BENZOVINDIFLUPYR (261)

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EXPLANATION

Benzovindiflupyr was scheduled for residue evaluation as a new compound by the 2014 JMPR at the Forty-fifth Session of the CCPR (2013). The toxicological review was conducted in 2013, which established an ADI of 0–0.05 mg/kg bw and an ARfD of 0–0.1 mg/kg bw. Additional toxicological data were provided for the metabolites SYN546039 and SYN545720. Benzovindiflupyr was defined by the WHO panel as the only toxicologically significant compound in animals, plants and the environment.

Benzovindiflupyr is a broad-spectrum fungicide belonging to the chemical class of pyrazole carboxamides. Benzovindiflupyr acts as an inhibitor of the fungal complex II respiratory chain, where it inhibits the succinate dehydrogenase enzyme (succinate dehydrogenase inhibitor, SDHI) by blocking the ubiquinone-binding sites in the mitochondrial complex.

The Meeting received information from the manufacturer on identity, metabolism, storage stability, residue analysis, use pattern, residues resulting from supervised trials on soya beans, fate of residue during processing, and livestock feeding studies.

IDENTITY

ISO common name:	benzovindiflupyr
Chemical name	
IUPAC:	N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. In the IUPAC convention the symbol 1RS,4SR represents a racemic mixture of 1R,4S and 1S,4R [Syngenta, 2014b],
CAS:	1*H*-pyrazole-4-carboxamide, N-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-
CAS Registry No:	1072957-71-1
CIPAC No:	-
Synonyms and trade names:	SYN545192; CSCD064398; N-[(1RS,4SR)-9-(dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (name used in the WHO monograph) N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide; N-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide; N-[11-(dichloromethylene)tricyclo[6.2.1.02,7]undeca-2,4,6-trien-3-yl]-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide 3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid ((1RS,4SR)-9-dichloromethylene-1,2,3,4-tetrahydro-1,4-methano-naphthalen-5-yl)-amide SMILES: Cn1cc(c(n1)C(F)F)C(=O)Nc2cccc3c2C4CCC3C4=C(Cl)Cl
Structural formula:	Structure for PAI (99.4% purity) confirmed by UV-VIS, IR, ^{1}H-NMR and MS [Oggenfuss, 2011, SYN545192_10087]

	or
	or
Molecular formula:	$C_{18}H_{15}Cl_2F_2N_3O$
Molecular weight:	398.2 g/mol

Benzovindiflupyr contains chiral centres at both the bridgehead carbon atoms potentially resulting in four stereoisomeric forms. However, the bicyclic ring is a rigid structure, and therefore only two stereoisomers exist (an enantiomeric pair). Technical benzovindiflupyr consists of a racemic mixture of two enantiomers SYN546526 and SYN546527, at a ratio of 50:50. SYN546526 represents N-[(**1R,4S**)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN546527 represents N-[(**1S,4R**)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide [Syngenta, 2014b]. The enantiomers were unresolved using the chromatographic solvent systems in the reports. Both enantiomers were measured within the same single chromatographic peak and they were collectively reported as one concentration [Syngenta 2014b]. Both enantiomers are fungicidally active. No toxicological studies were performed on the individual enantiomers.

PHYSICAL AND CHEMICAL PROPERTIES

Pure active ingredient (PAI)

Parameter	Result	References	Guidelines/method
Purity	99.4% (PAI)		
Appearance:	odourless white powder at 25 °C purity 99.4% (PAI)	[Das, 2010a, SYN545192_10057	organoleptic and visual assessment
Vapour pressure:	measured 2.0×10^{-5} Pa at 70 °C 7.6×10^{-5} Pa at 80 °C 3.9×10^{-4} Pa at 90 °C extrapolated 3.2×10^{-9} Pa at 25 °C	[Weissenfeld, 2010, SYN545192_10067]	OECD 104 gas saturation method

Parameter	Result	References	Guidelines/method
	purity 99.4% (PAI) The vapour pressure at 25 °C was extrapolated from high temperature measurements to allow the completion of the various experiments within a suitable period of time [Syngenta, 2014a, SYN545192_10468].		
Melting point:	148.4 °C ± 0.5 °C purity 99.4% (PAI)	[Kühne, 2010b, SYN545102_10060]	OECD 102 thermal analysis (differential scanning calorimetry)
Octanol/water partition coefficient:	log K_{ow} = 4.3 ± 0.3 at 25 °C ± 1 °C in pure water (pH 6.59) purity 99.4% (PAI) The log K_{ow} value calculated from the ratio of solubilities (19 g/L: 0.98 mg/L) is 4.3 which agrees well with the value determined using the OECD 107 [Syngenta, 2014a, SYN545192_10468].	[Vijayakumar, 2010b, SYN545102_10070]	OECD 107 shake flask method
Solubility:	0.98 mg/L RSD 8.9% in pure water at pH 6.7–7.5, at 25 °C purity 99.4% (PAI)	[Vijayakumar, 2011, SYN545102_10072]	OECD 105 column elution method
	solubility at 25 °C ± 0.5 °C purity 97.7% (TGAI) Acetone 350 g/L Dichloromethane 450 g/L Ethyl acetate 190 g/L Hexane 270 g/L Methanol 76 g/L Octanol 19 g/L Toluene 48 g/L No data are available for PAI [Syngenta, 2014a, SYN545192_10468].	[Vijayakumar, 2010a, SYN545102_10066]	CIPAC MT 157.3 flask method
Specific gravity:	1.466 g/cm^3 at 20.4 °C D^{20}_4 = 1.466 (rel. density) purity 99.4% (PAI)	[Kühne, 2010d, SYN545192_10068]	OECD 109 gas comparison pycnometer
Hydrolysis in water:	0.35 mg/L [pyrazole-^{14}C]-SYN545192 (radiochemical purity 98.7%) Hydrolytically stable in sterile buffer at pH 4, 5, 7 and 9 for up to 5 days at 50 °C in the dark. Stability was confirmed at all pH values for up to 30 days at 25 °C. No degradation products were detected by HPLC, TLC or HPLC-MS.	[Lowrie, 2009, SYN545192_10010]	OECD 111
Photolysis in water:	0.3 mg/L [phenyl-^{14}C]-SYN54519 (purity 98.5%) or 0.3 mg/L [pyrazole-^{14}C]-SYN545192 (purity 98.6%)	[Wardrope, 2011, SYN545192_10132]	OECD 316 EPA 161-2 OPPTS 835.224

Parameter	Result	References	Guidelines/method
	Moderate decline of parent to 74.8% TAR or 74.4% TAR for phenyl or pyrazole labels, respectively, after 15 days in sterile buffer at pH 7 at 25 °C ± 1 °C using a xenon arc lamp at 53.1 W/m^2 continuously DT_{50} = 44.2 days (average of both labels). Photodegradation of the phenyl labelled compound resulted in the production of multiple polar components (11.5% TAR) and diffuse radioactivity (6.3% TAR). No individual compound or region accounted for > 5% TAR. For the pyrazole label, the most significant degradates were NOA449410 at 8.5% TAR and SYN508272 at 2.6% TAR, both at 15 DAT. No other single degradate was observed at > 5% TAR. Photodegradation resulted in the production of $^{14}CO_2$, which reached a maximum mean value of 6.3% TAR in the phenyl samples and 1.1% TAR in the pyrazole samples at 15 DAT.		
Dissociation constant:	no dissociation at pH 2.0–12.0 purity 99.4% (PAI)	[Kühne, 2010e, SYN545192_10069]	OECD 112 titration method

Technical material (TGAI)

Parameter	Result	References	Guidelines
Purity	97.7% (TGAI)		
Appearance:	odourless off-white powder at 25 °C purity 97.7% (TGAI)	[Das, 2010b, SYN545192_10058]	organoleptic and visual assessment
Density:	1.42 g/cm^3) at 22.0 ± 1.0 ºC purity 97.7% (TGAI)	[O'Connor, 2013a, SYN545192_10444]	OECD 109 gas comparison pycnometer
Melting range:	145.7 °C (418.9 K), with RSD 0.07% purity 97.7% (TGAI)	[O'Connor, 2013b, SYN545192_10445]	OECD 102 differential scanning calorimetry
Thermal stability	PAI (purity 99.4%) decomposition started at about 285 °C before boiling occurred. No data are submitted for the TGAI.	[Kühne, 2010a, [SYN545192_10059]	OECD 103, thermal analysis (differential scanning calorimeter)
Stability:	TGAI (purity 97.7%) is stable in nitrogen or air at room temperature.	[Williams, 2011, SYN545192_10088]	OECD 113 differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

Formulations

Formulation authorised for use: WG (150 g/kg benzovindiflupyr + 300 g/kg azoxystrobin).

Benzovindiflupyr has not been evaluated by JMPS and therefore no FAO specifications for technical and formulated benzovindiflupyr have been published.

Table 1 List of reference compounds used in various study reports [Syngenta, 2014c]

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports	Found as or in
SYN545192 BVFP (parent)	benzovindiflupyr CSCD064398 IUPAC: N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide	rat goat (milk, liver, kidney, muscle, fat) hen (eggs, liver, muscle, skin with fat) tomato fruit wheat forage/hay/straw wheat grains soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat forage/hay/straw photolysis in water hydrolysis in water
SYN546206 (N-demethyl-BVFP)	CSCD711742; N-demethyl-benzovindiflupyr IUPAC: N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide MW 384.22	rat goat (liver, kidney, muscle, fat) hen (eggs, liver, muscle, skin with fat) tomato fruit wheat forage/hay/straw wheat grains soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat forage/hay/straw
SYN546039 (BVFP-OH)	CSCD695908 hydroxy-benzovindiflupyr isomer of SYN546040 IUPAC: N-[(1RS,2RS,4SR)-9-(dichloromethylene)-2-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 414.20	rat goat (milk, liver, kidney, muscle, fat) hen (eggs, liver, muscle, skin with fat) tomato fruit wheat forage/hay/straw wheat grains soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat forage/hay/straw
SYN546040 (BVFP-OH)	CSCD696468 hydroxy-benzovindiflupyr isomer of SYN546039	rat goat (milk, liver, kidney, muscle, fat

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports	Found as or in
	IUPAC: N-[(1RS,2SR,4SR)-9-(dichloromethylene)-2-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 414.20	hen (eggs, liver, muscle, skin with fat) tomato fruit wheat grains soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat forage/hay/straw
SYN546322	CSCD733585 hydroxy-benzovindiflupyr isomer of SYN546323 IUPAC: N-[(1RS,3SR,4SR)-9-(dichloromethylene)-3-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 414.20	not found in wheat
SYN546323	CSCD733586 hydroxy-benzovindiflupyr isomer of SYN546322 IUPAC: N-[(1RS,3RS,4SR)-9-(dichloromethylene)-3-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 414.20	not found in wheat
SYN546360	CSCD737493 hydroxy-benzovindiflupyr IUPAC: N-[(1RS,4SR)-9-(dichloromethylene)-8-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 414.20	rat goat (bile) hen (eggs, liver, muscle)
SYN546041 (N-demethyl-BVFP-OH)	CSCD695909 N-demethyl-hydroxy-benzovindiflupyr isomer of SYN546042 IUPAC: N-[(1RS,2RS,4SR)-9-(dichloromethylene)-2-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide	rat goat (milk, liver, kidney, muscle, fat hen (eggs, liver, muscle, skin with fat) wheat straw wheat grain

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports	Found as or in
		soya bean forage/hay soya bean seeds rotational crops: turnip roots/leaves, wheat forage/hay/straw
SYN546042 (N-demethyl-BVFP-OH)	CSCD695910 N-demethyl-hydroxy-benzovindiflupyr isomer of SYN546041 IUPAC: N-[(1RS,2SR,4SR)-9-(dichloromethylene)-2-hydroxy-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1*H*-pyrazole-4-carboxamide	rat goat (milk, liver, kidney, muscle, fat) hen (eggs, liver, muscle, skin with fat) soya bean hay soya bean seeds rotational crops: turnip roots/leaves, wheat forage/hay/straw
SYN546422	CSCD743668; IUPAC: N- [(1SR,3RS)-2-(dichloromethylene)-1-hydroxy-3-(2-hydroxy-ethyl)-indan-4-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide MW 432.3	goat (milk, liver, kidney, muscle, fat) not found in hen postulated in rat
SYN508272	CSCC210616 pyrazole amide IUPAC: 3- difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid amide MW 175.1	goat (milk, liver, kidney, muscle, fat) hen (eggs, liver, muscle, skin with fat) wheat hay/straw wheat grains soya bean forage soya bean hay rotational crops: turnip leaves, wheat forage/hay/straw photolysis in water common metabolite with other pyrazole fungicides like bixafen, fluxapyroxad, isopyrazam and sedaxane
NOA449410	pyrazole acid CSAA798670 IUPAC: 3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid	not detected in goat and hen commodities tomato fruit wheat hay/straw wheat grains soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports	Found as or in
		forage/hay/straw photolysis in water common metabolite with other pyrazole fungicides like bixafen, fluxapyroxad, isopyrazam and sedaxane
SYN545720	N-desmethylpyrazole-acid CSCD465008, R958945, IUPAC: 3-difluoromethyl-1*H*-pyrazole-4-carboxylic acid CASnr 151734-02-0 MW 162.1	not detected in rat, goat and hen commodities tomato fruit soya bean forage/hay soya bean seeds rotational crops: lettuce leaves, turnip roots/leaves, wheat forage/hay/straw (not found in photolysis in water) common metabolite with other pyrazole fungicides like bixafen, fluxapyroxad, isopyrazam and sedaxane

METABOLISM AND ENVIRONMENTAL FATE

The Meeting received information on the fate of benzovindiflupyr in livestock, plant commodities, soil and rotational crops. The test items used in the studies were benzovindiflupyr ^{14}C uniformly labelled in the phenyl ring or at the 5-position of the pyrazole ring as shown in Figure 1.

