also collected in triplicate at each time of sowing, and at the final harvest time of wheat sown at a PBI of 270 days. The samples were stored frozen as a backup and were not analysed.

Samples were homogenised and TRRs determined by sample combustion analysis, followed by liquid scintillation counting (LSC). Extraction and further analysis was carried out on samples where the radioactive residue was ≥ 0.01 mg/kg. Samples were extracted with aqueous acetonitrile solvent combinations (80 % v/v). Samples were extracted in multiple stages, the solvent: sample ratio remained constant (at 10:1 v/w) for wheat forage and immature lettuce (both 30-day PBI); the ratio decreased (to 5:1 v/w) for subsequent extractions of all other samples. Where required, the fourth and fifth extraction was performed using acetonitrile/water at 50 % v/v and a solvent: sample ratio of 5:1 v/w. Extracts were combined and concentrated using rotary evaporation, as required. Selected extracts were cleaned up using solid phase extraction (SPE) prior to LC-MS chiral analysis.

Samples from the 120 DALA wheat straw samples were characterised further. Residues in the combined aqueous acetonitrile samples were concentrated to remove the acetonitrile. The aqueous phase was acidified with dilute HCl and partitioned against diethyl ether three times. A portion of the aqueous layer was concentrated and hydrolysed with 0.1M HCl followed by 0.5M HCl and, in a separate experiment, a portion was hydrolysed with

0.5M NaOH followed by pectinase in sodium acetate: acetic acid buffer. Triplicate aliquots of the hydrolysed samples were taken for LSC analysis.

The post extraction solids from the 270 DALA wheat straw sample were characterised further using 'clean fractionation'. A portion of sample was extracted three times with methyl isobutyl ketone (MIBK): ethanol: water (16:34:50, v/v/v) plus sulphuric acid. This method provided an MIBK fraction (lignin), an aqueous fraction (hemicellulose), an acetone fraction and the final unextracted residue (cellulose). Extracts were adjusted to a known volume and triplicate aliquots taken for LSC analysis. The radioactive levels in the final unextracted residue were determined by oxidative combustion followed by LSC.

Identification of residues present in the principal fractions was conducted using HPLC-UV, with comparison to authentic reference standards of parent pydiflumetofen and its metabolites. Further identification was conducted using 1D-TLC. The following reference standards were used: metabolites which were detected- SYN545547 and SYN547891; metabolites which were not detected-SYN545720, SYN508272, NOA449410, SYN547890, SYN547892, SYN547893, SYN547894, SYN547895, and SYN547897.

Storage stability investigation - All samples not analysed immediately were stored in a freezer set to maintain $\leq -20^{\circ}\text{C}$. Storage stability was assessed for the principal crop fractions by comparing the initial TLC analysis (performed within 1 - 4 months of sampling) to the TLC chromatographic profile obtained on completion of the analysis. Representative radio chromatograms (TLC) of wheat hay & straw, immature lettuce and turnip foliage, 20-29 months (1.7 to 2.4 years) after initial analysis were considered (comparisons between TLC before and after the storage period).

Enantiomeric composition: Pydiflumetofen is a racemate. To consider the potential for differential metabolism of the 'S-' and 'R-' enantiomers of pydiflumetofen, the enantiomeric composition of pydiflumetofen residues in wheat straw was determined by LC-MS (and compared to the composition in spray solution).

Results and discussion

Total radioactive residue (TRR)

TRR levels are broadly similar across each label, for any given crop fraction. Extractability was high in all samples, accounting for between 83.2 and 96.8 % TRR.

For lettuce, the highest TRR was detected at the 30-day PBI in immature lettuce (0.019 mg/kg – pyrazole label). The TRR decreased over the following two PBIs. The TRR elucidated by direct quantification of the sample was <0.01 mg/kg for all samples of mature lettuce, and for the samples of immature lettuce at 120- and 270-day PBIs. Therefore, no further analysis was conducted on these samples.

For turnips, the highest TRR was detected at the 30-day PBI in foliage (0.014 mg/kg – pyrazole label). The TRR decreased over the following two PBIs. The TRR elucidated by direct quantification of the sample was <0.01 mg/kg for all samples of turnip root, and for the samples of turnip foliage at 120- and 270-day PBIs. Therefore, no further analysis was conducted on these samples.

For wheat, the highest TRR was detected at the 120-day PBI in wheat straw (0.219 mg/kg – pyrazole label). The residue picture for wheat is more complex than turnip and lettuce, and so each fraction will be discussed in turn:

- Wheat grain: Low levels of TRR (<0.01 mg/kg) were observed in all samples. No further analysis (e.g., characterisation) was performed.
- Wheat forage: The highest TRR was observed in the 30-day PBI sample (0.031 mg/kg – phenyl label). Residues declined from 0.031 to 0.013 mg/kg, and from 0.028 to 0.014 mg/kg, from the 30-day PBI to the 270-day PBI – for the phenyl and pyrazole labels, respectively. The TRR remained above 0.01 mg/kg for all PBIs, for both labels.
- Wheat hay: The highest TRR was observed in the 120-day PBI sample (0.108 mg/kg – pyrazole label). Residues declined from 0.064 to 0.039 mg/kg, and from 0.091 to 0.035 mg/kg, from the 30-day PBI to the 270-day PBI – for the phenyl and pyrazole* labels, respectively. *The TRR increased to the maximum of 0.108 at 120-day PBI, before declining to 0.035 mg/kg at the 270-day PBI. The TRR remained above 0.01 mg/kg for all PBIs, for both labels.

- **Wheat straw:** The highest TRR was observed in the 120-day PBI sample (0.219 mg/kg – pyrazole label). Residues declined from 0.174 to 0.114 mg/kg, and from 0.211 to 0.172 mg/kg, from the 30-day PBI to the 270-day PBI – for the phenyl and pyrazole* labels, respectively. *The TRR increased to the maximum of 0.219 at 120-day PBI, before declining to 0.172 mg/kg at the 270-day PBI. The TRR remained well above 0.01 mg/kg for all PBIs, for both labels. Residues in wheat straw are expected to remain significant at a PBI of 270 days.

The extracted radioactivity was analysed by chromatography. The extracted and identified residues are detailed in Table 7-145 and Tables 7-146 to 7-148 for each label.

Table 7-145 Total radioactive residues in rotational crop samples treated with [14C]- pydiflumetofen

Rotational interval (DAA ¹)	Crop commodity	Label ²	Extractable radioactivity		Non-extractable radioactivity		TRR	
							By Summation ³	By direct analysis ⁵
			%TRR	mg/kg	%TRR	mg/kg	mg/kg	mg/kg
30DAA	Wheat forage	Ph	96.8	0.030	3.2	0.001	0.031	0.023
		Py	96.8	0.027	3.2	0.001	0.028	0.026
	Wheat hay	Ph	91.9	0.060	8.1	0.005	0.064	0.065
		Py	90.0	0.082	9.9	0.009	0.092	0.091
	Wheat straw	Ph	86.9	0.151	12.2	0.021	0.174	0.167
		Py	88.2	0.187	11.3	0.024	0.211	0.203
	Wheat grain	Ph	-	-	-	-	NE	0.004
		Py	-	-	-	-	NE	0.008
	Immature lettuce	Ph	85.5	0.010	14.5	0.002	0.012	0.012
		Py	86.1	0.016	13.9	0.003	0.019	0.013
	Mature lettuce	Ph	-	-	-	-	NE	0.001
		Py	-	-	-	-	NE	0.007
	Mature turnip foliage	Ph	93.6	0.010	6.5	0.001	0.010	0.013
		Py	89.6	0.011	10.4	0.001	0.012	0.014
	Turnip tubers	Ph	-	-	-	-	NE	0.007
		Py	-	-	-	-	NE	0.008
120DAA	Wheat forage	Ph	89.2	0.010	10.7	0.001	0.010	0.010
		Py	91.1	0.025	8.9	0.002	0.027	0.030
	Wheat hay	Ph	85.2	0.050	14.8	0.009	0.059	0.060
		Py	84.5	0.091	15.5	0.017	0.108	0.111
	Wheat straw	Ph	85.9	0.131	14.1	0.022	0.153	0.151
		Py	85.9	0.188	14.1	0.031	0.219	0.218
	Wheat grain	Ph	-	-	-	-	NE	0.005
		Py	-	-	-	-	NE	0.007
	Immature lettuce	Ph	-	-	-	-	NE	0.005
		Py	-	-	-	-	NE	0.004
		Ph	-	-	-	-	NE	0.005

	Mature lettuce	Py	-	-	-	-	NE	0.004
	Mature turnip foliage	Ph	-	-	-	-	NE	0.004
		Py	-	-	-	-	NE	0.007
	Turnip tubers	Ph	-	-	-	-	NE	0.002
		Py	-	-	-	-	NE	0.003
270DAA	Wheat forage	Ph	96.3	0.013	3.7	<0.001	0.013	0.012
		Py	95.5	0.013	4.5	0.001	0.014	0.015
	Wheat hay	Ph	94.7	0.037	5.3	0.002	0.039	0.036
		Py	93.5	0.033	6.5	0.002	0.035	0.034
	Wheat straw	Ph	87.3	0.100	12.7 ⁴	0.014 ⁴	0.114	0.100
		Py	83.2	0.138	16.8 ⁴	0.028 ⁴	0.164	0.172
	Wheat grain	Ph	-	-	-	-	NE	0.003
		Py	-	-	-	-	NE	0.002
	Immature lettuce	Ph	-	-	-	-	NE	0.001
		Py	-	-	-	-	NE	0.006
	Mature lettuce	Ph	-	-	-	-	NE	0.001
		Py	-	-	-	-	NE	0.002
	Mature turnip foliage	Ph	-	-	-	-	NE	0.004
		Py	-	-	-	-	NE	0.007
	Turnip tubers	Ph	-	-	-	-	NE	0.002
		Py	-	-	-	-	NE	0.002

1 - DAA = days after radiochemical application.

2 - Ph = [phenyl-U-¹⁴C]-labelled experiment; Py = [pyrazole-5-¹⁴C]-labelled experiment.

3 - mg/kg calculated directly from summation of the radioactivity present in the extracts and debris.

4 - Straw (270DAA) was extracted further with 1M HCl and a “clean fractionation” technique to assess natural incorporation.

5 - The radioactive residue determined by direct quantification employing combustion/LSC – values less than 0.01 mg/kg were not extracted further.

Reasonable levels of identification were achieved for immature lettuce and turnip foliage samples, with ≥ 84.9 % and ≥ 53.1 % of the TRR identified, for the phenyl and pyrazole labels, respectively. Generally lower levels of identification were achieved for wheat samples, with ≥ 26.5 % and ≥ 37.9 % of the TRR identified, for the phenyl and pyrazole labels, respectively. The principal component of the residue was, in all cases, parent pydiflumetofen, accounting for between 44.4 and 77.2 % TRR for immature lettuce and turnip foliage samples; and between 18.6 and 77.8 % TRR for wheat (straw, hay and forage) samples.

Hydrolysis experiments conducted on 120 DALA wheat straw samples produced a number of extracts, each of which were analysed by HPLC. The acidified ether fractions were comprised of pydiflumetofen, SYN545547 and SYN547891 and low levels of multiple polar metabolites (individually ≤ 0.007 mg/kg). The aqueous fractions contained at least 18-22 multiple polar metabolites, none individually exceeding 0.010 mg/kg. Comparison of the HPLC chromatograms suggests that the major peaks observed in the aqueous fractions are common to both labels. The aqueous fraction from the [pyrazole-¹⁴C] label comprised of three, low level (total 1.2% TRR) components not observed in the [phenyl-¹⁴C] label.

‘Clean fractionation’ experiments on 270 DALA wheat straw samples released a further 9.2 % TRR (0.015 mg/kg) in the pyrazole label (MIBK fraction) – this was analysed by HPLC. All other fractions, for both labels, were ≤ 3.9 % TRR (≤ 0.006 mg/kg) and were not analysed further. No further analysis was conducted on the remaining unextracted solid.

Metabolites identified were SYN547891 and SYN545547. For immature lettuce and turnip foliage: the maximum identified metabolite was SYN547891, at 11.6 % TRR; absolute amounts of metabolite were low in all samples, for both labels – maximum at 0.001 mg/kg. For wheat (all matrices): the maximum % TRR of metabolite was SYN547891 at 13.3 % TRR, in wheat forage at 30 DALA. The contribution of SYN547891 and SYN545547 generally did not individually exceed 10 % TRR in the samples, except for the following: wheat forage at 30 DALA (both labels) and wheat hay at 270 DALA (pyrazole label). All identified metabolites were found in their free, non-conjugated form (hydrolysis steps were performed but these didn’t indicate any significant conjugation).

Storage stability investigations:

Representative radio chromatograms (TLC) of wheat hay & straw, immature lettuce and turnip foliage, 20-29 months (1.7 to 2.4 years) after initial analysis were considered (comparisons between TLC before and after the storage period). These representative TLC chromatograms supporting the storage stability work showed the major ‘spot’ of pydiflumetofen, and then weaker TLC spots for the metabolites SYN545547 and SYN547891. These are sufficient to show that there is no marked qualitative change in the samples over the period of the study, as far as can be seen in the context of the TLC work; these cannot be interpreted quantitatively.

The TLC storage stability results were only able to show the ‘weak’ TLC spots for the metabolites SYN545547 and SYN547891, and did not cover the low level peaks (indicated to be more polar in nature)/components that were unassigned (Tables 7-146 to 7-148) during the HPLC analysis and metabolic profiling. The metabolite profiles were obtained from samples stored for a maximum of 122 days (4 months).

HSE remarks: only limited information is available on stability of the residues from the investigations conducted on stability in this study. Whilst presenting some uncertainty, in view of the relatively long timescales of the study, the data are likely to be sufficient in the context of the conclusions surrounding the proposal for the residue definition for rotational crops. Many peaks present in the HPLC work were present at too low levels to enable identification. Only Pydiflumetofen (SYN545974, and metabolites SYN545547 and SYN547891 were identified in the rotational crop metabolism study. These three spots (parent and these two identified metabolites) were visible on the TLC radiochromatograms for the ‘post storage’ samples.

Enantiomer composition:

Pydiflumetofen is a racemate. In terms of enantiomeric conversion, the applicant has made the case (document N5 on isomeric composition) that chemically interconversions are not predicted based on mechanistic and structure related grounds. Interconversion of the enantiomers of SYN545974 is not considered feasible by any conventional chemical or biochemical process to which the compound will be exposed.

However, it is possible for differential metabolism of residues of pydiflumetofen to occur. The enantiomeric composition in the spray solution and in straw samples (120 & 270 DALA) was determined to see whether any change occurred. The enantiomeric fraction shifted from 0.5 in the spray solution to a maximum of 0.57 (270 DALA sample). The ‘S’ enantiomer of pydiflumetofen was more prevalent in the 270 DAA samples and the ‘R’ enantiomer of pydiflumetofen was more prevalent in the 120 DAA samples.

Based on these determinations, the % change in enantiomeric excess³ was estimated for wheat straw. This was calculated to be $\leq 10\%$ for the samples at 270 days (DAA). For the 120 DAA sample timing, one of the samples (pyrazole label) indicated an enantiomeric excess $> 10\%$ (13.4%). The direction of the enantiomer increase was different in the 270 DAA samples compared to the 120 DAA samples. HSE is not proposing to consider an assessment factor in the consumer risk assessment to consider the potential changes in isomer ratio/amounts in plants.

³ Enantiomeric excess is explained in the EFSA guidance on stereoisomers (2019, “Guidance of EFSA on risk assessments for active substances of plant protection products that have stereoisomers as components or impurities and for transformation products of active substances that may have stereoisomers”)

See also information on enantiomeric composition derived from published literature reports at the end of section B.7.2.1.

Table 7-146 Summary of identification and characterization of residues – 30-day PBI

30-day PBI	Immature lettuce foliage	Turnip foliage	Wheat forage	Wheat hay	Wheat straw
[Phenyl-¹⁴C]					
extractable TRR % (mg/kg)	85.5 (0.010)	93.6 (0.010)	96.8 (0.03)	91.9 (0.06)	86.9 (0.151)
pydiflumetofen	69.3 (0.009)	77.2 (0.008)	77.8 (0.024)	50.1 (0.032)	30.0 (0.052)
SYN545547	4.0 (<0.001)	ND	2.2 (0.001)	2.5 (0.002)	1.8 (0.003)
SYN547891	11.6 (0.001)	3.9 (<0.001)	12.0 (0.004)	6.2 (0.004)	6.1 (0.011)
Unassigned % (mg/kg)	3.4 (<0.001) ¹	10.2 (0.001) ³	10.6 (0.005) ⁵	38.9 (0.025) ⁷	43.1 (0.076) ⁹
Uncharacterised Extract †	-	-	-	2.4 (0.002)	3.6 (0.006)
total identified %	84.9 (0.010)	81.1 (0.009)	92.0 (0.029)	58.8 (0.038)	37.9 (0.066)
non extracted TRR % (mg/kg) ‡	14.5 (0.002)	6.5 (0.001)	3.2 (0.001)	8.1 (0.005)	12.2 (0.021)
total TRR% (mg/kg)	100 (0.012)	100 (0.010)	100 (0.031)	100 (0.065)	99.1 (0.172)
[Pyrazole-¹⁴C]					
extractable TRR % (mg/kg)	86.1 (0.016)	89.6 (0.012)	96.8 (0.027)	90 (0.082)	88.2 (0.187)
pydiflumetofen	76.7 (0.015)	44.4 (0.005)	59.1 (0.016)	23.8 (0.022)	26.0 (0.055)
SYN545547	2.3 (<0.001)	3.9 (<0.001)	3.5 (0.001)	1.7 (0.002)	2.3 (0.005)
SYN547891	6.8 (0.001)	4.8 (0.001)	13.3 (0.004)	3.1 (0.003)	5.5 (0.012)
Unassigned % (mg/kg)	2.1 (<0.001) ²	24.8 (0.002) ⁴	14.3 (0.004) ⁶	53.8 (0.051) ⁸	54.3 (0.116) ¹⁰
Uncharacterised Extract †	11.3 (0.002)	27.6 (0.003)	-	2.6 (0.002)	2.3 (0.005)
total identified %	85.8 (0.016)	53.1 (0.006)	75.9 (0.021)	28.6 (0.027)	33.8 (0.072)
non extracted TRR % (mg/kg) ‡	13.9 (0.003)	10.4 (0.001)	3.2 (0.001)	9.9 (0.009)	11.3 (0.024)
total TRR% (mg/kg)	100 (0.019)	100 (0.012)	100 (0.028)	99.9 (0.091)	99.5 (0.211)

† Extractable residues produced during processing that were too low for analysis; highest TRR – turnip foliage, for which, no single fraction comprised $>27.6\%$ TRR (>0.003 mg/kg).

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

¹ at least 1 individual component not exceeding 3.4 % TRR (0.001 mg/kg)

² at least 1 individual component not exceeding 2.1 % TRR (0.001 mg/kg)

³ at least 1 individual component not exceeding 10.2 % TRR (0.001 mg/kg)

⁴ at least 5 individual components not exceeding $>10.2\%$ TRR (>0.001 mg/kg)

⁵ at least 5 individual components not exceeding $>2.9\%$ TRR (>0.001 mg/kg)

⁶ at least 6 individual components not exceeding $>3.5\%$ TRR (>0.001 mg/kg)

⁷ at least 21 individual components not exceeding $>5.0\%$ TRR (>0.003 mg/kg)

⁸ at least 30 individual components not exceeding >7.3 % TRR (>0.007 mg/kg)

⁹ at least 1 individual component not exceeding 3.4 % TRR (0.001 mg/kg)

¹⁰ at least 1 individual component not exceeding 3.4 % TRR (0.001 mg/kg)

Table 7-147 Summary of identification and characterization of residues – 120-day PBI

120-day PBI	Wheat forage	Wheat hay	Wheat straw
[Phenyl-¹⁴C]			
extractable TRR % (mg/kg)	89.2 (0.01)	85.2 (0.05)	85.9 (0.131)
pydiflumetofen	37.3 (0.004)	42.9 (0.025)	33.7 (0.051)
SYN545547	2.4 (<0.001)	1.5 (0.001)	1.4 (0.002)
SYN547891	4.9 (<0.001)	4.5 (0.003)	5.3 (0.008)
Unassigned % (mg/kg)	33.7 (0.001) ¹	28.0 (0.019) ³	42.8 (0.068) ⁵
Uncharacterised Extract †	-	4.2 (0.002)	-
total identified %	44.6 (0.004)	48.9 (0.0029)	40.4 (0.061)
non extracted TRR % (mg/kg) ‡	10.7 (0.001)	14.8 (0.009)	14.1 (0.022)
total TRR% (mg/kg)	99.9 (0.011)	100 (0.059)	100 (0.153)
[Pyrazole-¹⁴C]			
extractable TRR % (mg/kg)	91.1 (0.025)	84.5 (0.091)	85.9 (0.188)
pydiflumetofen	22.5 (0.006)	52.2 (0.056)	28.7 (0.063)
SYN545547	2.3 (0.001)	2.3 (0.002)	4.4 (0.010)
SYN547891	3.0 (0.001)	5.5 (0.006)	2.2 (0.005)
Unassigned % (mg/kg)	63.0 (0.013) ²	27.3 (0.029) ⁴	46.4 (0.103) ⁶
Uncharacterised Extract †	2.5 (0.001)	5.3 (0.006)	-
total identified %	27.8 (0.008)	60.0 (0.064)	35.3 (0.078)
non extracted TRR % (mg/kg) ‡	8.9 (0.002)	15.5 (0.017)	14.1 (0.031)
total TRR% (mg/kg)	100 (0.027)	100 (0.108)	100 (0.219)

† Extractable residues produced during processing that were too low for analysis; highest TRR – wheat hay, for which, no single fraction comprised >5.3% TRR (>0.006 mg/kg).

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

¹ at least 12 individual components not exceeding >5.3 % TRR (>0.001 mg/kg)

² at least 29 individual components not exceeding >9.2 % TRR (>0.002 mg/kg)

³ at least 20 individual components not exceeding >4.7 % TRR (>0.003 mg/kg)

⁴ at least 17 individual components not exceeding >4.3 % TRR (>0.005 mg/kg)

⁵ at least 27 individual components not exceeding >6.0 % TRR (>0.009 mg/kg)

⁶ at least 33 individual components not exceeding >4.6 % TRR (>0.010 mg/kg)

Table 7-148 Summary of identification and characterization of residues – 270-day PBI

270-day PBI	Wheat forage	Wheat hay	Wheat straw
[Phenyl-¹⁴C]			
extractable TRR % (mg/kg)	96.3 (0.013)	94.7 (0.037)	87.3 (0.1)
pydiflumetofen	59.7 (0.008)	76.1 (0.03)	32.2 (0.037)
SYN545547	3.1 (<0.001)	3.6 (0.001)	2.0 (0.002)
SYN547891	7.5 (0.001)	9.7 (0.004)	5.5 (0.006)
Unassigned % (mg/kg)	16.8 (0.001) ¹	7.3 (0.003) ³	44.2 (0.05) ⁵
Uncharacterised Extract †	-	3.1 (0.001)	6.2 (0.008)
total identified %	70.3 (0.009)	89.4 (0.035)	39.7 (0.045)
non extracted TRR % (mg/kg) ‡	3.7 (<0.001)	5.3 (0.002)	12.7 (0.014)
total TRR% (mg/kg)	100 (0.014)	100 (0.039)	100 (0.114)
[Pyrazole-¹⁴C]			
extractable TRR % (mg/kg)	95.5 (0.013)	93.5 (0.033)	83.2 (0.138)
pydiflumetofen	21.9 (0.003)	67.9 (0.024)	18.6 (0.030)
SYN545547	ND	5.6 (0.002)	1.5 (0.002)
SYN547891	4.6 (0.001)	12.2 (0.004)	4.6 (0.008)
Unassigned % (mg/kg)	62.4 (0.009) ²	9.3 (0.003) ⁴	67.0 (0.107) ⁶
Uncharacterised Extract †	-	3.5 (0.001)	16.9 (0.026)
total identified %	26.5 (0.004)	85.7 (0.030)	24.7 (0.004)
non extracted TRR % (mg/kg) ‡	4.5 (0.001)	6.5 (0.002)	16.8 (0.028)
total TRR% (mg/kg)	100 (0.014)	103 (0.035)	100 (0.166)

† Extractable residues produced during processing that were too low for analysis; highest TRR – wheat straw, for which, no single fraction comprised >3.9 % TRR (>0.009 mg/kg).

‡ Radioactivity remaining in the debris after extraction with acetonitrile and aqueous acetonitrile.

¹ at least 6 individual components not exceeding >4.1 % TRR (>0.001 mg/kg)

² at least 15 individual components not exceeding >7.0 % TRR (>0.001 mg/kg)

³ at least 20 individual components not exceeding >4.7 % TRR (>0.003 mg/kg)

⁴ at least 17 individual components not exceeding >4.3 % TRR (>0.005 mg/kg)

⁵ at least 17 individual components not exceeding >4.6 % TRR (>0.005 mg/kg)

⁶ at least 19 individual components not exceeding >5.8 % TRR (>0.009 mg/kg)

The present study describes the metabolism of pydiflumetofen in succeeding crops, following one foliar spray application directly to the soil. Lettuce, turnip, and wheat were planted 30, 120 and 270 days after application. Immature lettuce, turnip foliage, wheat straw, wheat hay and wheat straw were harvested and analysed. Mature

lettuce, turnip roots and wheat grain were also harvested; however, overall levels of TRR were low and no further identification was conducted.

The study represents 2N with regard to the maximum seasonal application according to critical GAP (1 x 200 g as/ha). However, the study is clearly underdosed when the potential for accumulation of pydiflumetofen residues is taken into account. Please see section 2.7.7 in Volume 1 for a further discussion relating to this.

The main observations from the identification work are the following:

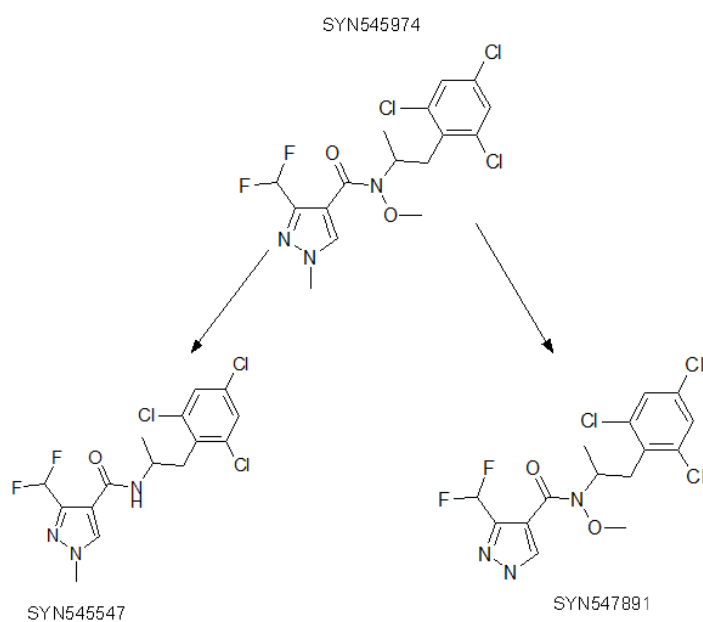
- Total residues declined between the 30 DALA and 270 DALA samples; this is true despite an increase in the TRR from the 30 DALA to 120 DALA wheat straw samples.
- Uptake of residue in wheat grain, turnip root and mature lettuce was low; with max TRR of 0.008 mg/kg (for wheat grain and turnip tubers, for 30 DALA samples – pyrazole label).
- Parent pydiflumetofen was the principal residue detected in all samples (18.6 – 77.8 % TRR); the maximum absolute residue of parent pydiflumetofen was 0.063 mg/kg in wheat straw at 120 DALA (pyrazole label).
- SYN545547 and SYN547891 were detected in each of the samples at lower levels than the parent; found at up to 0.005 mg/kg and 0.012 mg/kg, respectively (maximum TRR was 13.3 % TRR for SYN547891 in wheat forage, 30 DALA, pyrazole label – this corresponded to a low absolute amount – 0.004 mg/kg).
- For wheat samples, the proportion of the metabolites SYN545547 & SYN547891 remained broadly similar across all plant back intervals.

Unidentified components were present in all samples and, in total, accounted for between 2.3 and 67.0 % TRR; with the highest level found in 30 DALA wheat straw (0.116 mg/kg, pyrazole label). No individual unidentified component was present in wheat straw at >0.010 mg/kg (6.7-4.6 % TRR, in wheat straw). In turnip foliage there was an estimated highest level of unidentified residue (individual component) of 10.2 % TRR (0.001 mg/kg). Due to the low levels of TRR found, the radioactive peaks were present at low mg/kg amounts, even when at >5% TRR.

The principal biotransformations observed were:

- Demethylation of the pyrazole ring to produce SYN547891.
- Reduction of the parent molecule producing SYN545547.

Figure 7-15 Proposed metabolic pathway of pydiflumetofen in succeeding crops



B.7.6.2. Magnitude of residues in rotational crops

Across four studies, eight field trials have been conducted to investigate the magnitude of pydiflumetofen residues in succeeding crops, four trials in NEU/UK and four in SEU. In each trial a single application was made to bare soil using A19649B (an SC formulation) at a nominal rate of 500 – 600 g a.s./ha. In the first two studies, nine representative rotated crops (kale, tomato, maize, soybean, fresh bean, strawberry, spinach, carrot, radish) were planted at nominal intervals of 30, 120, 270 and 330 days. In the last two studies, three representative rotated crops (spinach, carrot and barley) were planted back into the treated plots at nominal intervals of 30, 60 and 365 days.

B.7.6.2.1. Study 1

Report:	K-CA 6.6.2/01: [REDACTED], (2018)
Title:	Adepidyn – Residue Study on Rotational Crops in Northern France and Germany during 2016 – 2017.
Report No:	Report Number CEMR-7709, (Syngenta Report No. TK0289435).
Document No:	VV-469769 (Syngenta File No. A19649B 10353)
Guidelines:	Commission of the European Communities, Crop Rotation Studies; 7524/VI/95 (rev. 2). Commission of the European Communities, General Recommendations for the Design, Preparation and Realisation of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals. OECD Test Guideline 504: Residues in Rotational Crops (Limited Field Studies).
Guideline deviations:	None
GLP:	Yes

Materials and methods

The purpose of the study was to determine the magnitude of residues of pydiflumetofen in/on rotated field crops (succeeding crops) after one spray application with A19649B, a suspension concentrate (SC) formulation containing 200 g/L pydiflumetofen to both treated plots. One application was made to bare soil at a nominal rate of 600 g a.s./ha (actual application rates ranged from 562 – 611 g a.s./ha); followed by sowing or planting of the rotational crops. Prior to replanting, the soil was harrowed at a depth of between 5-10 cm. The test soils used in the trials were: clay loam (Northern France; trial reference S16-04583-01) and loamy sand (Germany; trial reference S16-04583-02).

The study included two supervised residue trials conducted in Northern Europe (Northern France and Germany) during the 2016 & 2017 season. Nine representative crops were planted in each of the trial locations, at nominal plant back intervals of 30, 120, 270 and 330 days – see Table 7-149 and Table 7-150 for full application and sampling details.

Table 7-149 Application details

Crops	Plant back intervals (days)		Application rate (g a.s./ha)	
	Northern France (S16-04583-01)	Germany (S16-04583-02)	Northern France (S16-04583-01)	Germany (S16-04583-02)
Kale, Strawberry, Maize	32	30	599	594
	125	118	588	609
	268	273	611	592
	330	334	595	562
Tomato, Fresh bean, soybean	32	30	593	594
	125	118	577	609

Spinach, Carrot, Radish	268	273	605	592
	330	334	599	562
	32	30	595	594
	125	118	566	609
	268	273	611	592
	330	334	601	562

Table 7-150 Crop and sampling details

Representative crop group ^a	Crop	Variety		Application and sampling details	
		Northern France	Germany	Crop Part	Harvest Time (BBCH)
Leafy vegetables and Brassicas	Kale	<i>Reflex</i>	<i>Winneton</i>	Leaves	49
	Spinach	<i>Samos</i>	<i>Molokai</i>	Immature leaves	43-51
				Mature leaves	49-65
Root and tuber vegetables	Carrot	<i>Hekla</i>	<i>Yaguna F1</i>	Roots, tops with foliage	49
	Radish	<i>Apache</i>	<i>Alese</i>	Roots, tops with foliage	49
Cereals	Maize	<i>Quincey</i>	<i>P7378</i>	Whole cobs, remaining plant	89
Oilseeds and Pulses	Soybean ^b	<i>SG Fider</i>	<i>Adsov</i>	Forage	79-81
	Fresh bean ^c	<i>Flagiano</i>	<i>Primel</i>	Fresh seed, whole plant	74-79
				Dry seed	89
Fruits and Fruiting Vegetables	Strawberry	<i>Charlotte</i>	<i>Honeoye</i>	Fruit	87-89
	Tomato	<i>Montfaret</i>	<i>Harzfuer</i>	Fruit	74-89

a: Representative rotational crop is in reference to 'super' crop groups; as detailed in the OECD 504 guidance on Residues in Rotational Crops.

b: For soybean, the plants did not develop sufficiently in either trial in order to obtain usable samples of seed. Forage was collected in the Northern France trial only.

c: Fresh seed and dry were collected in the German trial only. The crop did not develop sufficiently to obtain usable seed samples in the trial conducted in Northern France.

Samples were frozen within 9 hours of sampling. Samples were then shipped and stored frozen at <-18 °C for a maximum of 7 months. Sufficient storage stability data has been reported in section B.7.1 to support all crops considered in this rotational field trial. Sample extracts were stored for a maximum of 9 days before analysis; procedural recovery values for these analyses are acceptable.

The majority of collected samples comprised of an appropriate weight and contained an appropriate minimum no. of units; with the following exceptions: soybean seeds, and fresh/dry beans in the trial conducted in Northern France. These samples were not analysed. Additionally, a reduced weight of sample was collected for strawberry

from the Northern France plot however this only impacted the retain samples. Weather conditions were generally acceptable; however, the weather conditions were less than optimum for spinach in Northern France – a number of samples had reduced weights, and a number of retained samples were not collected – overall, no impact is expected on the validity of the trial. The minimum no. of samples was acceptable in each case and all treated samples were >1 kg.

Soil samples were also collected from both control and treated plots but were not analysed in any case. No pesticides containing pydiflumetofen (or with a similar structure) were applied in the previous three seasons or as maintenance pesticides on any of the test or control plots – no interference is expected.

Residues were analysed using method 'GRM061.03A'. Method GRM061.03A has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (wheat grain and potato), high protein (dry beans), and dry (wheat straw) commodities. However, the method validation data does not cover all the plant commodities in the rotational crop trials – only tomatoes and beans (dry) are covered by the method validation data. A reduced validation data set is required for the other crops considered in the rotational field studies. Samples were spiked at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The following number of samples were prepared at each fortification level:

- > 3 samples: spinach (leaves), spring barley (whole plant, grain, straw), carrot (root, top)
- 2 samples: bean (fresh seed, dry seed, remaining plant), kale, tomato (fruit), maize (whole cob, remaining plant), soybean (forage), strawberries (fruit), radish (roots, tops)
- 1 sample: soybean (seed)

Mean recoveries were within the acceptable range (70-110%) except for maize (whole cob) at 0.01 mg/kg and maize (whole cob), soybean (forage), bean (dry seed) and radish (tops) at 0.1 mg/kg. However, these are acceptable as the recoveries are only slightly outside of the acceptable range. The %RSD was $\leq 20\%$. Matrix matched standards were used for quantification for all crop commodities, except for carrot (roots) in Study CEMR 7709 as matrix effects were not deemed significant. Example chromatograms have been provided showing the method is specific and no significant interference (>30% of the LOQ) is observed.