Figure 1 Benzovindiflupyr ^{14}C labelled in the phenyl ring or the pyrazole ring

Animal metabolism

The Meeting received information on the metabolic fate of benzovindiflupyr in ruminants (lactating goats) and poultry (laying hens). The metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2013.

Lactating goats

One lactating goat (Saanen × Toggenburg) per radiolabel was dosed orally once daily for 7 consecutive days with a gelatin capsule containing [phenyl-^{14}C]-benzovindiflupyr or [pyrazole-^{14}C]-benzovindiflupyr [Green, 2012, SYN545192_10184 and additional info in Syngenta, 2014c]. The actual mean daily dose administered was 41 (range 34–50) and 32 (range 30–34) ppm in the dry feed for the phenyl and pyrazole label, respectively. Average feed consumption was 1.5 kg/animal (phenyl label) and 1.9 kg/animal (pyrazole label experiment) as dry weight feed. Average dose rates were 0.89 and 0.98 mg/kg bw/day for the phenyl and pyrazole label, respectively. The age of the goats was 3.0 and 4.5 years for the phenyl and pyrazole label, respectively. Body weights were 70 and 63 kg at the start of the study and 66 and 59 kg at the end of the study for the phenyl and pyrazole label, respectively. Milk was collected twice daily; urine and faeces were collected once daily. Average milk production during application was 2.5 and 2.1 kg/day for the phenyl and pyrazole label experiments, respectively. Aliquots of the PM and AM milk were mixed and this composite 24 h sample was used to generate cream and skimmed milk for that time period following centrifugation. The goats were sacrificed approximately 12 hours after the administration of the final dose and liver, kidney, muscle (hindquarter, forequarter and tenderloin), fat (subcutaneous, omental and perirenal), blood, GI tract and contents, bile were taken post mortem. Samples were stored frozen at –20 °C for 82–139 days until analysis.

Homogenised samples were analysed by combustion LSC. The mean total recovery of the dosed radioactivity was 90% and 86% for the goats dosed with phenyl and pyrazole radiolabelled forms, respectively. The majority of the radioactivity was recovered in faeces (79%/73% TAR, phenyl/pyrazole). The remainder of the dose was recovered in urine (4.5%/5.2% TAR phenyl/pyrazole), GI tract contents (6.8%/7.2% TAR), cage wash (< 0.28% TAR), milk (< 0.16% TAR) and liver (0.33%/0.22% TAR), while only very low levels were found in kidney, muscle, blood or fat (≤ 0.01% TAR each).

Total radioactive residues are summarised in Table 2. The highest radioactivity concentrations in edible tissues were found in the liver (1.3/0.70 mg/kg eq), kidney (0.28/0.18 mg/kg eq), followed by fat (0.098/0.070 mg/kg eq) and muscle (0.070/0.032 mg/kg eq). Total radioactive residues in milk from the phenyl label dosed goat reached a plateau concentration of approximately 0.046 mg/kg eq following 96 hours dosing. Total radioactive residues in milk from the pyrazole label dosed goat reached a plateau concentration of approximately 0.035 mg/kg eq following 72 hours dosing. Residue levels in cream and skimmed milk derived from combined PM and AM milk samples (24 hour samples) from within the plateau milk residue period are summarised in Table 3. The ratio of cream total radioactive residue to skimmed milk total radioactive residue ranged from 3.7:1 to 4.8:1 in all analysed samples of both radiolabelled experiments except one sample (phenyl: 144 hour sampling) where the ratio was larger (12.2:1). No explanation for the increase in ratio in this single sample was evident. No constituent ^{14}C-residue analysis of the separated cream and aqueous (whey) fractions was undertaken [Syngenta 2014b].

Homogenised samples of liver, kidney and composite muscle were extracted sequentially with acetonitrile (1×), acetonitrile/water 4:1 v/v (1–4×), acetonitrile/water 3:7 v/v (1–2×), water (1×) and acetonitrile (1×). Composite fat was extracted first with dichloromethane (1×) and subsequently with the other solvents. Whole milk (144 hrs) was extracted with dichloromethane (3×), ethyl acetate (2×) and acetone (1×). Extracts and remaining solids were analysed by (combustion) LSC. Extracted residues were, where appropriate, combined and fractionated by liquid/liquid partition. Liver and kidney extracts were subject to β-glucuronidase (pH 5, 37 °C, 18 hrs) to cleave glucuronide and sulphate conjugates and subsequently partitioned with ethyl acetate. Fractions containing significant radioactive residues were subject to TLC with bio-imaging analysis to enable quantification and identification of residues. Residues were identified by co-chromatography with authentic reference standards for parent, SYN546206, SYN546039, SYN546040, SYN546041, SYN546042, SYN546360, SYN546422, SYN508272, NOA449410 and SYN545720.

Extractability of the radioactive residues was ≥ 94% TRR except that of liver (78–89% TRR). Identified radio-components present in extracted residues are summarised in Table 4.

Comparison of 2D-TLC chromatograms of pre- and post-hydrolysis fractions in liver and kidney shows that the released ^{14}C-exocons after hydrolysis originate predominantly from radio-components present on the origin (baseline) of the chromatogram. Unassigned radio-components that chromatograph away from the origin are unlikely to be conjugated metabolites for two reasons:

i) The solvent systems employed in the normal phase 2D-TLC analyses are quite non-polar in nature and the sulphate and glucuronide conjugated metabolites (or other logical conjugates) are intrinsically quite polar in nature and it would therefore be expected that those conjugates would be located on or very close to the origin of these chromatograms,

ii) Significant proportions of the "unassigned" chromatographed radio-components chromatograph well away from the origin of the 2D-chromatograms and in the region of identified "non-conjugated" and non-polar metabolites. The unassigned radio-components are therefore too non-polar to be characteristic of the conjugates characterised in i) above. [Syngenta, 2014c]

Solids remaining after extraction from liver (phenyl PES: 11% TRR, pyrazole PES: 22% TRR,) were further characterised in three parallel experiments. Incubation under mild acid hydrolysis conditions (0.1 M HCl, 37 °C, 4 hrs) rendered only very minor proportions (≥ 0.9% TRR) of the PES soluble. Following protein solubilisation using sodium dodecyl sulphate (SDS) and then a protein precipitation step, 6.8% TRR (phenyl) and 15% TRR (pyrazole) was shown to be associated with protein. Protease digestion (pH 7, 37 °C, 24 hrs + 48 hrs) solubilised almost all the PES. TLC analysis of the protease hydrolysates showed them to comprise a very complex mixture of highly polar metabolites of which no single one accounted for > 2.0% TRR (phenyl) or > 4.4% TRR (pyrazole).

Initial radio-component profiles of fractions of liver and whole milk samples were produced within 82–131 days of sacrifice. While there is no information on the first 82 days of storage, re-analysis of the milk fractions after completion of the analyses (730 days of total storage) demonstrated the radio-component profiles not to have changed significantly during the interim period of frozen storage. Re-analysis of the liver fractions after 694 days of total storage showed a different profile, indicating some change in composition during storage.

Note: The identified metabolite levels in muscle, fat and milk relate only to the free form of the metabolites. Since the unassigned fractions of these matrices were not subjected to glucuronidase treatment, it is not clear how much conjugated metabolites need to be added to these figures. It will however i) not be more than the sum of the "unassigned and baseline" figures and ii) in reality be closer to the polar "baseline" only figure due to the predominantly non-polar character of the "unassigned" residues precluding them from being conjugates.

Table 2 Total Radioactive Residues and extractability in samples from goats treated with [^{14}C]benzovindiflupyr

Radiolabel	Sample	Extracted Radioactivity		Post-extracted solids		TRR [a]
		%TRR	mg/kg eq	%TRR	mg/kg	mg/kg eq
Phenyl	Milk (144 h)	98	0.040	2.0	0.001	0.041
	Liver	89	1.1	11	0.14	1.3
	Kidney	96	0.27	4.0	0.011	0.28
	Composite muscle [b]	98	0.068	2.3	0.002	0.070
	Composite fat [c]	100	0.098	0.3	< 0.001	0.098
Pyrazole	Milk (144 h)	100	0.034	NA	NA	0.034
	Liver	78	0.54	22	0.16	0.70
	Kidney	94	0.18	5.9	0.011	0.18
	Composite muscle [b]	96	0.031	3.7	0.001	0.032
	Composite fat [c]	99	0.069	1.0	0.001	0.070

NA Not Applicable

[a] TRR in mg/kg eq calculated from radioactivity extracted and radioactivity in the PES.

[b] Forequarter, hindquarter and tenderloin muscle combined in the ratio 4:4:1 by weight to produce a single composite sample.

[c] Subcutaneous, omental and perirenal fat combined in the ratio 2:1:1 by weight to produce a single composite sample.

Table 3 Total Radioactive Residues in Cream and Skimmed Milk

Milk Sample Time Point	TRR am samples (mg/kg eq)		Ratio (C/S)	TRR am samples (mg/kg eq)		Ratio (C/S)
	Cream	Skimmed		Cream	Skimmed	
96 hours	0.20	0.041	4.8	0.12	0.029	4.2
120 hours	0.21	0.043	4.8	0.11	0.029	3.9
144 hours	0.31	0.025	12	0.13	0.034	3.7
156 hours	0.19	0.042	4.6	0.11	0.031	3.7

C/S = ratio cream / skimmed

Table 4 Characterisation and Identification of Components in samples from Goats Treated with [^{14}C]BVFP

	phenyl-^{14}C-BVFP					pyrazole-^{14}C-BVFP				
	Milk %TRR	Liver %TRR	Kidney %TRR	Muscle %TRR	Fat %TRR	Milk %TRR	Liver %TRR	Kidney %TRR	Muscle %TRR	Fat %TRR
TRR mg/kg eq [a]	0.041	1.3	0.28	0.070	0.098	0.034	0.70	0.18	0.032	0.070
parent	5.5	11	13	24	41	7.3	10	7.5	25	44
SYN546206	ND	0.4	0.9	1.3	0.8	ND	0.5	0.3	0.7	0.3
(N-demethyl-BVFP)		(ND)	(ND)				(0.2)	(0.1)		
SYN546039	24	50	23	39	36	22	38	22	39	25
(BVFP-OH)		(45)	(ND)				(35)	(7.8)		
SYN546040	2.3	5.4	3.5	8.1	3.5	0.4	1.3	0.9	1.8	0.9
(BVFP-OH)		(4.3)	(0.1)				(1.0)	(0.3)		
SYN546360 [f]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SYN546041	7.5	6.2	7.6	2.8	0.7	8.2	6.9	10.5	4.4	1.1
(N-demethyl-BVFP-OH)		(5.7)	(1.1)				(6.3)	(5.0)		
SYN546042	6.6	3.0	7.0	4.4	0.8	7.1	0.9	1.8	1.0	0.3
(N-demethyl-(BVFP-OH)		(1.9)	(ND)				(0.6)	(1.1)		
SYN546422	25	4.4	19	8.2	1.6	20	6.0	16	8.9	1.5
		(3.5)	(1.6)				(5.6)	(4.5)		
SYN508272	NA	NA	NA	NA	NA	2.5	0.2	0.7	3.3	0.3
NOA449410	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND
SYN545720	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND
Unassigned [b]	13	2.2	3.9	0.7	1.0	16	2.3	11	6.5	10
Baseline [c]	2.3	2.7	5.9	2.0	0.6	3.5	3.6	7.1	5.3	1.4
Other fractions [d]	8.7	3.3	2.3	2.5	2.5	7.0	4.5	2.6	2.5	4.9
PES [e]	2.0	11	4.0	2.3	0.3	NA	22	5.9	3.7	1.0
Total	97	100	89	95	88	95	97	86	102	91

ND Not detected (< 0.001 mg/kg eq)
NA Not applicable for the label indicated
() = The values within parentheses indicate the proportion of the TRR that is in the conjugated forms. Values without parentheses are the sum of both the free and conjugated forms.
[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction
[b] Unassigned radio components which chromatographed away from the origin in normal phase 2D-TLC.
Milk comprising at least 4–8 discrete components, no single one ≥ 6.5% TRR (≥ 0.003 mg/kg eq)
Liver post-enzyme hydrolysis, comprising at least eight discrete components, no single one ≥ 0.7% TRR (≥ 0.006 mg/kg eq).
Kidney post-enzyme hydrolysis comprising at least 10–19 discrete components, including a poorly resolved region of 14 components which accounted for a total of 8.5% TRR (0.016 mg/kg). All other unidentified components were < 1.3% TRR (< 0.004 mg/kg eq).
Muscle, comprising at least 2–3 discrete components, no single one ≥ 5.2% TRR (≥ 0.002 mg/kg eq).
Fat, comprising at least 2–4 discrete components, no single one ≥ 6.2% TRR (≥ 0.004 mg/kg eq).

[c] Polar material on the origin of the radio-chromatogram using normal phase 2D-TLC
[d] Extracted residues in fractions that were not analysed because levels of radioactivity were too low for analysis
Milk (2 fractions), No single fraction comprised ≥ 6.9% TRR (≥ 0.002 mg/kg eq)
Liver (3–4 fractions), No single fraction comprised ≥ 2.4% TRR (≥ 0.030 mg/kg eq)
Kidney (3 fractions). No single fraction comprised ≥ 1.7% TRR (≥ 0.004 mg/kg eq)
Muscle (5 fractions). No single fraction comprised ≥ 2.4% TRR (≥ 0.002 mg/kg eq).
Fat (4 fractions). No single fraction comprised ≥ 1.6% TRR (≥ 0.001 mg/kg eq).
[e] Radioactivity remaining in the solids after extraction
[f] The presence of SYN546360 in goat bile was shown by HPLC-MS. Its location in 2D-TLC chromatograms (the primary technique used for quantitative analysis of Milk and Tissue fractions) was not established in the goat metabolism study. However comparison of goat 2D-TLC chromatograms for milk and goat tissue fractions with those of the rat biotransformation study (where a metabolite standard of SYN546360 was shown to chromatograph between SYN546039 and SYN546206) demonstrated that either no residues or if present only a very minor residue (Goat liver: 0.4% TRR; 0.005 mg/kg eq) could be attributed to this metabolite. Its presence in milk and goat tissues can therefore be assumed to be negligible [Syngenta, 2014b].

Laying hens

Five laying hens (ISA Warren) per radiolabel were dosed orally once daily for 14 consecutive days via gelatin capsules containing [phenyl-U-^{14}C]-benzovindiflupyr or [pyrazole-5-^{14}C]-benzovindiflupyr [Lowrie and Kadow, 2012, SYN545192_10170 and additional information in Syngenta, 2014c]. The actual mean daily dose administered based on measured feed consumption of individual birds was 16–20 and 17–20 ppm in the dry feed, for the phenyl and pyrazole experiments, respectively. Average feed consumption ranged from 0.13–0.15 kg/animal (phenyl treatment) and 0.12–0.14 kg/animal (pyrazole experiment) on dry weight basis. Body weights ranged from 1.6–1.9 kg at the start and 1.7–2.1 kg at the end of the dosing period. Average dose rates were 1.4 mg/kg bw/day for the phenyl and pyrazole labels, each. Hens were pullets (young hens < 1 year old and greater than 23 weeks) and demonstrated good egg laying performance. Excreta were collected daily; eggs were collected twice daily. Eggs were separated into yolk and white. The hens were sacrificed approximately 12 hours after the administration of the final dose and liver, kidney, leg and thigh muscle, breast muscle, peritoneal fat, subcutaneous fat with skin attached, blood, bile, partially formed eggs, carcass and GI tract and contents were taken post mortem. Samples were stored frozen at –20 °C for 314–426 days until analysis.

Homogenised samples were analysed by combustion LSC. The kidneys, carcass and bile samples were not analysed. The average recovery of radioactivity was 88% and 93% TAR from birds of the phenyl and pyrazole label experiment, respectively. Of this on average 88% and 92% TAR was accountable in the hen excreta plus cage wash, while 0.28%/1.1% TAR was found in the gastrointestinal tract contents, 0.10%/0.13% TAR in egg yolks + whites, < 0.04% in liver, < 0.02% in muscle samples, < 0.01% in fat samples.

Total radioactive residues are summarised in Table 5. The highest radioactivity concentrations in edible tissues were found in the liver (0.19/0.25 mg/kg eq), followed by fat (0.033/0.045 mg/kg eq) and muscle (0.025/0.036 mg/kg eq). Mean total radioactive residues in egg yolks achieved a plateau concentration of 0.17 mg/kg eq (phenyl label) after 240 hrs dosing and 0.18 mg/kg eq (pyrazole label) after 168 hours dosing. Mean total radioactive residues in egg whites achieved a plateau concentration of 0.04 mg/kg eq (phenyl label) after 168 hrs dosing and 0.03 mg/kg eq (pyrazole label) after 120 hours dosing.

Homogenised composite samples from the five birds per radiolabel were prepared for liver, fat, muscle, egg yolk and egg white. Sub-samples of liver, composite muscle, and egg white were extracted sequentially with acetonitrile (1×), acetonitrile/water 4:1 v/v (2–3×), acetonitrile/water 3:7 v/v (1–4×), water (1×) and acetonitrile (1×). Egg yolk and composite fat with skin were extracted first with dichloromethane (1×) and subsequently with the other solvents. Extracts and remaining solids were analysed by (combustion) LSC. Extracted residues were, where appropriate, combined and fractionated by liquid/liquid partition. Aqueous fractions from liver extracts were subject to β-glucuronidase (pH 5, 37 °C, 18 hrs) to cleave glucuronide and sulphate conjugates and subsequently partitioned with diethyl ether. Fractions containing significant radioactive residues were subject to TLC with bio-image analysis to enable quantification and identification of residues. Residues were

identified by chromatographic comparison with authentic synthetic reference standards of parent, SYN546206, SYN546039, SYN546040, SYN546041, SYN546042, SYN546360, SYN508272, NOA449410, SYN545720.

Following solvent extraction, residue extractabilities were ≥ 83% TRR for egg yolk and egg white and 68–73% TRR for skin with subcutaneous fat. Residue extractability was lowest in liver (48–49% TRR) and composite muscle (24–37% TRR). Identified radio-components present in extracted residues are summarised in Table 6.

Comparison of 2D-TLC chromatograms of pre- and post-hydrolysis fractions in liver shows that the released [^{14}C]exocons after hydrolysis originate predominantly from radio-components present on the origin (baseline) of the chromatogram. Unassigned radio-components that chromatograph away from the origin are unlikely to be conjugated metabolites for two reasons:

i) The solvent systems employed in the normal phase 2D-TLC analyses are quite non-polar in nature and the sulphate and glucuronide conjugated metabolites (or other logical conjugates) are intrinsically quite polar in nature and it would therefore be expected that those conjugates would be located on or very close to the origin of these chromatograms,

ii) Significant proportions of the "unassigned" chromatographed radio-components chromatograph well away from the origin of the 2D-chromatograms and in the region of identified "non-conjugated" and non-polar metabolites. The unassigned radio-components are therefore too non-polar to be characteristic of the conjugates characterised in i) above. [Syngenta, 2014c]

Solids remaining after extraction from liver (PES 51–52% TRR) and muscle (PES 63–76%TRR) in both radiolabelled experiments were further characterised in three parallel experiments. Liver and muscle PES were characterised to be similar in nature. Incubation under mild acid hydrolysis conditions (0.1 M HCl, 37 °C, 4 hrs) rendered only very minor proportions of the PES soluble. Following protein solubilisation using sodium dodecyl sulphate solution (SDS) and then a protein precipitation step, almost all of PES were shown to be associated with protein. Protease digestion (pH 7, 37 °C, 24 hrs + 48 hrs) solubilised almost all the PES. Analysis of protease hydrolysates showed them to comprise almost entirely of radio-components that were highly polar in nature.

All tissues, egg yolk and egg white samples were initially extracted and a 2D-TLC radio-component profile obtained from the principal residue-containing fractions within 104–107 days of sacrifice. The same fractions were again analysed using the same 2D-TLC conditions at the end of the analytical phase of the study (after 413–559 days of total storage). While there is no information on the first 104 days of storage, the results demonstrated that there had been no significant changes in the respective radio-component profiles during the interim storage period.

Note: The identified metabolite levels in muscle, skin/fat, egg yolk and egg white relate only to the free form of the metabolites. Since the unassigned fractions of these matrices were not subjected to glucuronidase treatment, it is not clear how much conjugated metabolites need to be added to these figures. It will however i) not be more than the sum of the "unassigned and baseline" figures and ii) in reality be closer to the polar "baseline" only figure due to the predominantly non-polar character of the "unassigned" residues precluding them from being conjugates.

Table 5 Total Radioactive residues and extractability in samples from laying hens treated with [^{14}C]benzovindiflupyr

Radiolabel	Sample	Extracted Radioactivity		Post-extracted solids		TRR [a]
		%TRR	mg/kg eq	%TRR	mg/kg eq	mg/kg eq
[Phenyl-^{14}C]-BVFP	Liver	48	0.090	52	0.098	0.19
	Egg Yolk	85	0.14	15	0.024	0.16
	Egg White	98	0.033	2.3	0.001	0.034
	Muscle	24	0.006	76	0.019	0.025
	Skin and Fat	68	0.022	32	0.011	0.033
[Pyrazole-^{14}C]-BVFP	Liver	49	0.12	51	0.13	0.25
	Egg Yolk	83	0.15	17	0.030	0.18

Radiolabel	Sample	Extracted Radioactivity		Post-extracted solids		TRR [a]
		%TRR	mg/kg eq	%TRR	mg/kg eq	mg/kg eq
	Egg White	98	0.031	2.3	0.001	0.032
	Muscle	37	0.013	63	0.023	0.036
	Skin and Fat	73	0.033	27	0.012	0.045

[a] TRR calculated by summation of extracted radioactivity and post extracted solids (PES)

Table 6 Characterisation and Identification of components in samples from laying hens treated with [^{14}C]-BVFP

	phenyl-[^{14}C]-BVFP					[pyrazole ^{14}C]-BVFP				
	Liver %TRR	Egg yolk %TRR	Egg White %TRR	Muscle %TRR	Skin and fat %TRR	Liver %TRR	Egg yolk %TRR	Egg White %TRR	Muscle %TRR	Skin and fat %TRR
TRR mg/kg eq [a]	0.19	0.16	0.034	0.025	0.033	0.25	0.18	0.032	0.036	0.045
parent	0.2	14	11	3.3	38	0.2	14	12	3.3	42
SYN546206 (N-demethyl-BVFP)	0.2 (< 0.1)	3.8	2.8	0.4	0.7	0.9 (0.5)	3.4	2.7	0.6	1.4
SYN546039 (BVFP-OH)	1.3 (0.6)	14	22	2.2	3.1	2.3 (0.5)	12	21	2.4	5.2
SYN546040 (BVFP-OH)	0.2	5.8	11	0.4	0.4	0.6	3.9	8.1	0.4	1.0
SYN546360	0.4 (0.2)	2.9	1.2	0.4	ND	0.5 (0.3)	2.5	1.7	0.4	ND
SYN546041 (N-demethyl-BVFP-OH)	1.6 (0.2)	12	11	3.5	1.8	2.3 (0.2)	11	11	2.7	2.2
SYN546042 (N-demethyl-BVFP-OH)	1.7	12	9.0	3.0	1.0	2.6	9.3	6.6	2.3	1.2
SYN546422 [j]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SYN508272	NA	NA	NA	NA	NA	0.6 (0.2)	0.9	4.0	4.4	1.1
NOA449410	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND
SYN545720	NA	NA	NA	NA	NA	ND	ND	ND	ND	ND
Unassigned [b]		7.7	15.2	6.0	11.4		8.0	17.5	9.3	7.4
Organo soluble [c]	1.6					2.8				
Organo soluble [d]	1.5					2.5				
Water soluble [e]	23					18				
Baseline [f]	2.6	4.2	5.1	1.3	1.6	1.6	6.2	2.5	4.0	1.2
Remainder [g]	< 0.1	2.1	2.8	0.5	1.8	1.1	1.3	2.5	0.3	1.5
Other fractions [h]	2.1	9.9	2.3	1.5	1.6	1.7	11	< 0.1	2.2	1.6
PES [i]	52	15	2.3	76	32	51	17	2.3	63	27
Total	88	104	95	98	93	88	100	91	96	92

ND = Not detected (< 0.001 mg/kg eq)
NA = Not applicable for the label indicated
() = The values within parentheses indicate the proportion of the TRR that is in the conjugated forms. Values without parentheses are the sum of both the free and conjugated forms.
[a] TRR calculated by summation of extracted radioactivity and post extracted solids (PES)
[b] Unassigned radio-components which chromatographed away from the origin in normal phase 2D-TLC
Egg yolk, comprising at least 11–12 discrete components, no single one ≥ 1.3% TRR (≥ 0.002 mg/kg eq)
Egg white, comprising at least 6–10 discrete components, no single one ≥ 4.9% TRR (≥ 0.002 mg/kg eq)
Muscle, comprising at least 5–6 discrete components, no single one ≥ 3.7% TRR (≥ 0.001 mg/kg eq)
Fat/skin, comprising at least 4–6 discrete components, no single one ≥ 6.0% TRR (≥ 0.002 mg/kg eq)
[c] Liver unassigned radio-components in pre-enzyme hydrolysis organo soluble fraction, which chromatographed away from the origin in normal phase 2D-TLC, comprising at least 8–11 discrete components, no single one ≥ 0.6% TRR (≥ 0.001 mg/kg eq)
[d] Liver unassigned radio-components in post enzyme hydrolysis organo soluble fraction which chromatographed away from the origin in normal phase 2D-TLC, comprising at least 10–16 discrete components, no single one ≥ 0.4% TRR (≥ 0.001 mg/kg eq)
[e] Liver unassigned highly polar radio-components in post enzyme hydrolysis water soluble partition phase, which

remained at the origin in normal phase 1D-TLC, with a small proportion moving slightly in a more polar solvent system normal phase 1D-TLC.
[f] Polar material on origin of the radio-chromatogram using normal phase 2D-TLC
[g] Diffuse areas of radioactivity within the chromatogram which cannot be assigned to discrete radioactive components
[h] Extracted residues in fractions that were not analysed because levels of radioactivity were too low for analysis.
Liver (2 fractions), no single fraction comprised ≥ 1.4% TRR (≥ 0.003 mg/kg eq)
Egg yolk (4 fractions), no single fraction comprised ≥ 6.1% TRR (≥ 0.011 mg/kg eq)
Egg white (4 fractions), no single fraction comprised ≥ 1.2% TRR (≤ 0.001 mg/kg eq)
Muscle (4 fractions), no single fraction comprised ≥ 1.5% TRR (≤ 0.001 mg/kg eq)
Fat/skin (4 fractions), no single fraction comprised ≥ 1.6% TRR (≤ 0.001 mg/kg eq)
[I] Post extraction solids
[j] A reference standard of SYN546422 was not chromatographed in the hen study fractions. However, the location of this metabolite in 2D-TLC is very characteristic as it consistently chromatographs between SYN546040 and SYN546042 (just below SYN546040) in the goat metabolism study. In the hen metabolism study no radio-components were observed in this region of the corresponding 2D-TLC analyses of egg and tissue fractions. It was therefore concluded that SYN546422 was not present in the hen egg and tissues [Syngenta, 2014b].