Overall, the method is acceptably validated in spinach (leaves), spring barley (whole plant, grain, straw), carrot (root), carrot (tops), tomatoes and beans (dry) with an LOQ of 0.01 mg/kg. For bean (fresh seed, remaining plant), kale, maize (whole cob, remaining plant), soybean (forage, seed), strawberries (fruit) and radishes (roots and tops), the method can be considered fit for regulatory purposes.

Procedural recovery data was provided and is discussed below. Only one determination was made at each fortification level, per crop matrix.

Acceptable procedural recovery data within the range 70-110 % was provided for the following crop matrices at both fortification levels (0.01 and 0.1 mg/kg): kale, bean (fresh seed), strawberry (fruit), spinach (immature leaves), carrot (root and top w/ foliage) and radish (root and top w/ foliage).

The procedural recovery for several crop matrices marginally exceeded 110 %, for either one or both fortification levels, these are: tomato, maize (whole plant), soybean (forage), bean (dry seed and remaining plant) and spinach (mature leaves). The procedural recoveries for maize (whole cob) were the highest at 115 % and 121 % for the 0.01 and 0.1 mg/kg levels, respectively. As a result of this, the residue results for the aforementioned crops may be marginally overestimated. Nevertheless, the residue results are considered acceptable and can be used to estimate the magnitude of residues in rotational crops. As noted above, the procedural recoveries obtained were individual recoveries (one per crop/fortification level), and the guideline levels of acceptable recoveries cite ranges of acceptable mean % levels for validation work.

Results and discussion

For both trials, all control residues were <0.01 mg/kg for all crop matrices. The results in treated samples are summarised in Table 7-151.

Table 7-151 Results from rotational crop field trials following application at ~600 g a.s/ha to bare soil and four plant back intervals

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		Northern France (S16-04583-01)	Germany (S16-04583-02)
Kale leaves (BBCH 49 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Tomato mature fruit (BBCH 87-89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Maize whole cobs (BBCH 89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Maize remaining plant (BBCH 89 – NCH)	30	<0.01	<0.01
	120	0.02	<0.01
	270	<0.01	<0.01
	330	0.01	<0.01
Soybean forage (BBCH 73-76)	30	0.01	NS
	120	0.01	NS
	270	0.01	NS
	330	0.01	NS
Soybean seed	-	NS	NS
Bean – fresh seed (BBCH 79 – NCH)	30	NS	<0.01
	120	NS	<0.01
	270	NS	<0.01
	330	NS	<0.01
Bean – dry seed (BBCH 89 – NCH)	30	NS	<0.01
	120	NS	<0.01
	270	NS	<0.01
	330	NS	<0.01
Bean -remaining plant (BBCH 89 – NCH)	30	NS	<0.01
	120	NS	<0.01
	270	NS	<0.01
	330	NS	<0.01
Bean -Whole plant (BBCH 74-75)	30	<0.01	NS
	120	<0.01	NS

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		Northern France (S16-04583-01)	Germany (S16-04583-02)
	270	<0.01	NS
	330	<0.01	NS
Strawberry (BBCH 87-89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Spinach (BBCH 43)	30	0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	0.02
	330	<0.01	<0.01
Spinach (BBCH 49 – NCH)	30	0.05	<0.01
	120	0.01	0.02
	270	0.01	0.03
	330	0.01	<0.01
Carrot roots (BBCH 49 – NCH)	30	0.02	<0.01
	120	0.02	<0.01
	270	0.02	<0.01
	330	0.01	<0.01
Carrot tops with foliage (BBCH 49 – NCH)	30	0.01	0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Radish roots (BBCH 49 – NCH)	30	0.04	<0.01
	120	0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Radish tops with foliage (BBCH 49 – NCH)	30	0.03	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01

NS – not sampled

Residues of pydiflumetofen were detected at or above the LOQ (0.01 mg/kg) in some succeeding crops. The highest residues of pydiflumetofen were found in spinach leaves (0.05 mg/kg, 30d PBI), radish roots and tops (0.04 and 0.03 mg/kg 30d PBI, respectively), carrot roots (0.02 mg/kg at 30, 120 and 270 PBI) soya bean forage (0.01 mg/kg at all PBI) and maize remaining plant (0.02 mg/kg, 120d PBI).

Residues were below LOQ (0.01 mg/kg) in kale leaves, tomatoes, strawberries, whole maize cobs, fresh or dry beans at any of the plant back intervals.

B.7.6.2.2. Study 2

Report:	K-CA 6.6.2/02: [REDACTED] (2018)
Title:	Pydiflumetofen - Residue Study on Rotational Crops in Southern France and Spain during 2016-2017.
Report No:	Report Number CEMR-7710, (Syngenta Report No. TK0289439).
Document No:	VV-470802 (Syngenta File No. A19649B 10359)
Guidelines:	Commission of the European Communities, Crop Rotation Studies; 7524/VI/95 (rev. 2). Commission of the European Communities, General Recommendations for the Design, Preparation and Realisation of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals. OECD Test Guideline 504: Residues in Rotational Crops (Limited Field Studies).
Guideline deviations:	Yes – some impact from these, see below for details.
GLP:	Yes

Materials and methods

The purpose of the study was to determine the magnitude of residues of pydiflumetofen in/on rotated field crops (succeeding crops) after one spray application with A19649B, a suspension concentrate (SC) formulation containing 200 g/L pydiflumetofen to both treated plots. One application was made to bare soil at a nominal rate of 600 g a.s./ha (actual application rates ranged from 529 – 643 g a.s./ha); followed by sowing or planting of the rotational crops. Prior to replanting, the soil was harrowed at a depth of between 5-10 cm. The test soils used in both trials was loamy (Southern France, trial reference S16-04584-01; Spain, trial reference S16-04584-02).

The study included two supervised residue trials conducted in Southern Europe (Southern France and Spain) during the 2016 & 2017 season. Nine representative crops were planted in each of the trial locations, at nominal plant back intervals of 30, 120, 270 and 330 days – see Table 7-152 and Table 7-153 for full application and sampling details.

Table 7-152 Application details

Crops	Plant back intervals (days)		Application rate (g a.s./ha)	
	Southern France (S16-04584-01)	Spain (S16-04584-02)	Southern France (S16-04584-01)	Spain (S16-04584-02)
Kale, Tomato, Maize	32	31	596	577
	116	126	592	643
	287	253	576	593
	340	339	611	544
Fresh bean, soybean, Strawberry	32	31	596	585
	116	126	592	598
	287	253	576	529
	340	339	611	548
Spinach, Carrot, Radish	32	31	596	596
	116	126	592	616
	287	253	576	547
	340	339	611	536

Table 7-153 Crop and sampling details

Representative crop group ^a	Crop	Variety		Application and sampling details	
		Southern France	Spain	Crop Part	Harvest Time (BBCH)
Leafy vegetables and Brassicas	Kale	<i>Winterbor F1</i>	<i>Toscana</i>	Leaves	49
	Spinach	<i>Samos F1</i>	<i>Gigante de Invierno</i>	Immature leaves	43
				Mature leaves	49
Root and tuber vegetables	Carrot	<i>Lagos F1</i>	<i>Mantesa Forte</i>	Roots, tops with foliage	49
	Radish	<i>Radis de 18 jours</i>	<i>Punta Redonda</i>	Roots, tops with foliage	49
Cereals	Maize	<i>LG30597</i>	<i>P9400</i>	Whole cobs, remaining plant	89
Oilseeds and Pulses	Soybean	<i>Palladore</i>	<i>Judia Mung</i>	Seed, forage	79-89
	Fresh bean	<i>Mogex</i>	<i>Contender</i>	Fresh seed, whole plant	79
				Dry seed	89
Fruits and Fruiting Vegetables	Strawberry ^b	<i>Mara des bois</i>	<i>Morangos</i>	Fruit	89
	Tomato	<i>Rio</i>	<i>Manitor</i>	Fruit	89

a: Representative rotational crop is in reference to 'super' crop groups; as detailed in the OECD 504 guidance on Residues in Rotational Crops.

b: Southern France only. No samples were taken in the Spanish trial due to poor crop development.

Samples were frozen within 8 hours of sampling. Samples were then shipped and stored frozen at <-18 °C for a maximum of 11 months. Sufficient storage stability data has been reported in section B.7.1 to support all crops considered in this rotational field trial. Sample extracts were stored for a maximum of 6 days before analysis; procedural recovery samples were stored for the same length of time, and were acceptable (see below). An appropriate sample weight was obtained for the majority of samples with the exception of the following (the affected residue values have been highlighted in Table 7-154):

- **S16-04584-01**
 - **Strawberry:** low sample weight for sample at 330 DALA (only 0.635 kg instead of 1 kg, no retained sample); nevertheless, sample size was considered sufficient for analysis. Additionally, all residues at all PBIs were <LOQ; therefore, a reduced sample weight at 330 DALA is not expected to have any impact.
- **S16-04584-02**
 - **Kale:** no control sample, no treated sample at 120 or 330 day PBI.
 - **Strawberry:** no samples at any PBI.
 - **Soybean:** no forage samples at 30 or 270 day PBI; no sufficient seed sample for any PBI.
 - **Fresh bean:** no control samples of dry seed, no treated sample at 120 or 330 day PBI.
 - **Spinach:** no control sample, no sample of immature or mature leaf at 120 or 330 day PBI.

- **Carrot:** no treated sample (root and foliage with top) at 30 or 270 day PBI.

Soil samples were also collected from both control and treated plots, but were not analysed in any case. No pesticides containing pydiflumetofen (or with a similar structure) were applied in the previous three seasons or as maintenance pesticides on any of the test or control plots – no interference is expected. There were no exceptional weather events that impacted the validity of the study.

Residues were analysed using method 'GRM061.03A'. Method GRM061.03A has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (wheat grain and potato), high protein (dry beans), and dry (wheat straw) commodities. However, the method validation data does not cover all the plant commodities in the rotational crop trials – only tomatoes and beans (dry) are covered by the method validation data. A reduced validation data set is required for the other crops considered in the rotational field studies. Samples were spiked at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The following number of samples were prepared at each fortification level:

- > 3 samples: spinach (leaves), spring barley (whole plant, grain, straw), carrot (root, top)
- 2 samples: bean (fresh seed, dry seed, remaining plant), kale, tomato (fruit), maize (whole cob, remaining plant), soybean (forage), strawberries (fruit), radish (roots, tops)
- 1 sample: soybean (seed)

Mean recoveries were within the acceptable range (70-110%) except for maize (whole cob) at 0.01 mg/kg and maize (whole cob), soybean (forage), bean (dry seed) and radish (tops) at 0.1 mg/kg. However, these are acceptable as the recoveries are only slightly outside of the acceptable range. The %RSD was $\leq 20\%$. Matrix matched standards were used for quantification for all crop commodities in the study. Example chromatograms have been provided showing the method is specific and no significant interference ($>30\%$ of the LOQ) is observed.

Overall, the method is acceptably validated in spinach (leaves), spring barley (whole plant, grain, straw), carrot (root), carrot (tops), tomatoes and beans (dry) with an LOQ of 0.01 mg/kg. For bean (fresh seed, remaining plant), kale, maize (whole cob, remaining plant), soybean (forage, seed), strawberries (fruit) and radishes (roots and tops), the method can be considered fit for regulatory purposes.

Procedural recovery data was provided and is discussed below. Only one determination was made at each fortification level, per crop matrix.

Acceptable procedural recovery data within the range 70-110 % was provided for the following crop matrices at both fortification levels (0.01 and 0.1 mg/kg): tomato, maize (remaining plant), soybean (seed), bean (fresh seed, dry seed, remaining plant), strawberry, spinach (immature and mature leaves), carrot (root, tops w/ foliage), radish (roots).

The procedural recovery for several crop matrices marginally exceeded 110 %, for either one or both fortification levels, these are: kale, maize (whole cob), soybean (forage), radish (tops w/ foliage). The procedural recovery for soybean (forage) was the highest at 117 % for the 0.1 mg/kg level. As a result of this, the residue results for the aforementioned crops may be marginally overestimated. Nevertheless, the residue results are considered acceptable and can be used to estimate the magnitude of residues in rotational crops. As noted above, the procedural recoveries obtained were individual recoveries (one per crop/fortification level), and the guideline levels of acceptable recoveries cite ranges of acceptable mean % levels for validation work.

Results and discussion

For both trials, all control residues were <0.01 mg/kg for all crop matrices; with the exception of kale, and spinach (immature and mature leaf), for which no control samples were available in trial S16-04584-02. However, as all available control residues are <0.01 mg/kg, it can be assumed that all control residues are $<LOQ$. Additionally, treated samples were not available for crops corresponding to some of these missing control samples. The results in treated samples are summarised in Table 7-154.

Table 7-154 Results from rotational crop field trials following application at ~600 g a.s/ha to bare soil and four plant back intervals

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		Southern France (S16-04584-01)	Spain (S16-04584-02)
Kale leaves (BBCH 49 – NCH)	30	<0.01	<0.01 ^a
	120	<0.01	NS
	270	<0.01	<0.01 ^a
	330	<0.01	NS
Tomato (BBCH 89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Maize whole cobs (BBCH 89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Maize remaining plant (BBCH 89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Soybean forage (BBCH 79-81)	30	<0.01	NS
	120	<0.01	<0.01
	270	<0.01	NS
	330	<0.01	<0.01
Soybean seed (BBCH 89 – NCH)	30	<0.01	- ^b
	120	<0.01	NS
	270	<0.01	- ^b
	330	<0.01	NS
Bean – fresh seed (BBCH 79 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Bean – dry seed (BBCH 89 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Bean -remaining plant (BBCH 79 – NCH)	30	<0.01	<0.01
	120	<0.01	<0.01
	270	<0.01	<0.01

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		Southern France (S16-04584-01)	Spain (S16-04584-02)
	330	<0.01	<0.01
Strawberry (BBCH 89 – NCH)	30	<0.01	NS
	120	<0.01	NS
	270	<0.01	NS
	330	<0.01	NS
Spinach (BBCH 43)	30	<0.01	<0.01 ^a
	120	<0.01	NS
	270	<0.01	<0.01 ^a
	330	<0.01	NS
Spinach (BBCH 49, NCH)	30	<0.01	<0.01 ^a
	120	<0.01	NS
	270	<0.01	<0.01 ^a
	330	<0.01	NS
Carrot roots (BBCH 49 – NCH)	30	0.02	NS
	120	0.03	<0.01
	270	0.01	NS
	330	0.02	<0.01
Carrot tops with foliage (BBCH 49 – NCH)	30	0.01	NS
	120	0.01	<0.01
	270	<0.01	NS
	330	<0.01	<0.01
Radish roots (BBCH 49 – NCH)	30	0.03	<0.01
	120	0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01
Radish tops with foliage (BBCH 49 – NCH)	30	0.02	<0.01
	120	0.01	<0.01
	270	<0.01	<0.01
	330	<0.01	<0.01

NS – not sampled

a: No control sample available.

b: Insufficient sample size.

Residues of pydiflumetofen were detected at or above the LOQ (0.01 mg/kg) in some succeeding crops. The highest residues of pydiflumetofen were found in radish roots and carrot roots (0.03 mg/kg, 30d and 120d PBI, respectively). Residues >0.01 mg/kg were found at all PBIs for carrot roots. The remaining residues were found in radish and carrot tops with foliage (0.02 and 0.01 mg/kg 30d PBI, respectively)

Residues were below LOQ (0.01 mg/kg) in kale, tomato, maize (whole cobs and remaining plants), soybean (seed and forage), bean (fresh seed, dry seed and remaining plant), strawberry, spinach (immature and mature leaf) at

all plant back intervals. No residues greater than LOQ were found in any sample taken in the Spanish trial – although many samples were not taken, due to poor crop development.

B.7.6.2.3. Study 3

Report:	K-CA 6.6.2/03: [REDACTED], [REDACTED] (2016)
Title:	SYN545974 – Residue Study on Rotational Crops in Southern France and Italy during 2013 – 2015.
Report No:	Report Number S13-01023, (Syngenta Report No. TK0178755).
Document No:	VV-415410 (Syngenta File No. A19649B 10235)
Guidelines:	Commission of the European Communities, Crop Rotation Studies; 7524/VI/95 (rev. 2). Commission of the European Communities, General Recommendations for the Design, Preparation and Realisation of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals. OECD Test Guideline 504: Residues in Rotational Crops (Limited Field Studies).
Guideline deviations:	No
GLP:	Yes

Materials and methods

The purpose of the study was to determine the magnitude of residues of pydiflumetofen in/on rotated field crops (succeeding crops) after one spray application with A19649B, a suspension concentrate (SC) formulation containing 200 g/L pydiflumetofen to both treated plots. One application was made to bare soil at a nominal rate of 500 g a.s./ha (actual application rates ranged from 484-503 g a.s./ha); followed by sowing or planting of the rotational crops. Prior to replanting, the soil was harrowed at a depth of between 7-10 cm. The test soils used in the trials were: loam (Southern France, trial reference S13-01023-01) and sandy loam (Italy, trial reference S13-01023-02).

The study comprised of two supervised residue trials conducted in Southern Europe (Southern France and Italy) during the 2013, 2014 & 2015 seasons. Three representative crops were planted in each of the trial locations, at nominal plant back intervals of 30, 60 and 365 days – see Table 7-155 and Table 7-156 for full application and sampling details.