Overview of the metabolic pathway in livestock

Metabolism studies conducted with ruminants (lactating goats) and poultry (laying hens) and based on oral dosing, show that the metabolic pathways in ruminants and poultry are similar. Metabolism observed arose via:

- N-demethylation of the pyrazole ring to form SYN546206
- mono hydroxylation on the alicyclic ring to form the SYN546039 and SYN546040 isomers
- mono hydroxylation on the phenyl ring to form SYN546360
- N-demethylation of the pyrazole ring and subsequent mono hydroxylation of the alicyclic ring or mono hydroxylation of the alicyclic ring and subsequent N-demethylation of the pyrazole ring to form SYN546041 and SYN546042 isomers
- oxidative opening of the alicyclic ring to form SYN546422 (ruminants only)
- cleavage between the pyrazole and phenyl rings of parent or mono hydroxylated metabolites SYN546039/SYN546040 to form SYN508272
- conjugation of N-demethylated metabolite SYN546206, predominantly as its glucuronide conjugate
- conjugation of dihydroxylated metabolite SYN546422, predominantly as its glucuronide and/or sulphate conjugate (ruminants only)
- conjugation of mono hydroxylated metabolites SYN546039/SYN546040 and SYN546360, predominantly as their glucuronide and/or sulphate conjugates
- conjugation of mono hydroxylated N-demethylated metabolites SYN546041/SYN546042, predominantly as their glucuronide and/or sulphate conjugates
- conjugation of cleavage metabolite SYN508272 predominantly as its glucuronide conjugate.

SYN545192

SYN546360 [a]

SYN546039

SYN546040

SYN546206

SYN508272 [b]

SYN546041

SYN546042

SYN546422 [c]

a = Identified in hen tissues and eggs and goat bile as a very minor metabolite.
b = Identified in hen and goat commodities as a very minor metabolite
c = Identified in goat commodities only
Dotted arrows indicate an alternative pathway to continuous arrows.
All metabolites were present in their free (non-conjugated) forms in milk, egg, muscle and fat containing tissues; possible conjugates have not been released in the metabolism study. In liver and kidney all metabolites were present in their free and conjugated forms. The conjugates were characterised as glucuronides and/or sulphate esters.

Figure 2 Metabolic pathway of benzovindiflupyr in livestock

Plant metabolism

The Meeting received information on photolysis on leaves and the metabolic fate of benzovindiflupyr after foliar application on fruits (tomatoes), cereals (wheat) and pulses & oilseeds (soya beans).

Photolysis on leaves

To determine whether leaf photolysis is an appreciable route of degradation, the leaves of six green bean plants were treated topically with an unspecified formulation containing ^{12}C and ^{14}C-pyrazole-labelled-benzovindiflupyr [Syngenta, 2014d]. Four suitable healthy leaves of each plant were marked with a 5 cm diameter circle. The leaves on three plants were sprayed with the formulated material without adjuvant. The leaves on three other plants were sprayed with the formulated materials with adjuvant. Three different treatments were performed:

- The treated leaves of two of the plants (one with and one without adjuvant) were placed underneath artificial sunlight derived from a xenon lamp irradiation source. The treated leaves were placed as flat as possible underneath a quartz sheet and illuminated with a 14 hr light and 10 hr dark cycle.
- Two of the plants (one with and one without adjuvant) were placed in the greenhouse with a 14 hr light and 10 hr dark cycle.
- Two of the plants (one with and one without adjuvant) were placed outdoors between 3–10 July 2008, with the treated leaves facing south where possible.

Treated leaves from each plant were sampled at 2 hrs after treatment and at DAT=1, 4 and 7. The leaves underneath the Suntest were scorched brown and dried in some cases, especially at the 7 DAT sampling. Whole leaves were extracted twice with methanol/water (70:30, v/v) and radioactivity in the extracts and remaining solids were quantified by (combustion) LSC.

Extract recoveries were 70–94% TAR, while remaining solids represented 0.1–4.4% TAR. The missing radioactivity was assumed to have been translocated into the areas of the plant and/or lost by evaporation. At DAT =7 total recovery was > 98% TAR.

HPLC chromatograms of the extracts demonstrated very minor degradation in all three situations at DAT=7. The minor degradates formed gave qualitatively similar chromatographic pattern. The highest level of degradation was found on the leaves grown in a greenhouse, either with or without adjuvant.

Metabolism in tomatoes

The metabolic fate of benzovindiflupyr was studied in indoor-grown tomatoes (variety F1 Orange Pixie, a dwarf variety) after four foliar spray applications to the plants [Oddy, 2011, SYN545192_10117]. Two radio-labelled forms were used in the study, [phenyl-^{14}C]-BVFP and [pyrazole-^{14}C]-BVFP (see Figure 1). Plants were grown in containers in sandy loam soil. The phenyl and pyrazole test items were applied at an actual rate of 0.138 + 0.132 + 0.129 + 0.128 kg ai/ha and 0.138 + 0.131 + 0.129 + 0.127 kg ai/ha, respectively, at weekly intervals. The spray solution was applied as an EC formulation to 6 week old plants (BBCH 71–72) in a spray volume of 600 L/ha using a lance and trigger sprayer with CO_2 propellant to give as even a cover as possible. Mature tomato fruits (176–663 g) were harvested 1 and 14 days after the final application. At each harvest interval fruits were surface washed immediately following sampling by immersing the fruit for 30 sec in acetonitrile (2×). Samples washes and samples were stored frozen at –18 °C for 17–23 days until extraction and 21–22 months until last analysis.

The washed frozen fruit was homogenised and sequentially extracted with acetonitrile (1×), acetonitrile/water 4/1 v/v (3×), acetonitrile/water 3/7 v/v (1×), water (1×), and acetonitrile (1×). Radioactivity in wash, extracts and post-extraction solids (PES) was determined by LSC and combustion/LSC. The total radioactive residues (TRR) in tomato fruits taken at 1 and 14 days after the final application are shown in Table 7. The TRRs in the samples derived from the pyrazole experiment were higher than those derived from the phenyl experiment. The majority of the radioactivity (65–79% TRR) could be washed off by acetonitrile. The total extractability of residues into wash and solvents for all samples was ≥ 99% TRR.

Proportionate aliquots of the acetonitrile wash and extracts 1–4 were combined; extracts 5–7 were not analysed further. The combined extracts were concentrated by evaporation and residues were

partitioned into diethyl ether/hexane (3:1, v/v). The resulting organic phase was characterised using 2D-TLC with phosphor image analysis. The remaining aqueous phase was not analysed further. Radiolabelled components were co-chromatographed with authentic reference standards of parent, SYN546206, SYN546039, SYN546040, SYN546041, SYN546042, NOA449410, SYN545720, SYN508272 using two dissimilar solvent systems. Results are shown in Table 8. In total a high level of identification was achieved in all tomato fruits (> 91.8% TRR).

The surface wash was analysed by 2D-TLC at the start and end of the analytical phase of the study to demonstrate storage stability. The original samples were re-analysed at the end of the analytical phase of the study, following 21–22 months of storage. Comparison of the initial and final radiolabelled component profiles obtained showed that no significant change in the profiles had occurred during the interim period of storage. Storage stability data do not exist for the 1 DALT 1 and 14 DALT extracts (confirmed, Syngenta, 2014b). However, since the majority of the radioactive residue (65–79% TRR) is derived from the surface wash, and 90.8–95.1% TRR is identified as parent, this is considered to have no impact on the results of this study.

Table 7 Total Radioactive Residues and extractability in tomatoes treated with [^{14}C]benzovindiflupyr

Radiolabel	Mature fruit Commodity conditions	Initial Fruit Wash		Washed Fruit				TRR [a]
				Extracted Radioactivity		Post-extracted solids		
		%TRR	mg/kg eq	%TRR	mg/kg eq	%TRR	mg/kg eq	mg/kg eq
[Phenyl-^{14}C]-BVFP	4× (0.128–0.138) kg ai/ha, DALT = 1	79	0.037	20	0.0094	0.8	0.0004	0.047
	4× (0.127–0.138) kg ai/ha, DALT = 14	77	0.071	22	0.020	0.5	0.0005	0.092
[Pyrazole-^{14}C]-BVFP	4× (0.128–0.138) kg ai/ha, DALT = 1	73	0.13	26	0.048	0.3	0.0005	0.18
	4× (0.127–0.138) kg ai/ha, DALT = 14	65	0.095	34	0.050	0.8	0.0012	0.15

[a] TRR determined by summation of radioactivity present in the surface wash, solvent extracts and debris

Table 8 Identification and characterisation of residues in tomatoes treated with [^{14}C]benzovindiflupyr

	Phenyl-^{14}C		Pyrazole-^{14}C	
	DALT = 1	DALT = 14	DALT = 1	DALT = 14
TRR, mg/kg eq	0.047	0.092	0.18	0.15
Component	% TRR	%TRR	% TRR	%TRR
parent	95	93	91	92
SYN546206 (N-demethyl-BVFP)	ND	0.4	0.4	0.5
SYN546039 (BVFP-OH)	ND	0.2	0.3	0.4
SYN546040 (BVFP-OH)	ND	ND	ND	0.1
SYN546041 (N-demethyl-BVFP-OH)	ND	ND	ND	ND
SYN546042 (N-demethyl-BVFP-OH)	ND	ND	ND	ND
SYN508272	NA	NA	ND	ND
NOA449410	NA	NA	0.2	0.2
SYN545720	NA	NA	0.1	0.1
Unassigned [a]	ND	0.7	0.7	0.7
Baseline [b]	ND	0.5	0.3	0.5

	Phenyl-^{14}C		Pyrazole-^{14}C	
	DALT = 1	DALT = 14	DALT = 1	DALT = 14
Other fractions [c]	3.6	2.0	4.7	5.4
PES [d]	0.8	0.5	0.3	0.8
Total	100	97	98	101

ND = Not detected, i.e. < 1.5 times the background radioactivity in samples with no radioactivity (< 0.0001 mg/kg eq)
NA = Not applicable to this radiolabel
[a] Unassigned radio-components which chromatographed away from the origin in 2D-TLC (normal phase) and comprised at least 3 compounds
[b] Polar material on origin of the radiochromatogram using 2D-TLC (normal phase).
[c] Extracted residues in fractions that were not analysed because levels of radioactivity were too low for analysis. These comprised solvent extracts 5–7 and the aqueous partition of extracts 1–4.
[d] Post-extraction solids; radioactivity remaining in the debris after extraction with solvent.

Metabolism in wheat

The metabolic fate of benzovindiflupyr was studied in indoor-grown spring wheat (variety Tybalt) after two foliar spray application to the plants [Simmonds and Mackenzie, 2011, SYN545192_10136]. Two radiolabelled forms were used in the study, [phenyl-^{14}C]-BVFP and [pyrazole-^{14}C]-BVFP (see Figure 1). Plants were grown in containers in sandy loam soil. The phenyl and pyrazole test items were applied at an actual rate of 2 times 0.14 kg ai/ha, at a 35 day interval. The first application was conducted at BBCH 31 and the second at BBCH 69. The spray solution was applied as an EC formulation in a spray volume of 300 L/ha using a lance and trigger sprayer with CO_2 propellant to spray from above. Samples were collected at BBCH 39 (forage, 9 days after the first application), BBCH 77 (hay, 10 days after the second application) and BBCH 89 (mature grain and straw, 40 and 41 days after the second application). The hay samples were laid out in the greenhouse to dry for 4 days. Sample sizes were 0.71–0.91 kg forage, 0.61–0.70 kg hay, 1.2–2.0 kg straw and 2.1 kg grain. Samples were stored frozen at –18 °C for 18–60 days until extraction and 19–23 months until analysis.

Wheat forage, hay and straw were homogenised and sequentially extracted with acetonitrile (1×), acetonitrile/water 4/1 v/v (2×–4×), acetonitrile/water 3/7 v/v (1×), water (1×) and acetonitrile (1×). Wheat grain was homogenised and sequentially extracted with acetonitrile (1×), acetonitrile/water 4/1 v/v (3×) and acetonitrile/water 1/1 v/v (2×). Radioactivity in extracts and post-extraction solids (PES) was determined by LSC and combustion/LSC. The total radioactive residues (TRR) in forage, hay, straw and grain are shown in Table 9. Residue levels derived from the two radiolabelled experiments were generally similar on a commodity basis. The total level of extractability was ≥ 97%TRR for all commodities.

Proportionate aliquots of the acetonitrile and acetonitrile/water (4/1) extracts were combined; the other fractions were not analysed further. The combined extracts were concentrated by evaporation and if necessary partitioned between water and diethyl ether/hexane (3:1, v/v).

All fractions were analysed by 2D-TLC with phosphor image analysis. Identification was conducted by co-chromatography with authentic reference standards of parent, SYN546206, SYN546039, SYN546040, SYN546322, SYN546323, SYN546041, SYN546042, NOA449410, SYN545720, SYN508272 using two dissimilar solvent systems. Results are shown in Table 10.

Selected straw and grain fractions were subjected to hydrolysis procedures involving the use of bovine rumen fluid, pectinase enzyme or mild base followed by pectinase enzyme to release the metabolites and to characterise the nature of the conjugates. Cleavage following pectinase hydrolysis (pH 5, 18 hrs at 30 °C) alone characterised the residue as a glycoside conjugate. Cleavage following mild base (0.05 M NaOH, 18 hrs, room temperature) and pectinase hydrolysis (pH 5, 18 hrs at 30 °C) characterised the residue as malonyl glycoside conjugate. Cleavage following bovine rumen fluid (pH 7, 24 hrs at 39 °C), effected greater hydrolytic conversion compared to pectinase and base pectinase. Hydrolysates were adjusted to pH 2–3 and partitioned into diethyl ether. The remaining aqueous phase was not analysed further. The resulting organic phase was characterised using 2D-TLC. Results

are shown in Table 10. Several of metabolites were present in the free and conjugated form. Significant proportions of the SYN546039 conjugates in straw and grain were characterised as glycoside and malonyl glycoside conjugates. In total > 86% TRR was identified for each commodity.

Combined extracts were analysed by TLC within 1 to 5 months of sample harvest and were analysed again following 19 to 23 months frozen storage to demonstrate storage stability. Comparison of the initial and final radio-component profiles obtained showed no significant change in the profiles had occurred during the interim period of storage.

Table 9 Total Radioactive Residues and Extractability in Wheat Treated with [^{14}C]benzovindiflupyr

	Wheat Commodity	Conditions	Extracted Radioactivity		Post-extracted solids		TRR [a]
			%TRR	mg/kg eq	%TRR	mg/kg eq	mg/kg eq
[Phenyl-^{14}C]-BVFP	Forage	1× 0.137 kg ai/ha; DAFT = 9	100	3.0	0.3	0.009	3.0
	Hay	2× (0.135–0.137) kg ai/ha; DALT = 11	98	4.8	1.5	0.074	4.9
	Straw	2× (0.135–0.137) kg ai/ha; DALT = 40	97	7.9	2.7	0.22	8.1
	Grain	2× (0.135–0.137) kg ai/ha; DALT = 40	97	0.12	2.6	0.003	0.12
[Pyrazole-^{14}C]-BVFP	Forage	1× 0.142 kg ai/ha; DAFT = 9	100	2.1	0.2	0.004	2.1
	Hay	2× (0.135–0.142) kg ai/ha; DALT = 11	99	6.3	1.1	0.070	6.4
	Straw	2× (0.135–0.142) kg ai/ha; DALT = 41	98	8.9	2.2	0.20	9.0
	Grain	2× (0.135–0.142) kg ai/ha; DALT = 41	97	0.090	2.7	0.002	0.092

[a] TRR derived from the summation of the radioactivity present in the solvent extracts and debris
DALT = Days after last treatment
DAFT = Days after first treatment

Table 10 Identification and characterisation of residues in wheat commodities treated with [^{14}C]benzovindiflupyr

	Forage	Forage	Hay	Hay	Straw	Straw	Grain	Grain
	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C
TRR mg/kg eq	3.0	2.1	4.9	6.4	8.1	9.0	0.12	0.092
Component	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
parent	100	103	89	93	81	84	87	84
SYN546206 (N-demethyl-BVFP)	0.3	0.3	0.9	0.8	1.4	1.1	2.3	2.0
					(0.1)	(ND)		
SYN546039 (BVFP-OH)	0.1	0.1	0.7	0.5	2.8	2.2	3.6	2.0
					(1.8)	(1.3)	(1.7)	(0.7)
SYN546040 (BVFP-OH)	ND	ND	ND	ND	< 0.1	ND	< 0.1	0.1
SYN546322	ND	ND	ND	ND	ND	ND	ND	ND
SYN546323	ND	ND	ND	ND	ND	ND	ND	ND
SYN546041 (N-demethyl-BVFP-OH)	ND	ND	ND	ND	0.3	0.3	0.8	0.5
					(0.2)	(0.3)	(0.5)	(0.3)
SYN546042	ND	ND	ND	ND	ND	ND	ND	ND

	Forage	Forage	Hay	Hay	Straw	Straw	Grain	Grain
	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C
(N-demethyl-BVFP-OH)								
SYN508272	NA	ND	NA	0.2	NA	0.4	NA	0.3
						(0.1)		(0.1)
NOA449410	NA	ND	NA	0.2	NA	0.4	NA	0.6
						(0.1)		
SYN545720	NA	ND	NA	ND	NA	ND	NA	ND
Unassigned [a]	1.0	0.8	2.2	1.8	2.1	2.0	3.7	3.3
Baseline [b]	1.2	1.0	1.3	1.5	5.3	6.1	2.1	3.8
Remainder [c]	0.0	0.2	0.8	0.6	0.3	1.3	0.1	0.4
Other fractions [d]	1.2	1.2	1.1	1.0	2.8	2.7	2.2	3.2
Unextracted [e]	0.3	0.2	1.5	1.1	2.7	2.2	2.6	2.7
Total	104	106	98	101	99	103	104	103

ND = Not detected, i.e. < 1.5 times the background radioactivity in samples with no radioactivity (< 0.00015 mg/kg eq)
NA = Not applicable to this radiolabel
( ) = The main figures are the sum of both the free and conjugated forms, the figures in parentheses indicate the proportion of the residue that was found in the conjugated form
[a] Unassigned radio-components which chromatographed away from the origin in 2D-TLC (normal phase)
Forage, phenyl label: comprising at least 4 discrete components, no single one > 0.6% TRR (> 0.018 mg/kg eq)
Forage, pyrazole label: comprising at least 4 discrete components, no single one > 0.5% TRR (> 0.011 mg/kg eq)
Hay, phenyl label: comprising at least 4 discrete components, no single one > 1.5% TRR (> 0.074 mg/kg eq)
Hay, pyrazole label: comprising at least 4 discrete components, no single one > 1.3% TRR > 0.083 mg/kg eq)
Straw, phenyl label: comprising at least seven discrete components, no single one ≥ 0.4% TRR (≥ 0.032 mg/kg eq)
Straw, pyrazole label: comprising at least six discrete components, no single one ≥ 0.5% TRR (≥ 0.045 mg/kg eq)
Grain, phenyl label: comprising at least eight discrete components, no single one ≥ 0.8% TRR (≥ 0.001 mg/kg eq)
Grain, pyrazole label: comprising at least seven discrete components, no single one ≥ 0.9% TRR (≥ 0.001 mg/kg eq)
[b] Polar material on origin of the radiochromatogram using 2D-TLC (normal phase)
Straw, pyrazole/phenyl label from fraction F4 (≤ 0.8% TRR, ≤ 0.065 mg/kg eq)and F5 (≤ 5.4% TRR, ≤ 0.489 mg/kg eq. Polar material from phenyl label F5, likely to comprise more than one radio-component. Polar material from pyrazole label F5: Further 2D TLC using a more polar solvent system (2D-TLC SSC) showed this to comprise at least three discrete components no single one of which ≥ 0.9% TRR (≥ 0.081 mg/kg).
[c] The remainder comprises diffuse areas of radioactivity within the chromatogram which cannot be assigned to discrete radioactive components.
[d] Extracted residues in fractions that were not analysed, because these fractions contained very low levels of radioactivity. No single fraction comprised > 0.9% TRR (> 0.027 mg/kg eq)in forage, > 0.7% TRR (> 0.044 mg/kg eq)in hay, ≥ 1.7% TRR (≥ 0.154 mg/kg eq)in straw, ≥ 2.5% TRR (≥ 0.002 mg/kg eq)in grains in either radiolabelled experiment.
[e] Radioactivity remaining in the debris after extraction with solvent.

Metabolism in soya beans

The metabolic fate of benzovindiflupyr was studied in indoor-grown soya beans (variety S12-C2) after two foliar spray application to the plants [Oddy, 2012, SYN545192_10102]. Two radiolabelled forms were used in the study, [phenyl-^{14}C]-BVFP and [pyrazole-^{14}C]-BVFP, subsequently referred to as phenyl and pyrazole test item (see Figure 1). Plants were grown in containers in sandy loam soil. The phenyl and pyrazole test items were applied at an actual rate of two times 0.12–0.13 kg ai/ha, at a 22 day interval. The first application was conducted at BBCH 55–60 and the second at BBCH 75. The spray solution was applied as an EC formulation in a spray volume of 300 L/ha using an automated track sprayer. Commodities were taken at BBCH 70 (forage, 11 days after first application), BBCH 85 (hay, 13 days after second application), and BBCH 89 (mature bean, 30 days after second application). Forage hay samples were left in the greenhouse to air dry for 6 days. Mature soya bean pods were dried for 2 days, after which the seeds were taken from the pods. Samples (0.11 kg fresh forage, 0.10 kg dry hay, 0.25 kg dry seeds) were stored frozen at –15 °C for 20–37 days until extraction and 29–31 months until analysis.

Soya bean forage was homogenised and sequentially extracted with acetonitrile (1×), acetonitrile/water 4:1, v/v (2×), acetonitrile/water 3:7, v/v (1×), water (1×) and acetonitrile (1×). Soya bean hay and beans were homogenised and sequentially extracted with hexane (3×), diethyl ether

(2×), acetonitrile (2×), acetonitrile/water 4:1 v/v (2×–7×), acetonitrile/water 3:7 v/v (1×–2×), water (1×), and acetonitrile (1×). Radioactivity in extracts and post-extraction solids (PES) was determined by LSC and combustion/LSC. Total radioactive residues (TRR) present in the soya bean commodities are shown in Table 11. Residue levels in the soya bean seeds derived from the pyrazole experiment were approximately three times higher than those in the phenyl experiment. The total level of extractability was ≥ 99%TRR (forage), ≥ 98%TRR (hay), ≥ 89% TRR (beans, phenyl experiment) and ≥ 98% TRR (beans, pyrazole experiment).

For soya bean forage proportionate aliquots of the acetonitrile and acetonitrile/water 4:1, v/v, extracts were combined and the resulting fraction (≥ 98% TRR) was analysed by 2D-TLC. An aliquot of the combined extracts was subjected to hydrolysis procedures involving the use of bovine rumen fluid (pH 7, 24 hrs, 39 °C) to release the metabolites. The hydrolysate was analysed by 2D-TLC.

For soya bean hay proportionate aliquots of hexane, diethyl ether and acetonitrile extracts were combined and resulting fractions containing 76% TRR (phenyl experiment) and 67% TRR (pyrazole experiment) were analysed by 2D TLC. Similarly, proportionate aliquots of the acetonitrile/water extracts were combined and the resulting fractions containing 18% TRR (phenyl experiment) and 26% TRR (pyrazole experiment) were analysed by 2D TLC and 1D-TLC. An aliquot of the combined acetonitrile/water extracts was subjected to hydrolysis procedures involving the use of bovine rumen fluid, pectinase enzyme (pyrazole experiment only), and mild base followed by pectinase enzyme (pyrazole experiment only) to release the metabolites and to characterise the nature of the conjugates. The hydrolysates were analysed by 2D-TLC.

Cleavage following bovine rumen fluid (pH 7, 24 hrs at 39 °C) was the most effective at hydrolysing baseline conjugate material and effected greater hydrolytic conversion compared to pectinase and base pectinase. Cleavage following pectinase hydrolysis (pH 5, 18 hrs at 30 °C) alone is the least effective hydrolysis method and characterised the residue as a glycoside conjugate. Cleavage following mild base (0.05 M NaOH, 18 hrs, room temperature) and pectinase hydrolysis (pH 5, 18 hrs at 30 °C) characterised the residue as malonyl glycoside conjugate.

For soya bean seeds, proportionate aliquots of hexane, diethyl ether and acetonitrile were combined. The resulting fraction (phenyl: 35% TRR; pyrazole: 10% TRR) was concentrated by evaporation to an oil, dissolved in hexane and partitioned into acetonitrile/water (9:1, v/v) to remove endogenous oil. The hexane phase (phenyl: 7.2% TRR; pyrazole: 1.2% TRR) was not further analysed. The aqueous acetonitrile fraction (phenyl: 24% TRR; pyrazole: 8.7% TRR) was analysed by 2D-TLC. Similarly, proportionate aliquots of the primary acetonitrile/water extracts were combined and the resulting fractions (phenyl: 48% TRR; pyrazole: 85% TRR) were analysed by 2D-TLC. An aliquot of the combined primary acetonitrile/water extracts was subjected to a protein precipitation clean-up step: addition of acetone and cooling for 18 hrs at 4 °C. The resulting supernatant (phenyl: 46% TRR; pyrazole: 82% TRR) was analysed by 2D-TLC and 1D-TLC. A separate aliquot of the combined primary acetonitrile/water extracts was concentrated and subjected to hydrolysis procedures involving the use of bovine rumen fluid (pH 7, 24 hrs at 39 °C). The resulting hydrolysate was partitioned between water and diethyl ether/hexane (9:1 v/v). The organic phase (phenyl: 41% TRR; pyrazole: 55% TRR) was analysed by 2D-TLC and 1D-TLC. The aqueous phase derived from the phenyl experiment (8.1% TRR) was not analysed further. The aqueous phase derived from the pyrazole experiment (30% TRR) was subjected to acid hydrolysis (0.5 M HCl, 6 hrs, 100 °C) and the resulting hydrolysate was partitioned into diethyl ether/hexane 4:1 v/v. The organo soluble fraction (22% TRR) was analysed by 2D-TLC. The aqueous phase (0.0% TRR) was not analysed further.

All fractions were analysed by 2D-TLC and/or 1D-TLC with phosphor image analysis. Identification was conducted by co-chromatography with authentic reference standards of parent, SYN546206, SYN546039, SYN546040, SYN546041, SYN546042, NOA449410, SYN545720, SYN508272, SYN545720-aspartic acid conjugate, SYN545720-monosaccharide conjugate using two dissimilar solvent systems. Results are shown in Table 12.

In soya bean forage and hay, high levels of identification of the extracted residues were achieved (≥ 83% TRR). A significant proportion of the conjugates of SYN546039 in hay was characterised as glycoside (minor proportion) and malonyl glycoside (major proportion) conjugates.

In soya bean seeds, high levels of identification of the extracted residues were achieved for the pyrazole experiment (67% TRR) but lower levels of identification were found for the phenyl experiment (41% TRR) where absolute residue levels were lowest. Significant proportions of the conjugated residue of SYN545720 in seeds were identified as the aspartic acid conjugate (12% TRR) and monosaccharide conjugate (11% TRR).

Combined extracts were analysed by TLC within 3 months of sample harvest and were analysed again following 29-31 months frozen storage to demonstrate storage stability. Comparison of the initial and final radio-component profiles obtained showed no significant change in the profiles during the interim period of storage.