Deviations

The initial barley crop planted in plot 2 in S13-01023-01 failed in the first round of drilling in June 2014. This resulted in barley being re-drilled into plot P3, in April 2015, in order to achieve the nominal 365 day plant back interval for this crop. It is possible that this deviation had an impact on the residue levels seen for barley at the 365 day PBI in trial S13-01023-01. The re-drilled barley was sown on a plot on which a succeeding crop had already been grown (either spinach, barley or carrot for the 66 day PBI samples). Whilst this likely mirrors ‘real-world’ growing conditions and crop rotation, it is not line with the remaining crops which were planted at a nominal 365 day PBI. Overall, the impact is not considered significant – the residue results observed for the re-drilled barley (<LOQ in grain, low level residues in straw) are in line with all trials conducted on cereal (wheat and barley) for the same PBI.

Table 7-155 Application details

Crops	Plant back intervals (days)		Application rate (g a.s./ha)	
	Southern France (S13-01023-01)	Italy (S13-01023-02)	Southern France (S13-01023-01)	Italy (S13-01023-02)
Spinach	27	27	494	484
	66	55	503	502
	346	338	489	487

Barley	27	27		494	484
	66	55		503	502
	375 ^a	338		503	487
Carrot	27	27		494	484
	66	55		503	502
	346	338		489	487

a: Due to failure of the initial barley crop on plot 2 of trial S13-01023-01, barley was re-drilled into plot P3 to achieve the nominal PBI of 365 days

Table 7-156 Crop and sampling details

Representative crop group ^a	Crop	Variety		Application and sampling details	
		Southern France	Italy	Crop Part	Harvest Time (BBCH)
Leafy vegetables and Brassicas	Spinach	<i>Falcon</i>	<i>Carmen</i>	Immature leaves	43
				Mature leaves	49
Cereals	Barley	<i>Prestige^b</i>	<i>Tunika</i>	Immature whole plant	41
				Grain and straw	89
Root and tuber vegetables	Carrot	<i>Mondibel F1</i>	<i>Bolero</i>	Roots, tops with foliage	49

a: Representative rotational crop is in reference to ‘super’ crop groups; as detailed in the OECD 504 guidance on Residues in Rotational Crops

b: Initial crop variety was ‘*Explorer*’ – the re-drilled variety is reported in the table.

Samples were frozen within 4 hours of sampling. Samples were then shipped and stored frozen at <-18 °C for a maximum of 16 months. Sufficient storage stability data has been reported in section B.7.1 to support all crops considered in this rotational field trial. Sample extracts were stored for a maximum of 8 days before analysis, procedural recovery samples were stored for the same length of time, and were acceptable (see below). An appropriate sample weight was obtained for all samples; with the exception of the 30 day PBI spinach sample in study S13-01023-01 – for which only ~0.5 kg was obtained, this is considered sufficient to allow robust analysis.

No pesticides containing pydiflumetofen (or any pesticides with a similar structure) were applied in the previous three seasons or; as maintenance pesticides on any of the test or control plots – no interference is expected. There were no exceptional weather events that impacted the validity of the study.

Residues were analysed using method ‘GRM061.03A’. Method GRM061.03A has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (wheat grain and potato), high protein (dry beans), and dry (wheat straw) commodities. However, the method validation data does not cover all the plant commodities in the rotational crop trials – only tomatoes and beans (dry) are covered by the method validation data. A reduced validation data set is required for the other crops considered in the rotational field studies. Samples were spiked at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The following number of samples were prepared at each fortification level:

- > 3 samples: spinach (leaves), spring barley (whole plant, grain, straw), carrot (root, top)
- 2 samples: bean (fresh seed, dry seed, remaining plant), kale, tomato (fruit), maize (whole cob, remaining plant), soybean (forage), strawberries (fruit), radish (roots, tops)
- 1 sample: soybean (seed)

Mean recoveries were within the acceptable range (70-110%) except for maize (whole cob) at 0.01 mg/kg and maize (whole cob), soybean (forage), bean (dry seed) and radish (tops) at 0.1 mg/kg. However, these are acceptable

as the recoveries are only slightly outside of the acceptable range. The %RSD was $\leq 20\%$. Matrix matched standards were used for quantification for all crop commodities in this study. Example chromatograms have been provided showing the method is specific and no significant interference ($>30\%$ of the LOQ) is observed.

Overall, the method is acceptably validated in spinach (leaves), spring barley (whole plant, grain, straw), carrot (root), carrot (tops), tomatoes and beans (dry) with an LOQ of 0.01 mg/kg. For bean (fresh seed, remaining plant), kale, maize (whole cob, remaining plant), soybean (forage, seed), strawberries (fruit) and radishes (roots and tops), the method can be considered fit for regulatory purposes.

Procedural recovery data was provided. Three determinations were made at each fortification level (0.01 or 0.1 mg/kg), per crop matrix, aside from barley straw and carrot tops (where there were two procedural recoveries at each fortification level). All mean recoveries were within the range 70-110 %.

Results and discussion

For both trials, all control residues were <0.01 mg/kg for all crop matrices. The results in treated samples are summarised in Table 7-157.

Table 7-157 Results from rotational crop field trials following application at ~ 500 g a.s/ha to bare soil and three plant back intervals

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		Southern France (S13-01023-01)	Italy (S13-01023-02)
Spinach (BBCH 43)	30	0.01	<0.01
	60	0.02	<0.01
	365	<0.01	<0.01
Spinach (BBCH 49 – NCH)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Carrot roots (BBCH 49 – NCH)	30	0.02	<0.01
	60	0.02	0.02
	365	<0.01	<0.01
Carrot tops with foliage (BBCH 49 – NCH)	30	<0.01	<0.01
	60	0.01	<0.01
	365	<0.01	<0.01
Barley immature whole plant (BBCH 41)	30	<0.01	0.02
	60	<0.01	<0.01
	365	<0.01 ^a	<0.01
Barley grain (BBCH 89 – NCH)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01 ^a	<0.01
Barley Straw (BBCH 89 – NCH)	30	0.06	0.02
	60	0.09	0.02
	365	0.01 ^a	0.01

a: Crop re-drilled after a succeeding crop had already been grown (succeeding crops of carrot, spinach or barley were planted and harvested before this crop was sown).

Residues of pydiflumetofen were detected at or above the LOQ (0.01 mg/kg) in some succeeding crops. The highest residues of pydiflumetofen were found in barley straw (0.06 and 0.09 mg/kg, for the 30d and 60d PBI, respectively). Residues >0.01 mg/kg were also found carrot roots in both trials (max – 0.02 mg/kg at 60d PBI). Greater than LOQ residues were also found in spinach (immature leaf), carrot tops, and barley whole plant.

Residues were below LOQ (0.01 mg/kg) in spinach (mature leaf) and barley grain at all plant back intervals.

B.7.6.2.4. Study 4

Report:	K-CA 6.6.2/04: [REDACTED], [REDACTED] (2016)
Title:	SYN545974 – Residue Study on Rotational Crops in the United Kingdom and Germany during 2013 – 2014.
Report No:	Report Number S13-01022, (Syngenta Report No. TK0178741).
Document No:	VV-415357 (Syngenta File No. A19649B 10234)
Guidelines:	Commission of the European Communities, Crop Rotation Studies; 7524/VI/95 (rev. 2). Commission of the European Communities, General Recommendations for the Design, Preparation and Realisation of Residue Trials; 7029/VI/95 (rev. 5, working document). OECD Guidelines for the Testing of Chemicals. OECD Test Guideline 504: Residues in Rotational Crops (Limited Field Studies).
Guideline deviations:	No
GLP:	Yes

Materials and methods

The purpose of the study was to determine the magnitude of residues of pydiflumetofen in/on rotated field crops (succeeding crops) after one spray application with A19649B, a suspension concentrate (SC) formulation containing 200 g/L pydiflumetofen to both treated plots. One application was made to bare soil at a nominal rate of 500 g a.s./ha (actual application rates ranged from 459-515 g a.s./ha); followed by sowing or planting of the rotational crops. Prior to replanting, the soil was harrowed at a depth of between 7-15 cm. The test soils used in the trials were: clay loam (United Kingdom; trial reference S13-01022-01) and loamy sand (Germany; trial reference S13-01022-02).

The study comprised of two supervised residue trials conducted in Northern Europe (United Kingdom and Germany) during the 2013-2014 season. Three representative crops were planted in each of the trial locations, at nominal plant back intervals of 30, 60 and 365 days – see Table 7-155 and Table 7-159 for full application and sampling details.

Table 7-158 Application details

Crops	Plant back intervals (days)		Application rate (g a.s./ha)	
	United Kingdom ^a (S13-01022-01)	Germany ^b (S13-01022-02)	United Kingdom (S13-01022-01)	Germany (S13-01022-02)
Spinach	63	29	510	515
	94	58	505	500
	400	383	459	490
Barley	28	29	510	515
	59	58	505	500
	365	383	459	490
Carrot	63	29	510	515
	94	59	505	500
	400	383	459	490

- a: All crops were initially drilled on the same date (02/05/14). The initial spinach and carrot crop failed due to heavy rainfall; these crops were re-drilled 35 days later on the same plot. This is not expected to have any impact on the study
- b: Plot 2 (nominal PBI 365) was originally drilled 19 days earlier. However, none of the representative crops emerged. The plot was prepared again and the crops re-drilled. No impact expected.

Table 7-159 Crop and sampling details

Representative crop group ^a	Crop	Variety		Application and sampling details	
		United Kingdom (S13-01022-01)	Germany (S13-01022-02)	Crop Part	Harvest Time (BBCH)
Leafy vegetables and Brassicas	Spinach	<i>Renegade ^b</i>	<i>Molokai</i>	Immature leaves	43
				Mature leaves	49
Cereals	Barley	<i>Optic</i>	<i>Simba</i>	Immature whole plant	41
				Grain and straw	89
Root and tuber vegetables	Carrot	<i>Chantenay</i>	<i>Laguna F1</i>	Roots, tops with foliage	49

- a: Representative rotational crop is in reference to 'super' crop groups; as detailed in the OECD 504 guidance on Residues in Rotational Crops
- b: The spinach crop in trial S13-01022-01 began to bolt prematurely, meaning it went straight from BBCH 14-18 → BBCH 60. Therefore, the crops were harvested at the BBCH 14-18 stage. Nevertheless, the timing of the harvest is in line with what could be expected for normal harvest; therefore, the collected samples are considered representative of immature spinach leaves.

Samples were frozen within 4 hours of sampling. Samples were then shipped and stored frozen at <-18 °C for a maximum of 13 months. Sufficient storage stability data has been reported in section B.7.1 to support all crops considered in this rotational field trial. Sample extracts were stored for a maximum of 1 day before analysis – therefore no consideration of extract storage stability is required. An appropriate sample weight was obtained for all samples; the retained control sample of barley grain in trial S13-01022-01 was less than the required 1 kg (0.64 kg) – no impact is expected as a result of this.

No pesticides containing pydiflumetofen (or any pesticides with a similar structure) were applied in the previous three seasons or as maintenance pesticides on any of the test or control plots – no interference is expected. There were no exceptional weather events that impacted the validity of the study.

Residues were analysed using method 'GRM061.03A'. Method GRM061.03A has been acceptably validated in accordance with SANCO/3029/99 rev. 4 for the determination of pydiflumetofen residues in high water (apples, lettuce, tomato, cabbage, fresh peas and cereal forage), high acid (grape), high oil (oilseed rape), high starch (wheat grain and potato), high protein (dry beans), and dry (wheat straw) commodities. However, the method validation data does not cover all the plant commodities in the rotational crop trials – only tomatoes and beans (dry) are covered by the method validation data. A reduced validation data set is required for the other crops considered in the rotational field studies. Samples were spiked at 0.01 mg/kg (LOQ) and 0.1 mg/kg (10 x LOQ). The following number of samples were prepared at each fortification level:

- > 3 samples: spinach (leaves), spring barley (whole plant, grain, straw), carrot (root, top)
- 2 samples: bean (fresh seed, dry seed, remaining plant), kale, tomato (fruit), maize (whole cob, remaining plant), soybean (forage), strawberries (fruit), radish (roots, tops)
- 1 sample: soybean (seed)

Mean recoveries were within the acceptable range (70-110%) except for maize (whole cob) at 0.01 mg/kg and maize (whole cob), soybean (forage), bean (dry seed) and radish (tops) at 0.1 mg/kg. However, these are acceptable as the recoveries are only slightly outside of the acceptable range. The %RSD was ≤20%. Matrix matched standards were used for quantification for all crop commodities in this study. Example chromatograms have been provided showing the method is specific and no significant interference (>30% of the LOQ) is observed.

Overall, the method is acceptably validated in spinach (leaves), spring barley (whole plant, grain, straw), carrot (root), carrot (tops), tomatoes and beans (dry) with an LOQ of 0.01 mg/kg. For bean (fresh seed, remaining plant), kale, maize (whole cob, remaining plant), soybean (forage, seed), strawberries (fruit) and radishes (roots and tops), the method can be considered fit for regulatory purposes.

Procedural recovery data was provided. Only one determination was made at each fortification level, per crop matrix, for spinach and barley; two determinations per level were made for carrot root and tops. All individual, or mean, recoveries were within the range 70-110 %.

Results and discussion

For both trials, all control residues were <0.01 mg/kg for all crop matrices. The results in treated samples are summarised in Table 7-160.

Table 7-160 Results from rotational crop field trials following application at ~500 g a.s/ha to bare soil and three plant back intervals

Crop Part	Plant-back Interval (nominal in days)	pydiflumetofen Residue Found (mg/kg)	
		United Kingdom (S13-01022-01)	Germany (S13-01022-02)
Spinach (BBCH 43) ^a	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Spinach (BBCH 49 – NCH)	30	NS	<0.01
	60	NS	<0.01
	365	NS	<0.01
Carrot roots (BBCH 49 – NCH)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Carrot tops with foliage (BBCH 49 – NCH)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Barley immature whole plant (BBCH 39-42)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Barley grain (BBCH 89 – NCH)	30	<0.01	<0.01
	60	<0.01	<0.01
	365	<0.01	<0.01
Barley Straw (BBCH 89 – NCH)	30	0.02	<0.01
	60	0.03	<0.01
	365	0.01	<0.01

NS: not sampled

a: For trial S13-01022-01, this is BBCH 14-18, crop is sufficiently representative of immature spinach leaves.

Residues of pydiflumetofen were detected at or above the LOQ (0.01 mg/kg) only in barley straw: for trial ‘S13-01022-01’ these were 0.02, 0.03 and 0.01 mg/kg, for the 30d, 60d and 365 day PBIs, respectively. Residues were below LOQ (0.01 mg/kg) in all other crop matrices at all PBIs. No residue above LOQ was found in trial ‘S13-01022-02’ for any crop.

B.7.6.2.1. Conclusion on rotational residues studies

To investigate residues in rotational crops, a nature of the residue study has been conducted in different crops representing three different crop categories, namely leafy vegetables, root and tuber vegetables and cereals. Additionally, four magnitude of residue studies have been conducted in the crop categories: root and tuber; cereals; leafy vegetables and brassicas; oilseeds/pulses and fruits & fruiting vegetables

In the nature of residues study, pydiflumetofen, either phenyl labelled, or pyrazole labelled, was applied at a nominal rate of 400 g a.s./ha to bare soil in a confined rotational crop setting. Whilst this application rate is double the seasonal application rate according to the critical GAP, it is clearly underdosed when taking account of accumulation of pydiflumetofen in soil (see section 2.7.7 of Volume 1). Rotational crops of lettuce, turnip and wheat were grown in containers; the crops were planted at different replant intervals ranging from 30 to 270 days after treatment (DAT). The work has enabled a profiling of an overall metabolic pathway suitable for rotational crops.

Whilst some minor differences were observed compared to primary crop metabolism; the main patterns of metabolism are the same for the rotational crop metabolism. The principal biotransformations observed were: demethylation of the pyrazole ring to produce SYN547891; and reduction of the parent molecule producing SYN545547. The principal residue was parent pydiflumetofen in all crops. SYN545547 and SYN547891 were detected in each of the samples at lower levels than the parent; found at up to 0.005 mg/kg and 0.012 mg/kg, respectively (maximum TRR was 13.3 % TRR for SYN547891 in wheat forage, 30 DALA, pyrazole label – this corresponded to a low absolute amount – 0.004 mg/kg). Unidentified components accounted for a larger proportion of the overall TRR than in primary crops, which could be indicative of a more metabolites formed in the rotational crop context (uptake from soil and onward metabolism in crops) compared to the primary crop situation. However, no individual unidentified fraction accounted for greater than 0.014 mg/kg (this was <10 % TRR, at 6.7 % TRR). The maximum %TRR for an unidentified level was 10.2%TRR (0.001 mg/kg), however this was in turnip leaves where the total radioactive residue in this crop part was low. The amounts of total radioactive residues in the rotational crops were overall generally low (<0.01 mg/kg wheat grain, up to only 0.02 mg/kg in lettuce/turnip, up to 0.03, 0.11 and 0.22 mg/kg in wheat forage, hay and straw respectively).

In the magnitude of residues studies, pydiflumetofen was applied at a nominal rate of either 500 or 600 g a.s./ha to bare soil. For the trials dosed at 500 g a.s./ha; the rotational crops were cultivated after intervals of approximately 30, 60 and 365 days. The trials dosed at 600 g a.s./ha; the rotational crops were cultivated after intervals of approximately 30, 120, 270 and 330 days. Samples were taken at both mature and immature growth stages for spinach and barley (whole plant). Only parent pydiflumetofen was analysed for. Parent pydiflumetofen was found in these studies to a varying extent, with a large number of positive findings of pydiflumetofen, up to a level of 0.09 mg/kg in barley straw. The highest finding in a non-cereal crop was 0.05 mg/kg in spinach. In most of the rotational crop field samples taken, residues of pydiflumetofen were <0.01 mg/kg. However, there were a large number of findings of pydiflumetofen in the various crops. Based on the levels of pydiflumetofen found in the various crop matrices, it would not be expected that other constituents of the residue, metabolites, would have been found at or above a level of 0.01 mg/kg, in these studies if they had included determination of any of the metabolites.