Table 11 Total Radioactive Residues and extractability in soya bean commodities treated with [^{14}C]benzovindiflupyr

Radiolabel	Soya bean commodity	Conditions	Extracted Radioactivity		Post-extracted solids		TRR [a]
			%TRR	mg/kg eq	%TRR	mg/kg eq	mg/kg eq
[Phenyl-^{14}C]-BVFP	forage	1× 0.121 kg ai/ha; DAFT = 11	99	3.3	1.0	0.033	3.4
	hay	2× (0.121–0.123) kg ai/ha; DALT = 13	98	14	2.3	0.32	14
	seeds	2× (0.121–0.123) kg ai/ha; DALT = 30	89	0.026	11	0.003	0.029
[Pyrazole-^{14}C]-BVFP	forage	1× 0.126 kg ai/ha; DAFT 11	99	4.0	0.9	0.037	4.1
	hay	2× (0.120–0.126) kg ai/ha; DALT 13	98	12	2.4	0.31	13
	seeds	2× (0.120–0.126) kg ai/ha; DALT = 30	98	0.099	2.1	0.002	0.10

[a] TRR derived from the summation of the radioactivity present in the solvent extracts and debris
DALT = Days after last treatment
DAFT = Days after first treatment

Table 12 Identification and characterisation of residues in soya bean commodities treated with [^{14}C]benzovindiflupyr

	Forage	Forage	Hay	Hay	Seeds	Seeds
	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C
TRR, mg/kg eq	3.4	4.1	14	13	0.029	0.10
Component	% TRR	% TRR	% TRR	% TRR	% TRR	% TRR
parent	83	85	72	67	31	15
SYN546206	1.4	1.6	1.5	1.6	4.6	2.0
(N-demethyl-BVFP)			(0.1)	(0.2)	(ND)	(2.0)
SYN546039	9.9	9.2	11	12	1.2	0.4
(BVFP-OH)	(9.0)	(8.5)	(10)	(12)	(1.0)	(0.4)
SYN546040	0.1	0.2	0.2	0.3	0.8	0.1
(BVFP-OH)	(< 0.1)	(0.1)	(0.2)	(0.3)	(0.4	(0.1)
SYN546041	0.4	0.5	0.6	0.7	3.2	1.1
(N-demethyl-BVFP-OH)	(0.3)	(0.4)	(0.5)	(0.6)	(3.2)	(1.1)
SYN546042	ND	ND	0.1	0.2	0.2	ND
(N-demethyl-BVFP-OH)			(0.1)	(0.2)	(0.2)	
SYN508272	NA	0.1	NA	0.1	NA	ND
		(< 0.1)		(0.1)		
NOA449410	NA	0.3	NA	0.3	NA	1.2
		(0.1)		(0.1)		(1.2)
SYN545720	NA	0.3	NA	0.3	NA	47

	Forage	Forage	Hay	Hay	Seeds	Seeds
	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C	Phenyl-^{14}C	Pyrazole-^{14}C
		(< 0.1)		(ND)		(30)
Unassigned [a]	ND	1.4	1.2	1.5	23	11
Baseline [b]	1.8	1.1	2.7	2.0	5.0	6.6
Remainder [c]	4.3	1.2	4.3	6.7	5.6	2.6
Other fractions [d]	1.6	1.8	1.0	1.7	8.9	4.3
PES [e]	1.0	0.9	2.3	2.5	11.1	2.1
Total	104	103	97	97	95	93

Not detected, i.e. < 1.5 times the background radioactivity in samples with no radioactivity (< 0.00013 mg/kg eq)
NA = Not applicable to this radiolabel
( ) = The main figures are the sum of both the free and conjugated forms, the figures in parentheses indicate the proportion of the residue that was found in the conjugated form
[a] Unassigned radio-components which chromatographed away from the origin in 2D-TLC (normal phase)
Forage phenyl label: no discrete components;
Forage pyrazole label: comprising at least 5 discrete components, no single one of which > 0.8% TRR (> 0.03 mg/kg eq);
Hay phenyl label: comprising at least 4 discrete components, no single one > 0.6% TRR (> 0.084 mg/kg eq);
Hay pyrazole label: comprising at least 6 discrete components, no single one > 0.6% TRR (> 0.075 mg/kg eq);
Seeds phenyl label: comprising at least 7 discrete components, no single one > 14.1% TRR (> 0.0041 mg/kg eq);
Seeds pyrazole label: comprising at least 5 discrete components, no single one > 2.5% TRR (> 0.0025 mg/kg eq)
[b] Polar material on origin of the radiochromatogram using 2D-TLC (normal phase)
[c] Diffuse areas of radioactivity within the chromatogram which cannot be assigned to discrete radioactive components.
[d] Extracted residues, produced during processing that were too low for analysis. No single fraction comprised > 1.4% TRR (> 0.06 mg/kg eq)in forage or > 1.2% TRR (> 0.151 mg/kg eq)in hay or > 3.5% TRR (> 0.0004 mg/kg eq)in seeds in either radiolabelled experiment.
[e] Post extraction solids; radioactivity remaining in the debris after extraction.

Overview of the metabolic pathway in plants

Metabolism studies conducted with crops representative of cereal/grass (spring wheat), fruits (tomato) and pulses/oilseeds (soya bean) and based on foliar treatment show that the metabolic pathways in the three crop groups are similar. The nature of the terminal residue depends on the relative importance of the various routes of metabolism and the nature and extent of the conjugation reactions. The level of metabolism of benzovindiflupyr was most extensive in pulses/oilseeds (soya bean). Metabolism observed arose via:

- N-demethylation of the pyrazole ring to form SYN546206
- mono hydroxylation on the alicyclic ring to form the SYN546039 and SYN546040 isomers
- subsequent N-demethylation of the pyrazole ring and mono hydroxylation of the alicyclic ring or subsequent mono hydroxylation of the alicyclic ring and N-demethylation of the pyrazole ring to form SYN546041 and SYN546042 isomers
- cleavage between the pyrazole and phenyl rings of parent or mono hydroxylated metabolites SYN546039/SYN546040 to form SYN508272 and subsequent hydrolysis of the amide functionality of SYN508272 to form NOA 449410 and subsequent demethylation to form SYN545720
- cleavage between the pyrazole and phenyl rings of the N-demethylated metabolites SYN546206 or SYN546041/SYN546042 to form SYN545720
- conjugation of N-demethylated metabolite SYN546206
- conjugation of mono hydroxylated metabolites SYN546039/SYN546040, predominantly as their glycoside and malonyl glycoside conjugates
- conjugation of mono hydroxylated and N-demethylated metabolites SYN546041/SYN546042
- conjugation of hydroxylated cleavage metabolites NOA449410 and SYN545720, the latter metabolite found predominantly as its aspartic acid and sugar conjugates.

In tomato fruits, wheat grains, wheat forage/hay/straw, soya bean forage/hay the major compound in the residue is parent. In soya bean seeds the major compound is the cleavage compound SYN545720 with significant amounts of parent present. The major pathway in soya bean seeds is cleavage between the pyrazole and phenyl rings combined with N-demethylation of the pyrazole moiety (either before or after cleavage) to form SYN545720 followed by conjugation of SYN545720 to aspartic acid and sugar. The overall metabolic pathways in rotated crops are similar to those observed in primary crops.

SYN545192

SYN546039
conjugates*

SYN546040
conjugates*

SYN546206
conjugates*

SYN508272
Conjugates*

SYN546041
conjugates*

SYN546042
conjugates*

NOA449410
Conjugates*

SYN545720
Conjugates*

---> Dashed arrows indicate alternative pathway.

* Metabolites found in both the free and conjugated forms.

Figure 3 Metabolic pathway of benzovindiflupyr in plant commodities

Environmental fate in soil

The Meeting received information on aerobic/anaerobic degradation in soil, field dissipation studies, photolysis in soil and confined/field rotational crop studies. In conformance with the JMPR manual 2009 for foliar treatments, only confined and field rotational crops studies were considered for the current evaluation. In addition, The Meeting considered photolysis studies in/on soil relevant for the present evaluation. The fate and behaviour of benzovindiflupyr in the environment was investigated using the [^{14}C]pyrazole and [^{14}C]phenyl labelled compound (see Figure 1).

Photolysis studies in/on soil

The photolysis of [^{14}C]phenyl labelled and [^{14}C]pyrazole labelled benzovindiflupyr was investigated on both dry and moist soil surfaces [McCorquodale and White, 2012, SYN545192_10186]. Soil characteristics are indicated in Table 13. At day 0 moist soil was adjusted to pF2 moisture tension. Dry soil tests used air dried soil. In each case, benzovindiflupyr was applied, at rates equivalent to 0.25 kg ai/ha, to thin layers (ca 2 mm) of loamy soil in individual photolysis vessels. The treated soils were irradiated using light from a xenon arc lamp. The emitted light was filtered to give a spectral distribution close to that of natural sunlight at a mean light intensity of 48–58 W/m^2. The samples were maintained at 20 ± 2 °C and were continuously irradiated for 30 days, the equivalent of 56–69 days summer sunlight at latitudes 30–50 °N. CO_2 and volatile organics were trapped with 2 mol (M) NaOH. In each test, duplicate irradiated samples were taken for analysis at 0, 5, 10, 15, 20, 25 and 30 days after treatment (DAT) and traps for volatiles were replaced with fresh solution. Dry soils received no moisture maintenance. Moist soils were checked daily and moisture adjusted by addition of water if required to maintain soil close to the target weight at pF2 moisture tension. Dark control samples were also prepared and maintained at 20 °C and analysed at 0 and 30 DAT.

Extraction procedures were started on the day of sampling. Each soil sample was extracted with 0.01 M $CaCl_2$ and twice with acetone. Extracts, traps and remaining post-extracted soil residue were analysed by (combustion) LSC. Extracts were analysed by HPLC. Selected extracts were analysed by 1D-TLC to confirm both the co-chromatography with reference standards and the quantification of the main components. HPLC-MS-MS was also used to provide qualitative confirmation of the identification of parent, SYN546039, SYN508272 and NOA449410.

A mass balance for each sample was determined by summation of the radioactivity recovered in the soil extracts, the total $^{14}CO_2$ evolved and the post-extracted soil residues. The mean mass balance from the irradiated soils was 99% and from the dark controls was 99%. Benzovindiflupyr degraded slowly under irradiated conditions with no significant degradation in the dark controls.

Under dry, irradiated conditions, the amount of parent declined over the irradiation period to levels of 88.4% and 74.4% TAR by 30 DAT in soil treated with the [^{14}C]phenyl and [^{14}C]pyrazole labelled parent compound, respectively. In moist, irradiated soil, the amount of parent declined over the course of the irradiation period to levels of 89% and 88% TAR by 30 DAT in the phenyl and pyrazole labelled experiments, respectively. The DT_{50} values were calculated using non-linear regression and single first-order kinetics (SFO) and are 144 days in dry soil and 244 days in moist soil. Corrected for latitude, the DT_{50} is 298 and 501 for dry and moist soil, respectively, at 30–50 °N (OECD).

In general, a similar degradation pattern was observed in both dry and moist irradiated soil. Photodegradation of benzovindiflupyr resulted in the cleavage of the carboxamide link to produce the pyrazole amide, SYN508272, and the pyrazole acid, NOA449410. Levels of SYN508272 and NOA449410 were higher in dry soil, with amounts reaching maximum mean values of 6.5% and 2.8% TAR, respectively. In moist soil, the maximum mean levels of SYN508272 and NOA449410 were 1.1% and 2.3% TAR, respectively. The demethylated pyrazole acid, SYN545720, was identified in soil extracts from dry soil only, and reached a maximum level of 0.6% TAR. The hydroxylated degradate, SYN546039, was detected at a mean maximum level of 3.7% TAR, but only in the moist irradiated soil treated with the phenyl label. Minor, unidentified components accounted for a combined mean maximum value of 3.5% TAR across all the samples. Carbon dioxide was a minor

(≤ 2.5% TAR) product of photolysis. Unextracted residues increased during the irradiation but did not exceed > 9.0% TAR in any of the samples.

Table 13 Physico-chemical characteristics of the test soil

Soil origin	Gartenacker Switzerland
Texture (USDA) [a]	loam
Particle size distribution (%) —Sand (2000–50 um) —Silt (50–2 um) —Clay (< 2 um)	 45 43 12
Organic carbon (%)	2.0
Organic matter [b] (%)	3.5
CEC (meq/100 g)	10.8
pH (in Water)	7.3
pH (in 0.01 M $CaCl_2$)	7.0
Moisture Holding Capacity at 0.33 bar [c] (% w/w)	28.8
Moisture Holding Capacity at pF2 (% w/w); study conditions	39.0
Maximum Water Holding Capacity (% w/w)	ns
Microbial biomass (mg carbon/100 g dry soil)	ns

[a] Classification according to United States Department of Agriculture
[b] Calculated from organic carbon content x 1.724
[c] Equivalent to pF = 2.5

Confined rotational crop studies

A confined rotational crop study was designed to provide information on the uptake and metabolism of benzovindiflupyr in rotational crops [Roohi and Adams, 2012, SYN545192_10246]. Two radiolabelled forms were used in the study: [phenyl-^{14}C]-benzovindiflupyr and [pyrazole-^{14}C]-benzovindiflupyr. For each radiolabelled form, a single spray application onto the surface of bare soil was performed employing an EC formulation (A170560) at a spray volume of 300 L/ha. The soil characteristics were: USDA sandy loam, pH (H_2O) 5.8, 3.2% om, CEC = 9.4 meq/100 g. The actual application rates achieved were 0.54 kg ai/ha and 0.53 kg ai/ha for the pyrazole and phenyl experiments respectively. After application, the soil was kept in containers under greenhouse conditions and at intervals of 30, 90 and 300 days after application three representative rotational crops were sown into the treated soil. The crops selected were lettuce (variety Tonova), spring wheat (variety Tybalt) and turnip (variety Tokyo Cross), respectively. For each plant back interval and for each radiolabelled experiment the following crop samples were taken: immature lettuce (BBCH 41–43), mature lettuce (BBCH 49), mature turnip roots and leaves (BBCH 49), wheat forage (BBCH 39), wheat hay (BBCH 39–69, sampled 59 days after planting); wheat straw and grain (BBCH 89). Soil samples (0–20 cm) were taken at the time of each sowing (i.e. DAT = 30, 90, 300). Lettuce samples (0.07–1.1 kg) were harvested by cutting just above the soil surface using a knife and any dead leaves and loose soil were removed. Turnips were harvested by hand and any loose soil was removed. The leaves (1.6–2.9 kg) and roots (0.55–1.1 kg) were separated using a knife. If necessary, large roots were cut into smaller pieces. Wheat forage (0.30–1.3 kg) was harvested by cutting the plants with scissors 1 cm above the soil surface. The forage was cut into pieces of 5 cm length. Wheat hay (0.13–0.73 kg) was harvested as wheat forage and then left to dry for 6–10 days in a glasshouse. Mature wheat was harvested as wheat forage and intact ears of grain were removed from the straw using scissors. Grain (0.34–0.70 kg) and chaff were separated and the chaff was added to the straw samples (0.34–0.55 kg). The samples were stored at –18 ºC for 6–45 months.

Soil samples were not analysed (confirmed, Syngenta, 2014b). Plant commodities were homogenised and the total radioactive residue (TRR) determined by combustion LSC. In the pyrazole experiment, with the exception of the 30 day mature lettuce and the 90 and 300 day grain, all samples contained radioactive residues > 0.01 mg/kg eq (see Tables 14–21). In the phenyl experiment, with the exception of the lettuce and grain, all samples contained radioactive residues > 0.01 mg/kg eq (see

Tables 14–21). Maximum levels were 0.030 mg/kg eq in lettuce leaves, 0.031 mg/kg eq in turnip roots, 0.026 mg/kg eq in turnip leaves, 0.11 mg/kg eq in wheat forage, 0.34 mg/kg eq in wheat hay, 0.77 mg/kg eq in wheat straw and 0.014 mg/kg eq in wheat grain.

The samples with radioactive residues > 0.01 mg/kg eq were sequentially extracted with solvents of increasing polarity: acetonitrile (1×), acetonitrile/water (4:1, v/v) (2–3×), acetonitrile/water (3:7, v/v) (1×), water (1×), acetonitrile (1×). For grain this sequence was extended with an additional sequence of acetonitrile/water (3:7, v/v) (1×), water (1×), acetonitrile (1×). Radioactivity in extracts and solids was determined by (combustion) LSC. The overall levels of extractability were high with > 80% TRR extracted in all commodities.

For each commodity, extracts containing significant levels of radioactivity were combined, concentrated and re-suspended in water (pH 2). This aqueous concentrate was then partitioned with hexane/diethyl ether (1:3 v/v) in the ratio 3:1 v/v with the aqueous fraction. The resulting organic phase was analysed by 1D- or 2D-TLC for quantification and identification of the components. Identified components in these organic partition fractions were thereby shown to be present in the free form (unconjugated). When the residue in the aqueous fraction was considered significant, a sub-sample was subjected to hydrolysis by rumen technology (bovine rumen fluid, pH 7, 24 hrs at 39 ºC, under nitrogen). The resulting hydrolysates were analysed by 2D-TLC and 1D-TLC methods. Identified components were thereby shown to have originally been present in the conjugated form in the initial sample extracts. Where significant radioactivity remained in the post-extraction solids, selected samples were subjected to additional extraction procedures employing 0.1 M HCl (at 37 ºC for 6 hrs) or 0.5 M HCl (at 90 ºC for 6 hrs) or sodium dodecyl sulphate (SDS), (at 90 ºC for 2 hours) in parallel experiments. Identification was by comparison with authentic reference standards of parent, SYN546206, SYN546039, SYN546040, SYN546041, SYN546042, NOA449410, SYN545720 and SYN508722 using two dissimilar solvent systems. Analytical standards for parent, SYN546039, SYN546042, NOA449410, SYN545720 and SYN508722 were confirmed to be stable during hydrolysis by rumen technology.

Results are shown in Tables 14–21. Overall, residue identification accountability was > 80%. In samples where the TRR was low (< 0.03 mg/kg eq) the level of identification was lower: averaging 70% for lettuce, 45% for turnip leaves. In wheat commodities accountability was lower: identification averaged 66% in wheat forage, 61% in wheat hay and 50% in wheat straw. Wheat grain had very low residues (< 0.01–0.014 mg/kg eq) and most extracts were not amenable to chromatographic examination.

The pattern of metabolites found in the samples from the pyrazole experiment was different from that found in the samples from the phenyl experiment because of the presence of metabolites that retained only the pyrazole ring (i.e. NOA449410, SYN545720 and SYN508272).

Storage stability

Chromatographic profiles of all initial crop extracts were obtained within 190 days of harvest. The original extracts were stored at ≤ –18 °C and were then re-analysed at 155 weeks after harvest (i.e. at the end of the analytical phase of the study). Selected samples were immature lettuce (analysed 94 and 1079 days after harvest), turnip leaves (analysed 42 and 1079 days after harvest), turnip leaves (analysed 90 and 1082 days after harvest), turnip roots (analysed 90 and 1082 days after harvest) and wheat straw (analysed 48 and 1016 days after harvest). Comparison of the initial and final radio-component profiles obtained from the selected samples showed that no significant change in the profiles had occurred during the interim period of storage.

Table 14 Residues in immature lettuce grown in soil Treated with [^{14}C]benzovindiflupyr

immature lettuce	30 day PBI		90 day PBI		300 day PBI	
harvest, DAT	DAT 74	DAT 75	DAT 148	DAT 149	DAT 350	DAT 351
harvest, DAS	DAS 44	DAS 45	DAS 58	DAS 59	DAS 50	DAS 51
harvest, BBCH	41–43	41–43	41–43	41–43	41–43	41–43
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg eq [a]	N/A	0.012	N/A	0.023	N/A	0.019

immature lettuce	30 day PBI		90 day PBI		300 day PBI	
harvest, DAT	DAT 74	DAT 75	DAT 148	DAT 149	DAT 350	DAT 351
harvest, DAS	DAS 44	DAS 45	DAS 58	DAS 59	DAS 50	DAS 51
harvest, BBCH	41–43	41–43	41–43	41–43	41–43	41–43
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (direct) mg/kg eq [b]	0.005	0.013	0.009	0.023	0.003	0.018
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	N/A	38 (N/A)	N/A	13 (N/D)	N/A	6.5 (N/D)
SYN546206 (N-demethyl-BVFP)	N/A	4.9 (N/A)	N/A	0.5 (N/D)	N/A	N/D (N/D)
SYN546039 (BVFP-OH)	N/A	4.3 (N/A)	N/A	2.6 (2.1)	N/A	3.6 (N/D)
SYN546040 (BVFP-OH)	N/A	N/D (N/A)	N/A	5.9 (N/D)	N/A	N/D (N/D)
SYN546041 (N-demethyl-BVFP-OH)	N/A	N/D (N/A)	N/A	N/D (N/D)	N/A	N/D (N/D)
SYN546042 (N-demethyl-BVFP-OH)	N/A	N/D (N/A)	N/A	N/D (N/D)	N/A	N/D (N/D)
SYN508272	N/A	N/D (N/A)	N/A	N/D (N/D)	N/A	N/D (N/D)
NOA449410	N/A	11 (N/A)	N/A	15 (10)	N/A	39 (32)
SYN545720	N/A	N/D (N/A)	N/A	38 (16)	N/A	34 (N/D)
Unassigned [c]	N/A	0.7	N/A	4.2	N/A	1.7
Baseline [d]	N/A	0.9	N/A	14	N/A	13
Remainder [e]	N/A	1.7	N/A	0.2	N/A	3.5
Other Fractions [f]	N/A	23	N/A	N/A	N/A	2.8
PES [g]	N/A	5.0	N/A	9.8	N/A	5.7
Total	N/A	89	N/A	103	N/A	109

The figures in Tables 14–21 are the total radioactive residue for each metabolite reported expressed as mg/kg parent equivalents. The main figures are the sum of both the free and conjugated forms. The figures in parentheses indicate the portion of the radioactive residue in the conjugated form(s).
N/A = Not applicable (Sample not analysed or metabolite not possible in phenyl label).
N/D = Not detected
DAT = Days after soil treatment
DAS = Days after sowing of the rotational crops
[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 15 Residues in mature lettuce grown in soil treated with [^{14}C]benzovindiflupyr

Mature lettuce	30 day PBI		90 day PBI		300 day PBI	
Harvest, DAT	DAT 88	DAT 89	DAT 158	DAT 159	DAT 362	DAT 363
Harvest, DAS	DAS 58	DAS 59	DAS 68	DAS 69	DAS 62	DAS 63
Harvest, BBCH	49	49	49	49	49	49
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	N/A	N/A	N/A	0.030	N/A	0.020
TRR (direct) mg/kg [b]	0.004	0.008	0.006	0.027	0.005	0.018
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	N/A	N/A	N/A	24 (N/D)	N/A	13 (N/D)
SYN546206 (N-demethyl-BVFP)	N/A	N/A	N/A	0.2 (N/D)	N/A	0.2 (N/D)

Mature lettuce	30 day PBI		90 day PBI		300 day PBI	
Harvest, DAT	DAT 88	DAT 89	DAT 158	DAT 159	DAT 362	DAT 363
Harvest, DAS	DAS 58	DAS 59	DAS 68	DAS 69	DAS 62	DAS 63
Harvest, BBCH	49	49	49	49	49	49
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
SYN546039 (BVFP-OH)	N/A	N/A	N/A	1.9 (1.5)	N/A	0.5 (N/D)
SYN546040 (BVFP-OH)	N/A	N/A	N/A	8.0 (2.9)	N/A	6.8 (N/D)
SYN546041 (N-demethyl-BVFP-OH)	N/A	N/A	N/A	N/D (N/D)	N/A	N/D N/D)
SYN546042 (N-demethyl-BVFP-OH)	N/A	N/A	N/A	N/D (N/D)	N/A	N/D (N/D)
SYN508272	N/A	N/A	N/A	N/D (N/D)	N/A	N/D N/D)
NOA449410	N/A	N/A	N/A	9.5 (7.2)	N/A	21 (19)
SYN545720	N/A	N/A	N/A	24 (10.9)	N/A	25 (N/D)
Unassigned [c]	N/A	N/A	N/A	7.4	N/A	10.6
Baseline [d]	N/A	N/A	N/A	9.7	N/A	14.4
Remainder [e]	N/A	N/A	N/A	ND	N/A	ND
Other fractions [f]	N/A	N/A	N/A	N/A	N/A	0.5
PES [g]	N/A	N/A	N/A	12	N/A	9.3
Total	N/A	N/A	N/A	97	N/A	101

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 16 Residues in turnip leaves grown in soil treated with [^{14}C]benzovindiflupyr

Turnip leaves	30 day PBI		90 day PBI		300 day PBI	
harvest, DAT	DAT 74	DAT 75	DAT 140	DAT 141	DAT 351	DAT 352
Harvest, DAS	DAS 44	DAS 45	DAS 50	DAS 51	DAS 51	DAS 52
Harvest, BBCH	49	49	49	49	49	49
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	0.010	0.021	0.014	0.027	0.008	0.022
TRR (direct) mg/kg [b]	0.012	0.020	0.015	0.031	0.012	0.024
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	37 (N/A)	26 (N/A)	17 (N/A)	26 (N/A)	22 (N/D)	10 (N/D)
SYN546206 (N-demethyl-BVFP)	2.8 (N/A)	2.4 (N/A)	1.1 (N/A)	2.1 (N/A)	9.2 (6.7)	1.2 (N/D)
SYN546039 (BVFP-OH)	2.0 (N/A)	2.4 (N/A)	1.3 (N/A)	1.5 (N/A)	3.4 (N/D)	1.0 (N/D)
SYN546040 (BVFP-OH)	2.5 (N/A)	3.0 (N/A)	2.5 (N/A)	1.9 (N/A)	2.7 (N/D)	0.9 (N/D)
SYN546041 (N-demethyl-BVFP-OH)	0.7 (N/A)	1.2 (N/A)	0.3 (N/A)	0.6 (N/A)	1.0 (N/D)	0.3 (N/D)

Turnip leaves	30 day PBI		90 day PBI		300 day PBI	
SYN546042 (N-demethyl-BVFP-OH)	1.9 (N/A)	N/D (N/A)	1.2 (N/A)	1.5 (N/A)	1.9 (N/D)	0.7 (N/D)
SYN508272	N/A	1.4 (N/A)	N/A	4.2 (N/A)	N/A	8.5 (7.4)
NOA449410	N/A	7.3 (N/A)	N/A	3.9 (N/A)	N/A	14 (8.1)
SYN545720	N/A	12 (N/A)	N/A	12 (N/A)	N/A	15 (N/D)
Unassigned [c]	2.1	0.5	1.7	4.1	1.6	14
Baseline [d]	1.4	1.2	0.9	1.2	12	11
Remainder [e]	ND	ND	0.9	1.5	19	3.5
Other fractions [f]	30	40	34	28	38	N/A
PES [g]	4.9	2.8	7.9	4.9	5.0	3.0
Total	85	99	67	93	116	82

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 17 Residues in turnip roots grown in soil treated with [^{14}C]benzovindiflupyr