The application rate in the magnitude of residues studies, at 500 or 600 g as/ha, are higher than the maximum seasonal application rate of 1 x 200 g as/ha (critical GAP assessed in this evaluation). However, these rotational crop field trials are clearly underdosed when soil accumulation due to persistence is accounted for. Please refer to Volume 1 section 2.7.7 which discusses the significance of residues of both parent pydiflumetofen and metabolites in rotational crops also taking account of the potential soil plateau rate of pydiflumetofen considering the potential for multi-year use.

Based on results obtained in the nature of the residue study conducted with two labels (phenyl-label, pyrazole label) and the follow up studies on magnitude of the residues, the studies have been used to guide the residue assessment for residues of pydiflumetofen in rotational crops arising from the proposed GAP uses. Please refer to Volume 1 for a full discussion. Above LOQ residues were found in carrot (root and top), radish (root and top), spinach (immature and mature), barley (straw and immature whole plant), soybean (forage) and maize (remaining plant). Please see Volume 1 section 2.7.7 for further discussion.

B.7.7. OTHER STUDIES

B.7.7.1. Effect on the residue level in pollen and bee products

The following study was completed (18/01/2017) prior to the introduction of agreed EU/GB guidance documents or test methods to address these data requirements (Jan 2020). Since the completion of the study, technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9) were implemented on the 1st of January 2020.

Although the guidance was not in place at the time the study was conducted, the guidance was in force at the time the application was made to GB. The study was conducted according to a draft version of the guidance document, the evaluation below has considered whether the study is sufficiently in line with the applicable guidance.

The proposed uses of the representative product are on cereal and oilseed rape crops. According to the guidance document, cereals (wheat, barley, oats, rye, durum wheat, spelt and triticale) are considered non-melliferous. Therefore, residue uptake in honey is unlikely to occur directly following treatment of the target cereal crops. However, oilseed rape is considered to be melliferous, and the proposed GAP includes the BBCH growth stages where flowering is likely to occur; therefore, consideration is required. Additionally, considering the persistent, and systemic, nature of the active, residues may be present in succeeding, melliferous crops.

The MRL assessment additional uses considered here (in this assessment report, alongside the cereals and oilseed rape representative uses) are carrots, parsley root and parsnips. Carrot and parsnips are not considered to be melliferous crops, although parsley root is considered to be melliferous. However the assessment conducted here for oilseed rape is expected to be ‘worst case’ in terms of assessment of potential residues in honey.

The residue definition for risk assessment must be considered for residues in honey. In the absence of a specific metabolism studies, consideration must be given to existing studies. The most critical information to consider is the nature of the residue in primary crops, rotational crops and the stability of the active under representative pasteurisation conditions. Parent pydiflumetofen was proposed as residue definition for risk assessment in crops (see Vol 1 section 2.7.3); it was also found to be stable under all representative processing conditions. The same applies for the enforcement residue definition. This is the same enforcement definition as for plants. It is noted that an analytical method for the determination of pydiflumetofen in honey is available (see Volume 3, CA B5).

Therefore, parent pydiflumetofen can be considered an adequate residue definition for risk assessment and enforcement in honey (see Vol 1 section 2.7.3).

There is no data available on residues specifically in the aerial parts of oilseed rape crops (e.g. flowers) following treatment with pydiflumetofen. The available data cover residues in oilseed rape seed, indicating residues in the range of <0.01 to 0.04 mg/kg in the seed; whole plant (oil seed rape) data showed high residues in whole plants (up to 3.53 mg/kg) on the day of application, which declined (to up to 2.1 mg/kg 7 or 14 days after application; up to 1.1 mg/kg 21 days after application and up to 0.48 mg/kg 42 days after application). The applicant has supported their submission with bee tunnel trials. Four trials are required to set MRLs, as outlined in SANTE/11956/2016 rev. 9.

B.7.7.1.1. Study 1

Report:	K-CA 6.10.1: [REDACTED] (2017)
Title:	SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016.
Report No:	Report Number S16-02006, (Syngenta Report No. TK0283818).
Document No:	VV-466889 (Syngenta File No. A8240D 12181)
Guidelines:	EC (1997) Guidance document 7029/VI/95 (rev. 5), general recommendations for the design, preparation and realization of residue trials. Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009. The Application of the OECD Principles of GLP to the Organisation and management of Multi- Site Studies, ENV/ JM/ MONO (2002) 9.

	The national GLP requirements are based on the OECD Principles of Good Laboratory Practice, which are accepted by regulatory authorities throughout the European Community on the basis of intergovernmental agreements. Federal Office for Consumer Protection and Food Safety, Guidance Document Part C4 – Honey version 0-2a.doc (2003)
Guideline deviations:	No
GLP:	Yes

Materials and methods

The purpose of the study was to determine the magnitude of residues of pydiflumetofen in honey following the exposure of honeybees to treated oilseed rape. The study comprised of three supervised (tunnel) residue trials conducted in Germany (Northern Europe) in 2016; the trials locations were at least 20 km apart, hence are sufficiently independent. Each trial location consisted of three replicate test plots and one control group. At each plot, a light plastic gauze tunnel was established which fully covered the oilseed rape. The tunnels covered an area of 200 m² each; this exceeds the minimum required area as set out in SANTE/11956/2016 rev. 9; hence it is acceptable.

The crop was treated with one spray application with A21857B, an emulsifiable concentrate (EC) containing 62.5 g/L pydiflumetofen. A21857B was applied as a tank mix with A8240D, a water dispersible granule formulation (WG) containing 500 g/L fludioxonil – this active has not been considered further. The application was made during full flowering (BBCH 63), at a nominal application rate of 200 g a.s./ha (actual application rate ranged from 203.0-205.6 g a.s./ha).

The honeybee hives were placed in the tunnels in the evening after the application; this is not in line with the newly published guidance, which states the application should be made the day after the hives are introduced. However, this deviation is not expected to cause a significant impact. One hive was set up per tunnel/plot, a fresh water source was provided. The condition of the colonies was recorded on the day of introduction and at the end of the trial. The honeybee queen was caged on one comb to obtain more honey. The comb inside the fixing comb was changed regularly. The honeybee colonies were fed with marked food combs to exclude the possibility of sampling the given food as honey. The health status of all colonies was good throughout the trial, all colonies were free of visible symptoms of diseases.

Sampling details

Samples of honey were collected when the water content of the honey reached ~20 % (this was confirmed by refractometer) – this condition was not reached in trial 02. The honey in trial 02 was marginally over 20 %, for each of the triplicate plots (A, B & C) the water content was 24.5 %, >25 % and 25 %, respectively. Replicate plots A and C are acceptable, the honey collected in plot B cannot be relied upon due to the uncertainty over the exact water content. The residue for trial 02 will be calculated based on the results from plots A and C only.

Weather data was reported for the trials and no exceptional events were reported. Changing weather was given as the reason for high water content in trial 02. This seems to be questionable, as the conditions in trials 01 and 03 are almost identical, and acceptable water content was reached for these trials. Nevertheless, the impact of this has been sufficiently addressed, see above.

Acceptable sample weights were collected for all control plots and most treated plots, with the exception of plot A in trials 01 and 03 – plot B in trial 03 was also less than the required amount (80 g instead of the required 100 g) – however, this is not expected to have a significant impact on the validity of the result. The residue for trials 01 and 03 will be calculated based on the mean of plots B and C only. Only one treated plot is required, therefore, as at least one plot is available in each trial, this is sufficient. In accordance with the guidance, 4 replicates of each sample should have been analysed. It is unclear how many replicate analyses were performed from the study report; in some cases, three replicates were analysed. As the results from three trial sites, each with three tunnels were consistently <LOQ, the possible lack of replicate analysis is not considered to have significantly impacted the validity of the study.

Samples were frozen within 5 hours of collection and were stored at $\leq -18^{\circ}\text{C}$ for a maximum of 41 days prior to analysis. Sample extracts were stored for a maximum of 4 days (at $1-10^{\circ}\text{C}$). Stability of the extracts was proven for 11 days by comparing the recoveries taken before and after storage. No stability data was provided for the frozen samples; however, stability data is not required for periods up to 30 days. Pydiflumetofen has been shown to be stable under frozen conditions for up to 23 months in all crop categories – this is sufficient to assume stability in bee matrices stored frozen for 41 days.

Results and analysis

Analysis was performed using LC-MS/MS method GRM061.03A. Validation data was provided for honey within the study report, this is reported in Volume 3 CA B5, section B5.1.25. The method was fully validated in accordance with SANCO 3029/99 rev 4. Acceptable procedural recovery data was also presented. Fludioxonil was also analysed; however, the retention times do not interfere with pydiflumetofen, hence no impact is expected.

The residues of pydiflumetofen in all treated honey samples were $<\text{LOQ}$ (0.01 mg/kg). No residues of pydiflumetofen at or above LOQ were found in the untreated honey samples.

n.b. Although the guidance document ‘SANTE/11956/2016 rev. 9’ was not in force at the time the study was conducted; the study complied with the guidance document to a satisfactory extent.

Table 7-161 Summary of data from residues in honey

Reference: SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016. XXXXXXXXXX (2017). **Report No S16-02006**. Syngenta Task No. TK0283818. Syngenta File No. VV-466889.

GLP: Yes Crop/crop group: Oilseed rape – flowering, for honey production
 Indoor/Outdoor: Outdoor, protected – light gauze tunnel Analytical method: GRM061.03A
 Formulation A21857B (tank mixed with A8240D) Limit of Quantification (mg/kg): 0.01
 Content of active substance: 62.5 g/L pydiflumetofen (and 500 g/L fludioxonil) Residues calculated as: SYN545974 (pydiflumetofen)

Trial No./ Location/ Year	Commodity/ Variety	Date of 1. Installation of hive/colony 2. First day of exposure 3. Sampling	Application rate per treatment			Growth stage at last treatment	Portion analysed	Residues (mg/kg) ^a		Days after exposure (days)	Remarks
			Plot	g a.s./ha	Water (L/ha)			Sample			
Trial – 01 75177, Pforzheim, Baden-Württemberg, Germany, 2015-16	Oilseed rape / ‘PR46W26’	1)19/04/2016 2)20/04/2016 3)10/05/2016	A	203.0	306	BBCH 63	Honey	Plot A ^b	-	20	Sample storage conditions: Max 41 days at ≤- 18 °C, max extract storage period – 11 days.
			B	204.6	307			Plot B	<0.01		
			C	205.6	308			Plot C	<0.01		
								Mean	<u><0.01</u>		
Trial – 02 72411, Bodelshausen, Baden-Württemberg, Germany, 2015-16	Oilseed rape / ‘Avatar’	1)19/04/2016 2)20/04/2016 3)10/05/2016	A	203.4	305	BBCH 63	Honey	Plot A	<0.01	22	
			B	204.8	307			Plot B ^a	-		
			C	203.0	305			Plot C	<0.01		
								Mean	<u><0.01</u>		
Trial – 03 76297, Stutensee, Baden-Württemberg, Germany, 2015-16	Oilseed rape / ‘W26’	1)19/04/2016 2)20/04/2016 3)10/05/2016	A	205.0	308	BBCH 63	Honey	Plot A ^b	-	27	
			B	204.2	306			Plot B	<0.01		
			C	204.2	306			Plot C	<0.01		
								Mean	<u><0.01</u>		

a: Insufficient detail on water content in honey, this value has not been included in the calculation of the mean.

b: Insufficient sample weight, these values have not been included in the calculation of the mean.

B.7.7.2. Literature Review

The below evaluation (under heading of ‘More detailed evaluation (of original literature review)’ is based on the submission of the original literature review, submitted to HSE with the main data submission in July 2020, which covered a search span up to November 2015. During the latter stages of evaluation, the applicant supplied an updated literature review to complete the literature review to cover the years up to the time of submission and more recent studies. An add-on to the below evaluation, based on the updated literature review, is therefore added after the ‘More detailed evaluation (of original literature review)’.

The applicant submitted a literature review in support of pydiflumetofen and its metabolites in the area of residues and consumer risk. HSE considers that acceptable search criteria have been applied to this literature review when considering the residues and dietary exposure areas.

From the original literature review it was concluded that, no issues were identified that would impact on the regulatory residues/consumer risk assessment. No studies were considered relevant in the field of residues (and dietary exposure) and therefore no reliability criteria needed to be applied.

No studies were ‘returned’ by the original search, likely due to pydiflumetofen being a new active substance. The search was done in November 2015, a few years before the submission to HSE (July 2020), so it possible that more recent studies could have been missed.

The updated literature review (dated 2022), yielded six papers of potential relevance in the areas of residues and metabolism being identified. HSE considered that two of these were of potential interest for HSE to consider the full publications, and a detailed summary of these papers (on enantiomeric composition) is summarised at the end of section B.7.2.1, together with a summary of another paper on enantiomeric composition that was included with the toxicology literature papers.

The write up of the updated literature review (submitted 2022) follows on below after the write up of the original one (submitted 2020).

More detailed evaluation (of original literature review)

The applicant provided a review for pydiflumetofen (and its metabolites) in accordance with the EFSA 2011 Guidance (EFSA Journal 2011; 9(2):2092).

The applicant did not produce any relevant published papers for additional evaluation by HSE for incorporation into this residues chapter. Therefore no papers from this literature review cited in the below list of ‘references relied upon’ relating to residues.

Literature review report for Pydiflumetofen: 22 Jan, 2021. Author “Syngenta - Jealott’s Hill International Research Centre. The report was made available as document M-CA, Section 9 “Metabolism and residues Data – Literature Data”. The report was based on earlier versions (dated 2016 and 2018) that had been updated (e.g. to clarify the metabolites included in the (2015) literature search).

Summary of methodology employed:

1. A very broad search was conducted in 18 scientific source databases for Pydiflumetofen (SYN545974) and its metabolites.
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).

Although the planned intention was to continue with the points 4 to 6 below (*in italics*), this wasn't applicable in this case, since paper titles were used for the rapid assessment.

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*
6. *Any relevant papers were highlighted and assessed for reliability.*

Timespan- scope of search.

Whilst an initial search using pydiflumetofen was done in April 2015, this was updated in November 2015, when the assessment of metabolites was also included. The date span of the search was to cover a period 45 years.

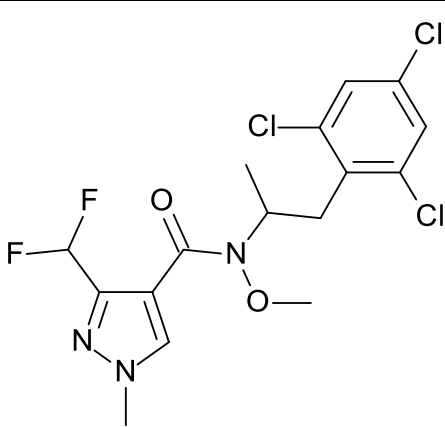
Regulation 1107/2009 states " Scientific peer-reviewed open literature, as determined by the Authority, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier shall be added by the applicant to the dossier."

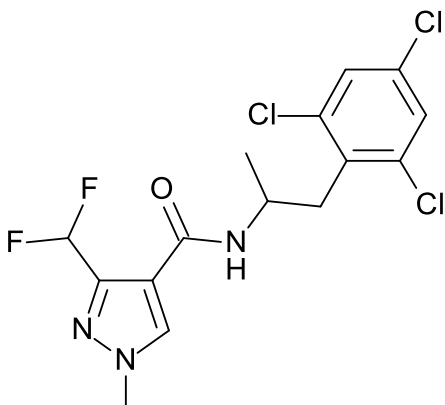
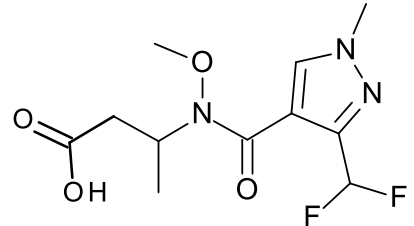
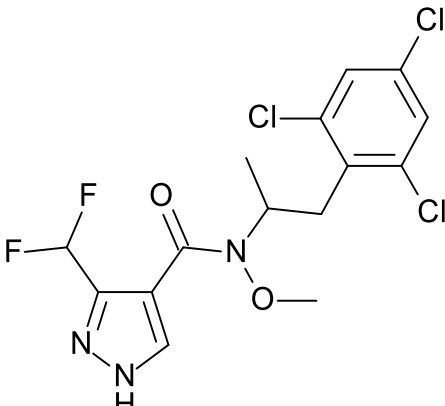
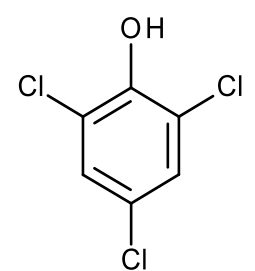
The date of submission of Pydiflumetofen was July 2020. Therefore the literature assessment has not checked final years (2016 to 2020) leading up to the submission of the package to HSE for assessment.

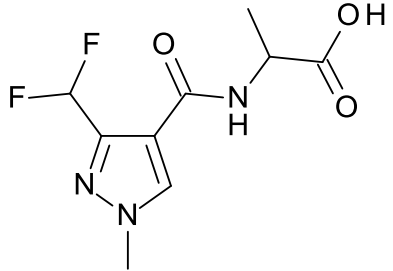
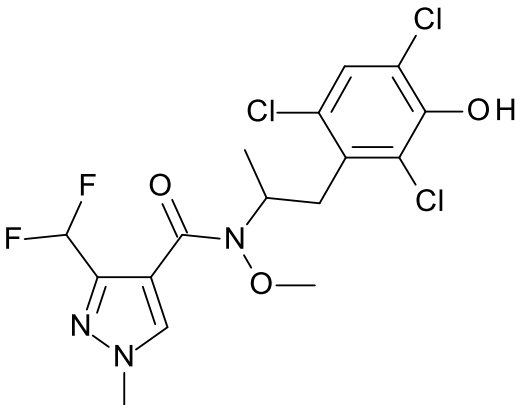
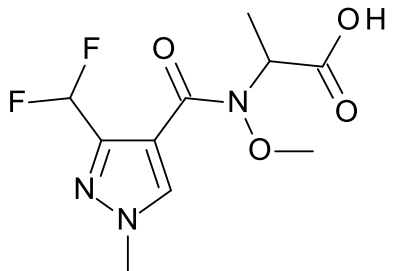
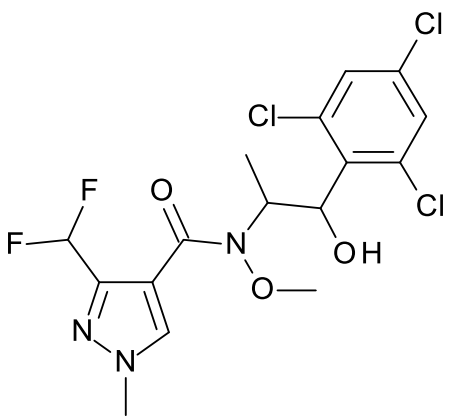
Search terms:

The search terms covered both the active substance Pydiflumetofen and specific metabolites, and including the Pydiflumetofen metabolites that are regarded as common SDHI metabolites. Pydiflumetofen is proposed to act as a succinate dehydrogenase inhibitor (SDHI) fungicide (see section B.3.6). Search terms were provided and encompassed code names for active substance and the proposed names for the pesticide products as well as codes for the metabolites.

The literature review covered the following compounds, Pydiflumetofen and metabolites. This includes the metabolites identified in the primary crop, rotational crop, ruminant and poultry metabolism studies.

Code Number (Synonyms)	Description	Structure
SYN545974 CSCD678790	Pydiflumetofen N-methoxy-N-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide 1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-	

Code Number (Synonyms)	Description	Structure
SYN545547 CSCD550897	<p><i>Context: found in plant metabolism studies(primary and rotational crops) and in livestock metabolism</i></p> <p>3-(difluoromethyl)-1-methyl-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]pyrazole-4-carboxamide</p>	
SYN548261	3-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]butanoic acid	
SYN547891 CSCV764139	<p><i>Context: found in plant metabolism studies(primary and rotational crops)and in and in livestock metabolism</i></p> <p>3-(difluoromethyl)-N-methoxy-N-[1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-1H-pyrazole-4-carboxamide</p>	
2,4,6-Trichlorophenol 2,4,6-TCP	<p>2,4,6-trichlorophenol</p> <p><i>Context: found in livestock metabolism</i></p>	

Code Number (Synonyms)	Description	Structure
SYN548264 CSCD548196 N-desmethoxy SYN548263	<i>Context: found in livestock metabolism</i> 2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]amino]propanoic acid	
SYN547897 CSCV764146	<i>Context: found in livestock metabolism</i> 3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-methyl-2-(2,4,6-trichloro-3-hydroxy-phenyl)ethyl]pyrazole-4-carboxamide	
SYN548263 CSCZ159698	<i>Context: found in livestock metabolism</i> 2-[[3-(difluoromethyl)-1-methyl-pyrazole-4-carbonyl]-methoxy-amino]propanoic acid	
SYN547948 CSCY608054	<i>Context: found in livestock metabolism</i> 3-(difluoromethyl)-N-[2-hydroxy-1-methyl-2-(2,4,6-trichlorophenyl)ethyl]-N-methoxy-1-methyl-pyrazole-4-carboxamide	

Code Number (Synonyms)	Description	Structure
Hydroxylated SYN545974	<p><i>Context: found in livestock metabolism</i></p> <p>Hydroxylated N-methoxy-N-[1-methyl-2-(2,4,6-trichlorophenyl)-ethyl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide</p>	
NOA449410 CSAA798670 R648993	<p><i>Context: found in livestock metabolism</i></p> <p>3-(difluoromethyl)-1-methylpyrazole-4-carboxylic acid</p>	
SYN508272 CSCC210616 R423363	<p><i>Context: found in livestock metabolism</i></p> <p>3-(difluoromethyl)-1-methylpyrazole-4-carboxamide</p>	

For completeness the search terms are listed below:

Search Strategy	
Initial SYN545974 search :	
L1	QUE (1228284-64-7 OR 1639015-49-8 OR 1639015-48-7)
L2	QUE (1485419-47-3 OR 1485419-44-0 OR (FUSHA(10A)FUNGICID?))
L3	QUE (SYN545974 OR (SYN(W)545974))
L4	QUE L1-3 FUSHA PLUS STEREOISOMERS
L5	QUE (1658468-84-8 OR 1561039-73-3 OR 1336797-48-8)
L6	QUE (1335518-65-4 OR 1245827-93-3 OR 1228286-43-8)
L7	QUE (1228284-63-6 OR 1204298-65-6 OR 1192017-82-5)
L8	QUE (1105713-22-1 OR 1004285-82-8 OR 960053-63-8)
L9	QUE (925689-10-7 OR 176969-34-9 OR 151734-02-0)
L10	QUE (SYN545547 OR SYN547894 OR SYN547892 OR SYN547893)
L11	QUE (NOA449410 OR SYN547895 OR SYN547890 OR SYN545720)
L12	QUE (SYN508272 OR SYN545357 OR SYN547896 OR SYN547897)
L13	QUE (SYN547891 OR SYN548263 OR SYN548264 OR SYN548265)
L14	QUE (SYN548279 OR (SYN(W)548279) OR (NOA(W)449410))
L15	QUE (SYN(W)(545547 OR 547894 OR 547892 OR 547893))
L16	QUE (SYN(W)(547895 OR 547890 OR 545720))
L17	QUE (SYN(W)(508272 OR 545357 OR 547896 OR 547897))
L18	QUE (SYN(W)(547891 OR 548263 OR 548264 OR 548265))

Search Strategy	
Top-up SYN545974 search :	
L1	QUE SPE=ON ABB=ON PLU=ON (1639015-49-8 OR 1639015-48-7 OR 1485419-47-3 OR 1485419-44-0 OR 1228284-64-7)
L2	QUE SPE=ON ABB=ON PLU=ON (DIFLUOROMETHYL(1W)METHOXY(1W)METHYL(2W)METHYL(4W) TRICHLOROPHENYL (W) ETHYL(1W)PYRAZOL?(1W)CARBOXAMID? OR DIFLUOROMETHYL(1W) METHYL(1W)PYRAZOL?(1W)CARBOXYLIC(W)ACID(W)METHOXY(1W)METHYL(4W)TRICHLORO(W) PHENYL(W)ETHYL(W)AMID?)
L3	QUE SPE=ON ABB=ON PLU=ON (ADEPIDYN OR FUSHA OR PYDIFLUMETOFEN# OR SYN545974 OR SYN(W)545974)
L4	QUE SPE=ON ABB=ON PLU=ON (L1 OR L2 OR L3)
L5	QUE SPE=ON ABB=ON PLU=ON (1658468-84-8 OR 1561039-73-3 OR 1336797-48-8 OR 1335518-65-4 OR 1245827-93-3 OR 1228286-43-8 OR 1228284-63-6 OR 1204298-65-6 OR 1192017-82-5 OR 1105713-22-1 OR 1004285-82-8 OR 960053-63-8 OR 925689-10-7 OR 176969-34-9 OR 151734-02-0)
L6	QUE SPE=ON ABB=ON PLU=ON (SYN545547 OR SYN547894 OR SYN547892 OR SYN547893 OR NOA449410 OR SYN547895 OR SYN547890 OR SYN545720 OR SYN508272 OR SYN545357 OR SYN547896 OR SYN547897 OR SYN547891 OR SYN548263 OR SYN548264 OR SYN548265)
L7	QUE SPE=ON ABB=ON PLU=ON (SYN548279 OR (SYN(W)548279) OR (NOA(W)449410) OR SYN(W) (545547 OR 547894 OR 547892 OR 547893) OR SYN(W)(547895 OR 547890 OR 545720) OR SYN(W)(508272 OR 545357 OR 547896 OR 547897) OR SYN(W)(547891 OR 548263 OR 548264 OR 548265))
L8	QUE SPE=ON ABB=ON PLU=ON (DICHLOROPHENYL(1W)METHYLETHYL(1W)DIFLUOROMETHYL(1W)METHOXY(1W)METHYL(1W)PYRAZOLE(1W)CARBOXAMIDE OR DIFLUOROMETHYL(1W) HYDROXY(1W)METHYL(2W)METHYL(4W)TRICHLOROPHENYL(W)ETHYL(1W)PYRAZOLE (1W) CARBOXAMIDE OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL#(1W)CARBOXAMID#)
L9	QUE SPE=ON ABB=ON PLU=ON (DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL#(1W)CARBOXYLIC (W)ACID OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL# (1W)CARBOXYLIC(W)ACID(2W) HYDROXY(1W)METHYLETHYL(W)AMIDE OR DIFLUOROMETHYL(1W) METHYL(1W)PYRAZOL # (1W) L(W)CARBONYL(1W)ALANINE)
L10	QUE SPE=ON ABB=ON PLU=ON (DIFLUOROMETHYL(1W)METHYL(2W)METHYL(4W) TRICHLOROPHENYL (W) ETHYL (1W) PYRAZOL#(1W)CARBOXAMIDE ORDIFLUOROMETHYL(1W) METHYLPYRAZOL#(1W)CARBOXAMIDE OR DIFLUOROMETHYL(1W)METHYLPYRAZOL # (1W) CARBOXYLIC(W)ACID)
L11	QUE SPE=ON ABB=ON PLU=ON (DIFLUOROMETHYL(1W)PYRAZOL#(1W)CARBOXYLIC(W)ACID OR DIFLUOROMETHYL(2W)HYDROXY(1W)METHYLETHYL(1W)METHOXY(1W)METHYL(1W) PYRAZOL# (1W)CARBOXAMIDE OR DIFLUOROMETHYL(3W)HYDROXYL(1W)METHYLETHYL(1W)METHYL (1W) PYRAZOL#(1W)CARBOXAMID#)
L12	QUE SPE=ON ABB=ON PLU=ON (METHYL(1W)DIFLUOROMETHYL(W)PYRAZOL#(1W) CARBOXYLIC (W)ACID OR TRICHLORO(1W)METHYL(1W)BENZENEETHANAMINE OR TRICHLORO(1W)METHYL(W) BENZENE (W) ETHANAMIN#)

SYN545974 specific metabolites search :

L1 QUE SPE=ON ABB=ON PLU=ON (960053-63-8 OR
DIFLUOROMETHYL(1W)METHYL(2W)METHYL(4W)
TRICHLOROPHENYL (W) ETHYL(2W)PYRAZOL?(1W)CARBOXAMID# OR SYN545547 OR
SYN(W)545547)

L2 QUE SPE=ON ABB=ON PLU=ON (3784-03-0 OR 2591-21-1 OR 95-95-4 OR 88-06-2 OR 89465-86-1
OR
77001-45-7)

L3 QUE SPE=ON ABB=ON PLU=ON (TRICHLOROPHENOL OR TRICHLOROPHENATE OR
TRICHLOROPHENOLATE OR TRICHLOROPHENOXIDE OR TRICHLORO(W)PHENOL OR
TRICHLOROPHENOXY OR TRICHLOROPHENIC(W)ACID OR
TRICHLORO(1W)HYDROXYBENZENE)

L4 QUE SPE=ON ABB=ON PLU=ON (DOWICIDE OR NSC(W)2266 OR NSC2266 OR PREVENTOL OR
TCP OR
2(W)4(W)6(W)TCP OR BTS(W)45186 OR BTS45186 OR NSC(W)2165 OR NSC2165 OR OMAL OR
PHENACHLOR)

L5 QUE SPE=ON ABB=ON PLU=ON (FUNGI!ID? OR MOLDICID? OR PESTI!ID? OR MICROBIO!ID?
OR
MICROBI!ID? OR BIO!ID? OR BI!ID? OR ANTIFUNG? OR ANTI(W)FUNG?)

L6 QUE SPE=ON ABB=ON PLU=ON L4(10A)L5

L7 QUE SPE=ON ABB=ON PLU=ON (1192017-82-5 OR SYN548264 OR SYN(W)548264)

L8 QUE SPE=ON ABB=ON PLU=ON
(DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL#(1W)CARBONYL
(W) AMINO (W) PROPANOIC(W)ACID OR
DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL#(1W)
CARBONYL (W) AMINO(W)PROPANOAT# OR
DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL#(1W)
YL(W)CARBONYL(1W)ALANIN#)

L9 QUE SPE=ON ABB=ON PLU=ON (SYN547891 OR SYN(W)547891 OR DIFLUOROMETHYL(1W)
METHOXY (2W)
METHYL(4W)TRICHLOROPHENYL(W)ETHYL(1W)PYRAZOL?(1W)CARBOXAMID?)

L10 QUE SPE=ON ABB=ON PLU=ON (SYN547897 OR SYN(W)547897 OR DIFLUOROMETHYL(1W)
METHYL
(1W)PYRAZOL?(1W)CARBOXYLIC(W)ACID(W)METHOXY(1W)METHYL(4W)TRICHLORO(1W)
HYDROXYL (W)PHENYL(W)ETHYL(W)AMID?)

L11 QUE SPE=ON ABB=ON PLU=ON (SYN548263 OR SYN(W)548263 OR
DIFLUOROMETHYL(1W)METHYL
(W) PYRAZOL?(1W)CARBONYL(W)METHOXY(W)AMINO(W)PROPANOIC(W)ACID OR
DIFLUOROMETHYL (1W) METHYL(W)PYRAZOL?(1W)CARBONYL(W)METHOXY(W)
AMINO(W)
PROPANOAT? OR DIFLUOROMETHYL(1W)METHYL(W)PYRAZOL?(1W)CARBONYL(1W)
METHOXY
(W)ALANIN#)

L12 QUE SPE=ON ABB=ON PLU=ON (SYN548261 OR SYN(W)548261 OR
DIFLUOROMETHYL(1W)METHYL
(W)PYRAZOL?(1W) CARBONYL(W)METHOXY(W)AMINO(W)(BUTANOIC OR BUTYRIC OR
PROPANECARBOXYLIC)(W)ACID OR
DIFLUOROMETHYL(1W)METHYL(W)PYRAZOL?(1W)CARBONYL
(W)METHOXY(W)AMINO(W)(BUTANOAT? OR BUTYRATE? OR PROPANECARBOXYLAT?))

L13 QUE SPE=ON ABB=ON PLU=ON (SYN547948 OR SYN(W)547948 OR DIFLUOROMETHYL(4W)
HYDROXY (1W)
METHYL(4W)TRICHLOROPHENYL(W)ETHYL(1W)METHOXY(1W)METHYL(W)
PYRAZOL? (1W)CARBOXAMID?)

L14 QUE SPE=ON ABB=ON PLU=ON (1639015-49-8 OR 1639015-48-7 OR 1485419-47-3 OR 1485419-
44-0 OR
1228284-64-7)

L15 QUE SPE=ON ABB=ON PLU=ON
(DIFLUOROMETHYL(1W)METHOXY(1W)METHYL(2W)METHYL(4W)
TRICHLOROPHENYL (W)ETHYL(1W)PYRAZOL?(1W)CARBOXAMID? OR
DIFLUOROMETHYL(1W)
METHYL (1W) PYRAZOL? (1W)CARBOXYLIC(W)ACID (W)METHOXY(1W)METHYL(4W)
TRICHLORO
(W) PHENYL(W)ETHYL(W)AMID?)

Search Strategy	
L16 OR	QUE SPE=ON ABB=ON PLU=ON (ADEPIDYN OR FUSHA OR PYDIFLUMETOFEN# OR SYN545974 OR SYN(W)545974)
L17	QUE SPE=ON ABB=ON PLU=ON (HYDROXY OR OXY?)(3W)(L14 OR L15 OR L16)
L18 OR L12	QUE SPE=ON ABB=ON PLU=ON ((L1 OR L2 OR L3) OR (L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L13) OR L17)
SYN545974 metabolites that are common SDHI metabolites search :	
L1 OR	QUE SPE=ON ABB=ON PLU=ON (176969-34-9 OR 1334398-13-8 OR NOA (W)449410 OR NOA449410 OR R(W)648993 OR R648993 OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL?(1W)CARBOXYLIC(W) ACID OR (PYRAZOL?(1W)CARBOXYLIC(W)ACID)(1A)(DIFLUOROMETHYL(1W)METHYL))
L2	QUE SPE=ON ABB=ON PLU=ON (METHYL(1W)DIFLUOROMETHYL(1W)PYRAZOL?(1W) CARBOXYLIC (W)ACID OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL? (1W)CARBOXYLAT? OR DIFLUOROMETHYL (1W)METHYLPYRAZOL?(1W)CARBOXYLIC(W)ACID)
L3 OR	QUE SPE=ON ABB=ON PLU=ON (925689-10-7 OR SYN(W)508272 OR SYN508272 OR R(W)423363 OR R423363 OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL?(1W)CARBOXYLIC(W)ACID(W)AMID? OR DIFLUOROMETHYL(1W)METHYL(1W)PYRAZOL?(1W)CARBOXAMID##)
L4 (1W)	QUE SPE=ON ABB=ON PLU=ON ((PYRAZOL?(1W)CARBOXAMIDE)(1A)(DIFLUOROMETHYL METHYL) OR DIFLUOROMETHYL(1W)METHYLPYRAZOL?(1W)CARBOXAMIDE##)
L5	QUE SPE=ON ABB=ON PLU=ON (L1 OR L2 OR L3 OR L4)
Plus	
L1	QUE (METABOL? OR RESIDUE# OR TRANSFORM? OR BIOTRANSFORM?)
L2	QUE (DEGRAD? OR BIODEGRAD? OR FATE# OR MRL OR MRLS)
L3	QUE (CONJUGAT? OR EXCRET? OR ELIMINAT?)
L4	QUE (FOOD# OR FEED# OR DIET# OR DIETARY OR CONSUMER? OR HUMAN#)
L5	QUE (CONTAMINAT? OR SAFE? OR EXPOS? OR ANALY? OR ASSES?)
L6	QUE (INTAKE? OR (IN(W)TAKE?) OR SURVEY? OR RISK?)
L7	QUE (TOXIC? OR STUDY? OR STUDIES?)
L8	QUE (L4 (10A) (L5 OR L6 OR L7))
L9	QUE (LIVESTOCK# OR COW# OR GOAT# OR CATTLE# OR BULLOCK#)
L10	QUE (BOVINE? OR BOVIDAE? OR BOS OR BULL# OR HEIFER? OR CAPRA#)
L11	QUE (SHEEP# OR EWE OR EWES OR RAM# OR SWINE# OR PIGLET#)
L12	QUE (PIG# OR SUIDAE? OR SUS OR OVIS OR OX OR OXEN)
L13	QUE (RUMINANT? OR HEN# OR CHICKEN# OR FOWL# OR TURKEY?)
L14	QUE (DUCK# OR GOOSE OR GEESE OR CAPON# OR POULTRY?)
L15	QUE (MEAT OR MILK OR EGG# OR TISSUE#)
L16	QUE (((BROKEN? OR BREAK?) (W) (DOWN OR UP)) OR BREAKDOWN?)
L17	QUE (BREAKSDOWN? OR UPTAKE? OR PROCESSING? OR BOUND?)
L18	QUE ((NON(W)EXTRACTAB?) OR (ROTATIONAL (3A)CROP#))
L19	QUE ((L1 OR L2 OR L3) OR L8 OR (L9 OR L10 OR L11 OR L12 OR L13 OR L14) OR (L15 OR L16 OR L17 OR L18))

Search- methodology applied and databases used:

Time period of search: November 2015 (scope over 45 years)

The search strategy was conducted using bibliographic databases conducted using STN as host provider. STN provides electronic access to a vast number of scientific and technical bibliographical databases. The applicant

included a justification of each of the databases used. Taken together, these covered a comprehensive source for which to conduct an overall search covering residues, metabolism and consumer risk assessment.

The following 18 (Host STN) databases were included:

MEDLINE, EMBASE, EMBASE, EMBASE, AGRICOLA, BIOSIS, CABA, HCAPLUS, FSTA, FROSTI, GEOREF, TOXCENTER, PQSCITECH, PASCAL, SCISEARCH, ANABST, HCHEMLIST, CROPU, CROPB.

A subset of the individual databases (Pascal, Medline, and Agricola) are those that EFSA provided as a list of reliable databases {sent to MSs by EFSA in March 2015}. With the overall list of databases used by this applicant, the search covers a good range (e.g. including a global range). The STN database searches are efficient means of retrieval of papers from a large number of database searches. The STN approach described and used by the applicant for pydiflumetofen and metabolites is considered to be a comprehensive approach. It is used by a number of registrants in the undertaking of literature reviews.

The applicant considered that the bibliographic databases would provide a comprehensive search to retrieve quality peer reviewed literature, and as search did not do further retrieval searches, such as web search (e.g. websites of conferences or organisations), search of journals' tables of contents, or search of reference lists of full-text journal articles (e.g. reviews).

Relevancy criteria:

The applicant set out a series of relevance criteria pertinent to the assessment of regulatory residues studies (as outlined below). These cover each of the respective data areas represented by the residues data requirements: storage stability of residues, metabolism (primary crops, livestock and rotational crops), magnitude of the residues studies, feeding studies, processing studies (hydrolysis and magnitude of residues studies), and rotational crop field studies.

The specific list of relevance criteria that were applied in the assessment of the literature:

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
Metabolism and residues data (CA 6.1 to 6.9)	
Summary	<p>The relevance criteria applied to determine whether a literature reference was relevant for the residues and metabolism sections of the active substance renewal process are given below.</p> <ol style="list-style-type: none"> 1. Well defined test material. e.g. are purity and batch data provided? 2. Applicable test species. e.g. is the crop a representative use; were relevant animal commodities used? 3. Study conditions should not differ significantly from guidelines and recommended protocols. e.g. did the study meet the relevant guidelines? 4. Trial site/test system not previously exposed to the test material or other contaminants e.g. was the compound used previously at the trial site; was the animal feed free from the compound? 5. Sufficient experimental information is provided to substantiate and evaluate whether the study conclusions and endpoints are robust. e.g. were storage intervals recorded; are weather conditions and plot histories available? 6. Validated Analytical methodology employed. e.g. were control samples used, acceptable recoveries obtained, clear example chromatograms given? 7. Study conditions do not interfere with the interpretation of the study results. e.g. starting processing material residue is robust and there is measurable residue in processed products?

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
CA 6.1 Storage Stability of Residues (plant and animal)	<u>Storage Stability Studies</u> <ol style="list-style-type: none"> 1. Well defined test material. e.g. are purity and batch data provided? 2. Applicable test species. e.g. is the crop a representative use; were relevant animal commodities used? 3. Study conditions should not differ significantly from guidelines and recommended protocols, and must be relevant to those in the submission. e.g. did the study meet the relevant guidelines and GAP? 4. Sufficient experimental information is provided to substantiate and evaluate whether the study conclusions and endpoints are robust. e.g. were storage intervals recorded; are weather conditions and plot histories available? 5. Validated analytical methodology employed e.g. were control samples used, acceptable recoveries obtained, clear example chromatograms given? 6. Study conditions do not interfere with the interpretation of the study results. e.g. starting processing material residue is robust and there is measurable residue in processed products?
CA 6.2 Metabolism, distribution and Expression of Residues (plant and animal) CA 6.6.2 Metabolism and Distribution in Succeeding Crops	<ol style="list-style-type: none"> 1. Well defined test material. e.g. if radiolabelled material was used, was an appropriate isotope used? 2. Applicable test species. e.g. ruminant, poultry, etc.? 3. Study conditions should not differ significantly from guidelines and recommended protocols, and must be relevant to those in the submission. e.g. is the application rate relevant and equivalent to maximum seasonal rate on rotated crops? 4. Trial site/test system not previously exposed to the test material or other contaminants e.g. is plot history supplied? 5. Sufficient experimental information is provided to substantiate and evaluate whether the study conclusions and endpoints are robust. e.g. Were metabolites identified by appropriate techniques? 6. Validated analytical methodology employed. e.g. were representative clear chromatograms provided to support metabolite identification? 7. Study conditions do not interfere with the interpretation of the study results. e.g. if the test item is photolabile was the study conducted outdoors?
CA 6.3 Magnitude of Residues Trials in Plants	<p>Published monitoring reports were not considered relevant due to the fact that it would not be possible to determine whether or not a misuse scenario had resulted in the residue levels reported.</p> <p><u>Crop Studies</u></p> <ol style="list-style-type: none"> 1. Well defined test material (including purity/content) 2. Applicable test species 3. Study conditions should not differ significantly from guidelines and recommended protocols. 4. Trial site/test system not previously exposed to the test material or other contaminants. 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. 7. Study conditions should not interfere with the interpretation of the study results. <p><u>Notes for above criteria</u></p> <ol style="list-style-type: none"> 1. Well defined test material (including purity/content) e.g. was the formulation comparable to the proposed representative formulation?

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
	<ol style="list-style-type: none"> 2. Applicable test species e.g. is it a representative use crop? 3. Study conditions should not differ significantly from guidelines and recommended protocols. e.g. Is the GAP relevant? Correct rate, application method, interval, PHI, spray volume, BBCH (if applicable), region, indoor/outdoor, control samples taken? e.g. were weather details available? e.g. were the control plots well separated from treated plots? e.g. was the field phase conducted according to GLP? e.g. were samples stored deep frozen? Were appropriate numbers of samples taken, e.g. 2kg of apples? e.g. was appropriate sampling methodology employed? Was the sample handling traceable? 4. Trial site/test system not previously exposed to the test material or other contaminants. e.g. Plot history supplied, e.g. evidence that compound not used that year or previous year, and information on other plant protection products (e.g. to check for common metabolites). 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. Examples as in 3 above and also, have they proposed an endpoint, e.g. MRL, what statistical methods have they used for this? 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. e.g. Was a validated method used, were acceptable recoveries obtained, were control samples analysed, were control samples 'clean', were representative clear chromatograms provided, Was the analytical phase conducted according to GLP? Were all components of the residue definition analysed for? Were samples analysed within a time period covered by storage stability data?
CA 6.4 Livestock Feeding Studies	<p>Same criteria as for crop studies, examples could be as above with the following additions.</p> <p><u>Livestock Feeding Studies Notes</u></p> <ol style="list-style-type: none"> 1. Well defined test material (including purity/content) 2. Applicable test species e.g. Ruminant, poultry, pig, fish, any edible animal. 3. Study conditions should not differ significantly from recommended protocols. e.g. is the dosing level extreme? e.g. was the application form appropriate, e.g. capsule? e.g. was the number of test species correct, e.g. three cows, nine hens? e.g. was the dosing period appropriate, e.g. minimum 28 days? e.g. were control animals included? e.g. were the animals healthy? e.g. were the animals acclimatized? 4. Trial site/test system not previously exposed to the test material or other contaminants. e.g. is it clear that additional animal feed did not contain treated substance? 5. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. 6. Study conditions should not interfere with the interpretation of the study results. 7. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc.

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
CA 6.5 Effects of Processing	<p><u>High Temperature Hydrolysis</u></p> <ol style="list-style-type: none"> Well defined test material (including purity/content) e.g. if radiolabelled test item was used, was an appropriate isotope used (e.g. ^{14}C and <u>not</u> ^3H)? e.g. if radiolabelled test item was used, was the labelling position(s) appropriate to capture potential metabolites? e.g. if radiolabelled test item was used, was the specific activity adequate to meet an LOQ of 0.01 mg/kg? N.B. If water solubility of test item is < 0.01 mg/L then no study is required and can be deemed non-relevant Applicable test system e.g. Was the test undertaken in a <u>sterilised</u> buffer medium? Study conditions should not differ significantly from guidelines and recommended protocols. e.g. Were the temperature and pH conditions applied typical of processing operations carried out on commodities relevant to the test item? e.g. were samples stored deep frozen? Trial site/test system not previously exposed to the test material or other contaminants. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. Examples as in 3 above e.g. Were metabolites identified by appropriate techniques (e.g. co-chromatography with known standards using two dissimilar chromatographic systems or by techniques capable of positive structural identification e.g. MS, NMR)? Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc. e.g. Were relevant control experiments carried out when harsher techniques (e.g. acid/base hydrolysis) were used to identify metabolites (i.e. to ensure metabolites identified are not merely artefacts)? e.g. were representative clear chromatograms provided to support metabolite identification? e.g. where sample analysis exceeded 6 months from sample collection was storage stability of samples demonstrated? Study conditions should not interfere with the interpretation of the study results. <p><u>Field Studies</u></p> <ol style="list-style-type: none"> Well defined test material (including purity/content) Applicable test species Study conditions should not differ significantly from guidelines and recommended protocols. Trial site not previously exposed to the test material or other contaminants. Sufficient experimental information provided to substantiate and evaluate whether the study conclusions and endpoints are robust. Study conditions should not interfere with the interpretation of the study results. Validated Analytical methodology employed, e.g. control samples used, acceptable recoveries obtained, clear example chromatograms etc.
CA 6.6 Residues in Rotational crops	Same criteria as for crop residue studies, examples could be subtly different, e.g. acceptable PBIs, crop types, again monitoring information should not be considered relevant.
CA 6.7 Proposed residue definition and MRLs	Residue definition would only be affected if data generated in another section, e.g. metabolism/tox. MRLs would only be affected if residues generated and this is covered under 6.3.

Data requirements(s) (indicated by the correspondent CA data point(s))	Criteria for relevance
CA 6.8 Proposed Safety Intervals	Not required. Any animal safety reports that might affect withholding periods would be covered in the review of literature in the Toxicology Section.
CA 6.9 Risk Assessment	Not required – any adverse findings for the risk assessment will have to be due to a data point from one of the other sections, and are therefore covered in other sections of this document.
CA 6.10 Other Studies	Not required.

Results and stepwise consideration:

Overall 5899 papers (excluding duplicates) were retrieved in the searches of the databases:

- From the initial database searches 52 titles were retrieved (in relation to Pydiflumetofen (parent) search).
- From the initial database searches 5844 titles were retrieved (in relation to Pydiflumetofen metabolites search).
- From the initial database searches 3 titles were retrieved (in relation to common SDHI metabolites search).

All of these ‘returns’ were excluded at the step of the rapid assessment when considering the paper title where rapid assessment sought to exclude those titles of ‘obviously irrelevant methods’ as per EFSA Journal 2011; 9(2) 2092). The applicant explained that “no external research has been published on the parent molecule SYN545974, the SYN545974 specific metabolites and the SYN545974 common SDHI metabolites”. The metabolite search which returned many thousands of hits did not contain any of the metabolites of pydiflumetofen relevant to metabolism and residues. There were many hits for common classes of chemistry e.g. trichlorophenols. The applicant stated that there were specific references to 2,4,6-trichlorophenol (which is a main metabolite in the livestock assessment) however, these were not relevant to metabolism and residues in animal matrices. The applicant did not state that abstracts were referred to, however it appears that it was possible to deduce that there were not relevant papers from the titles alone.

Therefore no further (more detailed) assessment for relevance were carried out, no full text was assessed by the applicant, and no assessment of reliability of studies needed to be performed.

In view of the dates of the search (November 2015 and over a span of 45 years), it is would not necessarily be expected that there might be expected hits, as pydiflumetofen is currently being assessed as a new active substance. The intention of the literature review is to consider studies published within the last ten years of publication before the time of submission of the dossier. The dates 2016 to 2020 have not been covered. July 2020 was the date of submission to HSE. It is possible that a more recent search could have disclosed some relevant/recent papers.

Conclusion: HSE concludes that regarding the literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to this residues risk assessment.

Comments by HSE residues evaluator.

1. The relevancy criteria seem specific to retrieving possibly only regulatory studies, rather than published studies of other types (research studies) that might include data or information useful to the understanding of residues or metabolism more generally: e.g. “Study conditions should not differ significantly from guidelines and recommended protocols. e.g. did the study meet the relevant guidelines?” Therefore residues considerations outside the scope of standard regulatory residues studies would not be expected to be part of the papers/titles retrieved. It would be useful in future to seek to broaden the scope of the search to be able to retrieve a wider range of peer reviewed published papers of interest to residues and the consumer risk assessment.
2. The AGES⁴ guidance in 2013 on relevancy criteria concluded that “The relevance criteria should not be too restrictive in order to avoid useful information being lost.” The applicant notes above that “no

⁴ EFSA supporting publication 2013:EN-511

Citation: Berger, E., Čoja, T., Dellantonio, A., Hrdina-Zödl, B., Hutzenlaub, N., Jölli, D., Müller, M., Prohaska, C. (2013). Case studies for the application of the Guidance of EFSA on Submission of scientific peer-reviewed open literature for the

external research has been published....” however the search criteria seem specific to retrieving regulatory assessment studies (such as those expected to be conducted to GLP and generated in accordance with OECD test guidelines rather than those which would be published in scientific peer reviewed literature).

3. Despite the above points, the relevance criteria applied seem similar in style to the suggested approach in EFSA 2011 Guidance (EFSA Journal 2011; 9(2):2092)) e.g. Table 1 on page 16 of the EFSA Guidance as a suggested style for relevancy criteria).

The dates that the searches were done were November 2015 (updated search for parent and also the search based on the metabolites) and the time span was over the long term (of 45 years). However the dates leading up to submission (2016 to 2020) have not been covered by this original literature review assessment. Regulation 1107/2009 states that “Scientific peer-reviewed open literature, as determined by the Authority, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier shall be added by the applicant to the dossier.” The short-fall was addressed by the submission of the updated literature review, see below.

Updated literature review (2022)

The applicant updated their original literature review by doing a further top up search to add to the original (considered in the above). This was to retrieve papers that were more up to the date, considering date of submission of the main dossier in July 2020 (span of the updated search was 01 November 2015 – 31 July 2020). It is noted that some of the papers retrieved were dated 2020 or 2021. The date of the searches was 2 August 2022.

The same stipulated criteria for relevance that were applied in the original literature review were applied in this update. It seems that the top up search was for parent pydiflumetofen (SYN545974), metabolites: both parent-specific metabolites and the common SDHI metabolites. The previous metabolite search (see original literature review) had retrieved nearly 6000 papers that did not contain any of the metabolites of pydiflumetofen relevant to metabolism and residues.

For the updated literature review, the following ‘add-ins’ were made to the search strategy (supplemental to the original search): see **search strategy** below.

The following 13 (Host STN) databases were included:

MEDLINE, EMBASE, EMBIOBASE, AGRICOLA, BIOSIS, CABA, HCAPLUS, FSTA, GEOREF, TOXCENTER, PQSCITECH, SCISEARCH, and ANABSTR. All of these were included in the original literature review (which covered 18 databases). A justification of the selection used was provided.

As before, the applicant considered that the bibliographic databases would provide a comprehensive search to retrieve quality peer reviewed literature, and as search did not do further retrieval searches, such as web search (e.g. websites of conferences or organisations), search of journals’ tables of contents, or search of reference lists of full-text journal articles (e.g. reviews).

Search Strategy	
SYN545974 second top-up search year 2022	
L1	QUE “Pydiflumetofen”
L2	QUE “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide” OR “3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide”
L3	QUE “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(2S)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide” OR “3-(difluoromethyl)-N-methoxy-1-methyl-N-[(2S)-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide”

approval of pesticide active substances under Regulation (EC) No 1107/2009, using substances for which dossiers are submitted under Regulation (EU) No 1141/2010. EFSA supporting publication 2013:EN-511

Search Strategy	
L4	QUE “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(2R)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide” OR “3-(difluoromethyl)-N-methoxy-1-methyl-N-[(2R)-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide”
L5	QUE “FUSHA” OR “ADEPIDYN” OR “MIRAVIS”
L6	QUE (L1 OR L2 OR L3 OR L4 OR L5)
L7	QUE “(2R)-2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]amino]propanoic acid” OR “(2R)-2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonylamino]propanoic acid”
L8	QUE “(2S)-2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]propanoic acid” OR “(2S)-2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]propanoic acid”
L9	QUE “(2R)-2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]propanoic acid” OR “(2R)-2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]propanoic acid”
L10	QUE “(3R)-3-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]butanoic acid” OR “(3R)-3-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]butanoic acid”
L11	QUE “(3S)-3-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]butanoic acid” OR “(3S)-3-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]butanoic acid”
L12	QUE “3-(difluoromethyl)-1-methyl-N-[(2S)-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-1-methyl-N-[(2S)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L13	QUE “3-(difluoromethyl)-1-methyl-N-[(2R)-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-1-methyl-N-[(2R)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L14	QUE “3-(difluoromethyl)-N-methoxy-N-[(2R)-1-(2,4,6-trichlorophenyl)propan-2-yl]-1H-pyrazole-4-carboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-N-[(2R)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]-1H-pyrazole-4-carboxamide”
L15	QUE “3-(difluoromethyl)-N-methoxy-N-[(2S)-1-(2,4,6-trichlorophenyl)propan-2-yl]-1H-pyrazole-4-carboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-N-[(2S)-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]-1H-pyrazole-4-carboxamide”
L16	QUE “3-(difluoromethyl)-N-methoxy-1-methyl-N-[(2S)-1-(2,4,6-trichloro-3-hydroxyphenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(2S)-1-[2,4,6-tris(chloranyl)-3-oxidanyl-phenyl]propan-2-yl]pyrazole-4-carboxamide”
L17	QUE “3-(difluoromethyl)-N-methoxy-1-methyl-N-[(2R)-1-(2,4,6-trichloro-3-hydroxyphenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(2R)-1-[2,4,6-tris(chloranyl)-3-oxidanyl-phenyl]propan-2-yl]pyrazole-4-carboxamide”
L18	QUE “3-(difluoromethyl)-N-[(1R,2S)-1-hydroxy-1-(2,4,6-trichlorophenyl)propan-2-yl]-N-methoxy-1-methyl-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(1R,2S)-1-oxidanyl-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L19	QUE “3-(difluoromethyl)-N-[(1R,2R)-1-hydroxy-1-(2,4,6-trichlorophenyl)propan-2-yl]-N-methoxy-1-methyl-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[(1R,2R)-1-oxidanyl-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L20	QUE “88-06-2” OR “2,4,6-trichlorophenol” OR “2,4,6-tris(chloranyl)phenol”
L21	QUE “3-(difluoromethyl)-1-methyl-N-[1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-1-methyl-N-[1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L22	QUE “3-(difluoromethyl)-N-methoxy-N-[1-(2,4,6-trichlorophenyl)propan-2-yl]-1H-pyrazole-4-carboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-N-[1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]-1H-pyrazole-4-carboxamide”
L23	QUE “3-(difluoromethyl)-N-methoxy-1-methyl-N-[1-(2,4,6-trichloro-3-hydroxyphenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[1-[2,4,6-tris(chloranyl)-3-oxidanyl-phenyl]propan-2-yl]pyrazole-4-carboxamide”
L24	QUE “3-(difluoromethyl)-N-[1-hydroxy-1-(2,4,6-trichlorophenyl)propan-2-yl]-N-methoxy-1-methyl-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[1-oxidanyl-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”

Search Strategy	
L25	QUE “3-(difluoromethyl)-N-methoxy-1-methyl-N-[rac-(1S,2S)-1-hydroxy-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[rac-(1R,2R)-1-oxidanyl-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L26	QUE “3-(difluoromethyl)-N-methoxy-1-methyl-N-[rac-(1S,2R)-1-hydroxy-1-(2,4,6-trichlorophenyl)propan-2-yl]-4-pyrazolecarboxamide” OR “3-[bis(fluoranyl)methyl]-N-methoxy-1-methyl-N-[rac-(1R,2S)-1-oxidanyl-1-[2,4,6-tris(chloranyl)phenyl]propan-2-yl]pyrazole-4-carboxamide”
L27	QUE “3-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]butanoic acid” OR “3-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]butanoic acid”
L28	QUE “2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]-methoxyamino]propanoic acid” OR “2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonyl-methoxy-amino]propanoic acid”
L29	QUE “1192017-82-5” OR “2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]amino]propanoic acid” OR “2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonylamino]propanoic acid”
L30	QUE “1192017-82-5” OR “(2S)-2-[[[3-(difluoromethyl)-1-methyl-4-pyrazolyl]-oxomethyl]amino]propanoic acid” OR “(2S)-2-[[3-[bis(fluoranyl)methyl]-1-methyl-pyrazol-4-yl]carbonylamino]propanoic acid”
L31	QUE (L6-30)
SAVE L31 PYDIPM/Q	

Results and stepwise consideration:

Overall 807 additional papers (excluding duplicates) were retrieved in the area of residues and metabolism in the updated (August 2022) ‘top-up’ literature review searches of the databases considering parent pydiflumetofen. MEDLINE retrieved 112 papers and HCAPLUS retrieved 371 papers; these two were the databases that returned the most retrieved titles.

801 of these 807 ‘returns’ were excluded at the step of the rapid assessment when considering the paper title where rapid assessment sought to exclude those titles of ‘obviously irrelevant methods’ as per EFSA Journal 2011; 9(2) 2092). The applicant did not state that abstracts were referred to, however it appears that it was possible to deduce that there were not relevant papers from the titles alone.

There were 6 titles retrieved that led to consideration of the full text documents (detailed assessment) by the applicant; of these 4 were decided to be excluded from any further consideration. These were:

1. EFSA (2019) Peer review of the pesticide risk assessment of the active substance pydiflumetofen. EFSA journal. European Food Safety Authority, (2019 Oct) Vol. 17, No. 10
2. EFSA (2019) Scientific support for preparing an EU position in the 51st Session of the Codex Committee on Pesticide Residues (CCPR). EFSA journal. European Food Safety Authority, (2019 Jul) Vol. 17, No. 7, pp. e05797. Electronic Publication Date: 31 Jul 2019
Journal code: 101642076. E-ISSN: 1831-4732. L-ISSN: 1831-4732.
3. Wu Junxue; Zhi Shenwei; Jia Chunhong; Zhu Xiaodan; Zhao Ercheng
Zhi Shenwei; Li Xinghai (2019) “Dispersive solid-phase extraction combined with dispersive liquid-liquid microextraction for simultaneous determination of seven succinate dehydrogenase inhibitor fungicides in watermelon by ultra high performance liquid chromatography with tandem mass spectrometry.” Journal of separation science, (2019 Dec) Vol. 42, No. 24, pp. 3688-3696.
Electronic Publication Date: 20 Oct 2019
Journal code: 101088554. E-ISSN: 1615-9314. L-ISSN: 1615-9306. This includes some monitoring results (samples of watermelon of unknown origin).

4. Miller, Nathan [Reprint Author]; Adams, Mike; Quesada-Ocampo, Lina (2020) “Evaluation of a New Fungicide for Management of Fusarium Wilt of Watermelon”. HortScience 52(9) (Supplement)—2017 SR-ASHS Annual Meeting—3–5 February 2017 [Only the efficacy of the fungicide was discussed in this paper, and no residues data]

Therefore, further to the exclusion of the above 4 papers, there were two remaining publications in the area of residues and metabolism not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance).

These are:

1. Wu Xiuming; Dong Fengshou; Xu Jun; Liu Xingang; Wu Xiaohu; Zheng Yongquan (2020). “Enantioselective separation and dissipation of pydiflumetofen enantiomers in grape and soil by supercritical fluid chromatography-tandem mass spectrometry” Journal of separation science, (2020 Jun) Vol. 43, No. 11, pp. 2217-2227.

Electronic Publication Date: 16 Mar 2020

Journal code: 101088554. E-ISSN: 1615-9314. L-ISSN: 1615-9306.

2. Wang, Zhen; Li, Rui; Zhang, Jing; Liu, Shiling; He, Zongzhe; Wang, Minghua (2021) Evaluation of exploitive potential for higher bioactivity and lower residue risk enantiomer of chiral fungicide pydiflumetofen. Pest management science, (1 Jul 2021) Vol. 77, No. 7, pp. 3419-3426.

E-ISSN: 1526-4998

The updated literature assessment stated that these were considered as relevant but not reliable. No further explanation of the reliability assessment was included in the report.

HSE requested these papers and following consideration decided to include a summary of these papers in the evaluation, as they assist in providing information on the enantiomeric composition of residues. The HSE summary of these papers has been included at the end of section B.7.2.1 (following a consideration of enantiomeric composition of residues in plants, since the applicant considered this aspect in the regulatory (GLP) residues data submitted in the areas of primary crop and rotational crop metabolism).

Conclusion: HSE concludes that regarding the updated literature search undertaken by the applicant, it is considered that the search is acceptable in terms of databases searched and the search criteria applied. See the HSE comments above (for the original literature review) regarding relevancy criteria.

Regarding the exclusion of papers by the applicant following the reliability assessment of the two above (‘relevant’) papers, the reliability assessment was not explained in the updated literature review report. There does not seem to be a standardised specific tool for estimation of the reliability of scientific peer reviewed open literature dealing with the pesticide residue behaviour. On this basis, HSE selected to consider the above two publications in more detail (on enantiomeric composition) and at the same time considered a publication on enantiomeric composition in one of the toxicology papers (that was retrieved in the toxicological search for papers). For a more detailed consideration of these three papers, please refer to the end of section B.7.2.1.

B.7.8. REFERENCES RELIED ON

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 6	[REDACTED]	25/02 /2021	Pydiflumetofen - Appendix G	N	N	N/A	SYN	N

			Metabolism Residue trials Report No. N/A Document No. VV-893256 , Test Facility Syngenta Limited Not GLP Unpublished					
KCA1 6.1/07	██████ ██████ ██████	2015	Storage Stability in Crops Stored Frozen for up to 23 months. Final Report Report No. S13-02224 (Syngenta Report No. TK0103785). Document No. VV-414120 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.1	██████	2016, final 2017	SYN545974 – Storage Stability of SYN545974 in Bovine Muscle, Liver, Milk, Fat and Chicken Eggs Report Number: 36552 Document No. VV-414208 Test Facility Charles River Laboratories Edinburgh Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.1	██████ ██████	2016	SYN545974 – Frozen Storage Stability of Residues of SYN508272, SYN548264, SYN547897 and SYN548263 in Animal Matrices Report No. CEMR-7064 Document No. VV-412637 Test Facility CEM Analytical Services Limited (CEMAS) GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.1	██████ ██████ ██████ ██████	2016	SYN545974 – Storage Stability of Residues of Conjugated 2,4,6 Trichlorophenol in Animal Matrices	N	Y	The study is necessary for this regulatory decision and is eligible	SYN	N

			Stored Frozen for up to Twelve Months Report No. PTRL Europe ID P 3669 G Document No. VV-414155 Test Facility PTRL Europe GmbH GLP Unpublished			for data protection		
KCA1 6.2.1/0 2		2014	SYN545974 - Metabolism of [¹⁴ C]-SYN545974 in Wheat. Report Number 33586 (Syngenta Report No. TK0123335) Document No. VV-411108 Test Facility Charles River Laboratories Edinburgh Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.2.1/0 3		2014	SYN545974 - Metabolism of [¹⁴ C]-SYN545974 in Tomatoes. Report Number 34592 (Syngenta Report No. TK0123336) Document No. VV-411024 Test Facility Charles River Laboratories Edinburgh Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.2.1/0 1		2015	SYN545974 - Metabolism of [¹⁴ C]-SYN545974 in Oilseed Rape Report Number 33587 (Syngenta Report No. TK0123337) Document No. VV-412847 Test Facility Charles River Laboratories Edinburgh Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.2.2		2015	SYN545974 - Metabolism of [¹⁴ C]-SYN545974 in the Laying Hen Report No. 33964 Document No. VV-414163	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

			Test Facility [REDACTED] [REDACTED] [REDACTED] GLP Unpublished					
KCA1 6.2.3	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]	2015	SYN545974 – Metabolism of [¹⁴ C]- SYN545974 in the Lactating Goat Report No. 33963 Document No. VV- 414236 Test Facility [REDACTED] [REDACTED] [REDACTED] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.5.3	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]	30/08 /2017	SYN545974 - Residue Study on Barley and Processed Specimens in Northern France, Germany and Poland in 2013 Report No. S13-02518 Document No. VV- 463141 , A17573A_10004 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.5.3 (Also submitt ed under KCA 6.3.15/ 3)	[REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED] [REDACTED]	30/08 /2017	SYN545974 - Residue Study on Wheat in Northern France and the United Kingdom in 2013 Report No. S13-02516 Document No. VV- 467692 , A17573A_10005 Test Facility Eurofins Agroscience Services Chem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.5.3 (Also submitt ed under KCA 6.3.13/ 2)	[REDACTED] [REDACTED] [REDACTED]	24/07 /2017	SYN545974 - Residue Study on Oilseed Rape and Processed Products in Northern France, Germany and the United Kingdom in 2014 Report No. CEMR- 6531 Document No. VV- 468119 ,	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

			A19649B_10334 Test Facility CEM Analytical Services, Ltd. GLP Unpublished					
KCA 6.3.13/ 4		2016	SYN545974 – Residue Study on Oilseed Rape in the United Kingdom and Northern France in 2013. Eurofins Agrosience Services Chem GmbH. Report No. S13-02259 Document No. VV-415279. A19649B_10230 GLP Unpublished	N	Y	Study to support new active approval in GB	SYN	N
KCA 6.3.14/ 1		2017	SYN545974 – Residue Study on Barley in North France, Germany, Poland, Hungary and the UK in 2016. Charles River Laboratories Edinburgh Ltd. Report No. 38034 Document No. VV- 467584 GLP Unpublished	N	Y	Study to support new active approval in GB	SYN	N
KCA 6.3.15/ 1		2017	SYN545974 – Residue Study on Wheat in North France, Germany, Poland, the Czech Republic and the UK in 2016. Charles River Laboratories Edinburgh Ltd. Report No. 38051 Document No. VV- 467609 GLP Unpublished	N	Y	Study to support new active approval in GB	SYN	N
Appen dix C 3.1.2.0 4-01		2017	SYN545974 – Residue Study on Carrot in Northern France, Germany, Poland and the United Kingdom in 2016. CEM Analytical Services Ltd, UK. Report No. CEMR- 7597 GLP Unpublished	N	Y -	Study to support new active approval in GB. Not relevant at this time, study submitted for MRL assessment	SYN	N
KCA1 6.4.1		2015	SYN545974- Magnitude of the	Y	Y	The study is necessary	SYN	N

			Residues in Tissue and Eggs Resulting from the Feeding of Three Dose Levels to Poultry 2014 Report No's. TK0103796, [REDACTED] [REDACTED] Document No. VV-414618 Test Facilities; [REDACTED]; [REDACTED], [REDACTED] GLP Unpublished			for this regulatory decision and is eligible for data protection		
KCA1 6.4.2 (Also submitted under KCA1 6.4)	[REDACTED]	2017	SYN545974 – Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administration of SYN545974 Report No. 35775 Document No. VV-414196 Test Facility [REDACTED] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.4.2	[REDACTED]	2016	SYN545974 – Magnitude of Residues in Tissues of Dairy Cows Following Multiple Oral administrations of SYN545974 Report No. 37460 Document No. VV-465348 Test Facility [REDACTED] GLP Unpublished	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA1 6.5.1	[REDACTED]	2014	[14 C] SYN545974 - Aqueous Hydrolysis at 90, 100 and 120°C Report No. 35072 Document No. VV-410228, [REDACTED] SYN545974_10110 Test Facility Charles River Laboratories	Y	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

			GLP Unpublished					
KCA 6.6.1/0 1		2015	SYN545974 –Uptake and Metabolism of [14C]-SYN545974 in Confined Rotational Crops Report Number 34316, (Syngenta Report No. TK0061717). Document No. VV-412848 Test Facility Charles River Laboratories Edinburgh Ltd GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 6.6.2/0 1		2018	Adepidyn – Residue Study on Rotational Crops in Northern France and Germany during 2016 – 2017. Report Number CEMR-7709, (Syngenta Report No. TK0289435). Document No. VV-469769 Test facility: CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 6.6.2/0 2		2018	Pydiflumetofen - Residue Study on Rotational Crops in Southern France and Spain during 2016-2017. Report Number CEMR-7710, (Syngenta Report No. TK0289439). Document No. VV-470802 Test facility: CEM Analytical Services Ltd (CEMAS) GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 6.6.2/0 3		2016	SYN545974 – Residue Study on Rotational Crops in Southern France and Italy during 2013 – 2015. Report Number S13-01023, (Syngenta Report No. TK0178755). Document No. VV-415410	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N

			Test facility : Eurofins Agroscience Services Chem GmbH. GLP Unpublished					
KCA 6.6.2/0 4	■■■■■ ■■■ ■■■	2016	SYN545974 – Residue Study on Rotational Crops in the United Kingdom and Germany during 2013 – 2014. Report Number S13- 01022, (Syngenta Report No. TK0178741). Document No. VV- 415357 Test facility : Eurofins Agroscience Services Chem GmbH. GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N
KCA 6.10.1	■■■■■ ■■■■■ ■	2017	SYN545974 and Fludioxonil – Residues in Honey Following Exposure of Bees to Treated Winter Oilseed Rape in Germany during 2016. Report Number S16- 02006, (Syngenta Report No. TK0283818). Document No. VV- 466889 Test facility : Eurofins Agroscience Services EcoChem GmbH GLP Unpublished	N	Y	The study is necessary for this regulatory decision and is eligible for data protection	SYN	N