Turnip roots	30 day PBI		90 day PBI		300 day PBI	
harvest, DAT	DAT 74	DAT 75	DAT 140	DAT 141	DAT 351	DAT 352
harvest, DAS	DAS 44	DAS 45	DAS 50	DAS 51	DAS 51	DAS 52
harvest, BBCH	49	49	49	49	49	49
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	0.019	0.028	0.026	0.031	0.011	0.014
TRR (direct) mg/kg [b]	0.020	0.027	0.022	0.028	0.011	0.013
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	90 (N/A)	81 (N/A)	69 (N/A)	72 (N/A)	71 (N/A)	64 (N/A)
SYN546206 (N-demethyl-BVFP)	5.0 (N/A)	5.8 N/A	5.1 (N/A)	5.6 (N/A)	5.8 (N/A)	8.0 (N/A)
SYN546039 (BVFP-OH)	(N/D) (N/A)	1.8 (N/A)	1.3 (N/A)	1.6 (N/A)	1.8 (N/A)	1.4 (N/A)
SYN546040 (BVFP-OH)	1.4 (N/A)	1.5 (N/A)	2.1 (N/A)	1.1 (N/A)	1.2 (N/A)	1.0 (N/A)
SYN546041 (N-demethyl-BVFP-OH)	(N/D) (N/A)	0.4 (N/A)	0.8 (N/A)	0.3 (N/A)	0.4 (N/A)	0.4 (N/A)
SYN546042 (N-demethyl-BVFP-OH)	(N/D) (N/A)	0.5 (N/A)	(N/D) (N/A)	0.5 (N/A)	0.2 (N/A)	0.6 (N/A)
SYN508272	N/A	N/D (N/A)	N/A	N/D (N/A)	N/A	0.1 (N/A)
NOA449410	N/A	3.5 (N/A)	N/A	1.9 (N/A)	N/A	6.1 (N/A)
SYN545720	N/A	0.4 (N/A)	N/A	0.4 (N/A)	N/A	0.8 (N/A)
Unassigned [c]	1.2	< 0.1	1.2	< 0.1	< 0.1	0.4
Baseline [d]	2.5	0.9	1.8	1.5	1.4	1.2
Remainder [e]	ND	ND	0.1	ND	ND	ND
Other fractions [f]	1.0	4.7	4.8	5.3	3.5	6.4
PES [g]	2.2	1.5	2.5	3.1	3.8	2.7
Total	103	102	89	93	89	93

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as

100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 18 Residues in wheat forage grown in soil treated with [^{14}C]benzovindiflupyr

wheat forage	30 day PBI		90 day PBI		300 day PBI	
harvest, DAT	DAT 74	DAT 75	DAT 138	DAT 139	DAT 348	DAT 349
harvest, DAS	DAS 44	DAS 45	DAS 48	DAS 49	DAS 48	DAS 49
harvest, BBCH	39	39	39	39	39	39
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	0.044	0.099	0.067	0.11	0.064	0.090
TRR (direct) mg/kg [b]	0.040	0.098	0.067	0.097	0.051	0.090
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	31 (N/D)	26 (N/D)	35 (N/D)	19 (N/D)	29 (N/D)	17 (N/D)
SYN546206 (N-demethyl-BVFP)	13 (N/D)	10 (N/D)	13 (N/D)	11 (2.5)	15 (N/D)	8.3 (N/D)
SYN546039 (BVFP-OH)	19 (15.8)	7.4 (5.1)	8.0 (4.7)	8.7 (6.9)	20 (16)	10 (8.5)
SYN546040 (BVFP-OH)	0.8 (N/D)	2.1 (1.4)	2.3 (N/D)	1.0 (N/D)	3.1 (N/D)	1.0 (N/D)
SYN546041 (N-demethyl-BVFP-OH)	6.1 (5.7)	1.2 (0.8)	0.6 (N/D)	1.5 (1.5)	2.1 (N/D)	N/D (N/D)
SYN546042 (N-demethyl-BVFP-OH)	N/D (N/D)	0.4 (N/D)	1.0 (N/D)	2.3 (1.8)	1.7 (1.0)	N/D (N/D)
SYN508272	N/A	5.4 (4.6)	N/A	2.4 (2.0)	N/A	5.7 (3.9)
NOA449410	N/A	6.1 (N/D)	N/A	12 (9.9)	N/A	14 (9.5)
SYN545720	N/A	1.3 (N/D)	N/A	12 (10)	N/A	12 (10)
Unassigned [c]	2.3	17	6.2	3.6	9.2	4.8
Baseline [d]	8.7	3.4	25	19	9.0	27
Remainder [e]	7.3	ND	0.7	2.5	0.3	ND
Other fractions [f]	4.4	1.3	N/A	4.2	N/A	N/A
PES [g]	4.4	5.9	6.8	5.3	5.5	5.5
Total	96	88	99	104	95	106

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 19 Residues in wheat hay grown in soil treated with [^{14}C]benzovindiflupyr

wheat hay	30 day PBI		90 day PBI		300 day PBI	
Harvest	DAT 89	DAT 89	DAT 149	DAT 149	DAT 359	DAT 359
Harvest, DAS	DAS 59	DAS 59	DAS 59	DAS 59	DAS 59	DAS 59
Harvest, BBCH	41-69	39-65	41-69	39-65	41-69	39-65
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	0.21	0.34	0.26	0.34	0.20	0.29

wheat hay	30 day PBI		90 day PBI		300 day PBI	
Harvest	DAT 89	DAT 89	DAT 149	DAT 149	DAT 359	DAT 359
Harvest, DAS	DAS 59	DAS 59	DAS 59	DAS 59	DAS 59	DAS 59
Harvest, BBCH	41-69	39-65	41-69	39-65	41-69	39-65
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (direct) mg/kg [b]	0.18	0.35	0.22	0.33	0.20	0.29
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	33 (N/D)	24 (N/D)	32 (N/D)	25 (N/D)	23 (N/D)	15 (N/D)
SYN546206 (N-demethyl-BVFP)	18 (N/D)	9.3 (N/D)	15 (N/D)	14 (2.6)	12 (N/D)	7.6 (N/D)
SYN546039 (BVFP-OH)	14 (11)	7.0 (5.1)	9.8 (7.3)	1.9 (N/D)	16 (12)	9.4 (7.0)
SYN546040 (BVFP-OH)	0.8 (N/D)	1.2 (0.7)	1.2 (N/D)	0.9 (N/D)	3.8 (1.8)	1.0 (N/D)
SYN546041 (N-demethyl-BVFP-OH)	4.6 (3.7)	1.6 (1.3)	0.8 (N/D)	0.5 (N/D)	2.2 (1.4)	0.6 (N/D)
SYN546042 (N-demethyl-BVFP-OH)	0.5 (N/D)	0.8 (0.5)	0.8 (N/D)	0.4 (N/D)	2.1 (1.3)	0.6 (N/D)
SYN508272	N/A	3.9 (3.3)	N/A	2.8 (2.3)	N/A	3.4 (2.9)
NOA449410	N/A	4.3 (3.1)	N/A	5.6 (4.9)	N/A	8.8 (6.7)
SYN545720	N/A	2.0 (1.6)	N/A	5.8 (5.4)	N/A	11 (9.8)
Unassigned [c]	4.7	14	3.8	12	7.7	14
Baseline [d]	8.6	13	18	30	7.1	14
Remainder [e]	ND	ND	ND	ND	1.5	1.2
Other fractions [f]	1.2	4.2	4.4	1.1	3.2	0.7
PES [g]	6.9	0.2	9.0	10	11	13
Total	92	86	95	111	90	100

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 20 Residues in wheat straw grown in soil treated with [^{14}C]benzovindiflupyr

wheat straw	30 day PBI		90 day PBI		300 day PBI	
Harvest	DAT 140	DAT 141	DAT 200	DAT 201	DAT 420	DAT 421
Harvest, DAS	DAS 110	DAS 111	DAS 110	DAS 111	DAS 120	DAS 121
Harvest, BBCH	89	89	89	89	89	89
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	0.71	0.77	0.34	0.33	0.20	0.23
TRR (direct) mg/kg [b]	0.71	0.77	0.26	0.31	0.23	0.22
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Parent	15 (N/D)	14 (N/D)	16 (N/D)	18 (N/D)	14 (N/D)	12 (N/D)
SYN546206 (N-demethyl-BVFP)	18 (N/D)	13 (1.4)	14 (N/D)	15.1 (1.3)	14 (1.1)	13 (N/D)
SYN546039 (BVFP-OH)	7.1 (4.2)	9.0 (6.2)	5.2 (2.2)	5.8 (3.9)	9.6 (7.4)	9.6 (8.0)
SYN546040 (BVFP-OH)	2.0 (1.2)	2.7 (1.8)	1.5 (N/D)	1.4 (0.8)	3.7 (1.7)	2.5 (1.6)
SYN546041 (N-demethyl-	3.5 (2.5)	3.9 (3.1)	1.0 (N/D)	2.1 (1.5)	2.2 (1.4)	1.9 (1.2)

wheat straw	30 day PBI		90 day PBI		300 day PBI	
Harvest	DAT 140	DAT 141	DAT 200	DAT 201	DAT 420	DAT 421
Harvest, DAS	DAS 110	DAS 111	DAS 110	DAS 111	DAS 120	DAS 121
Harvest, BBCH	89	89	89	89	89	89
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
BVFP-OH)						
SYN546042 (N-demethyl-BVFP-OH)	1.7 (1.00)	2.0 (1.5)	0.9 (N/D)	2.6 (2.0)	2.1 (1.4)	1.3 (0.8)
SYN508272	N/A	1.1 (0.8)	N/A	2.1 (1.9)	N/A	1.3 (1.0)
NOA449410	N/A	4.5 (3.6)	N/A	2.5 (2.2)	N/A	1.7 (1.0)
SYN545720	N/A	2.3 (2.0)	N/A	0.3 (N/D)	N/A	1.4 (1.1)
Unassigned [c]	12	20	15	9.3	17	14
Baseline [d]	15	17	16	12	12	13
Remainder [e]	5.5	1.9	2.3	0.9	ND	0.2
Other fractions [f]	1.5	1.3	3.0	1.6	4.1	5.3
PES [g]	9.5	9.5	12	12	13	19
Total	90	102	87	85	92	96

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Unassigned radio-components which chromatographed away from the origin using 2D-TLC SSD, comprising 1–41 discrete components (depending on matrix), none greater than 5.0% TRR (each < 0.01 mg/kg eq)
[d] Polar material remaining on the origin on the TLC plate using 2D-TLC SSD
[e] The remainder consists of areas of the chromatogram which cannot be assigned to discrete radioactive components.
[f] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[g] Radioactivity remaining in the solids after the initial solvent extractions.

Table 21 Residues in wheat grain grown in soil treated with [^{14}C]benzovindiflupyr

	30 day PBI		90 day PBI		300 day PBI	
Harvest	DAT 140	DAT 141	DAT 200	DAT 201	DAT 420	DAT 421
Harvest, DAS	DAS 110	DAS 111	DAS 110	DAS 111	DAS 120	DAS 121
Harvest, BBCH	89	89	89	89	89	89
	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C	phenyl-^{14}C	pyrazole-^{14}C
TRR (sum) mg/kg [a]	N/A	0.014	N/A	N/A	N/A	N/A
TRR (direct) mg/kg [b]	0.003	0.013	0.002	0.004	0.007	0.007
Component	%TRR	%TRR	%TRR	%TRR	%TRR	%TRR
Extracts [c]	N/A	80	N/A	N/A	N/A	N/A
PES [d]	N/A	20	N/A	N/A	N/A	N/A
Total	N/A	100	N/A	N/A	N/A	N/A

[a] TRR determined by summation of radioactivity present in the extracts and solids following solvent extraction and set as 100% TRR.
[b] TRR determined by direct quantification employing combustion/LSC.
[c] Extracts or aqueous partition fractions that were considered too low for analysis: < 5% TRR or < 0.01 mg/kg eq
[d] Radioactivity remaining in the solids after the initial solvent extractions.

Field rotational crop studies

Four rotational crop field trials have been conducted in the EU to investigate the magnitude of residues in rotational crops following a single application to bare soil at 0.200 kg ai/ha. Additionally, four rotational crop field trials have been conducted in the USA to investigate the magnitude of residues in rotational crops following three foliar applications at 14 day intervals and at a rate equivalent to 0.100 kg ai/ha per application to target crops of peanuts and soya beans.

Study 1

Two field crop rotation trials were carried out in Northern Europe during 2010 and 2011 [Ellis, 2012b, A17056B_10004]. Plots (72–75 m^2 each) were treated with a single application to bare soil at a nominal rate of 0.20 kg ai/ha using an EC formulation (A17056B). Plots received minimal cultivation with a harrow or rototill to 5 cm depth 8–13 days prior to drilling of the rotational crops. Rotational crops were planted into the treated soil at 28–30, 60–61 or 355–365 days after application. The 355–365 day rotation crops were planted on the same plot as the 28–30 day plots, after the first rotation crops were harvested and removed. Experimental conditions for trial S10-00261-01 and S10-00261-02 are indicated in Table 22. Crops sampled at immature stages were spinach (leaves) at BBCH 42–43 (DAT 81/71, 91/99, 420/420) and spring wheat (whole plant) at BBCH 39 (DAT 88/90, 98/107, 429/436) for trial 01/02. Mature samples were taken for spinach at BBCH 49 (DAT 91/86, 100/111, 434/432), for spring wheat (grain and straw) at BBCH 89 (DAT 144/155, 161/170, 505/525) and for carrot (root and tops with leaves) at BBCH 49 (DAT 149/131, 156/170, 491/467) for trial 01/02. Samples were taken randomly across each plot from at least 12 points. Spinach leaves were cut above ground using scissors, a knife or a sickle. Wheat forage whole plants were taken by hand or with scissors/secateurs or a sickle. Wheat grain and straw samples were produced by threshing the whole plant samples using a plot harvester. Carrot plants were taken by hand and the soil washed off. Sample sizes were in accordance with appendix V of the FAO manual: 1.0–2.0 kg spinach leaves from at least 12 plants, 1.0–1.4 kg wheat forage from at least 12 plants, 1.0–1.4 kg wheat grain and 0.50–0.73 kg wheat straw from at least 50 plants, 2.0–2.3 kg carrot roots and 1.0–1.2 kg carrot tops from at least 12 plants. Samples were stored frozen within 1–6 hrs after harvest at –18 ºC for a maximum period of 10 months until analysis. The storage period of 10 months is covered by the storage stability studies on parent, SYN546039 and SYN546206 in commodities with high water content, high starch content and wheat straw. During shipment, temperatures were out of range for short periods of less than 12 hrs. Since the samples remained frozen, this is considered to have no impact on the study.

Soil samples have not been analysed for parent or its soil metabolites (confirmed, Syngenta, 2014b). Wheat forage, grain and straw from the 30 and 60 day plant back intervals were analysed for parent and SYN546039 using HPLC-MS-MS method GRM042.03A with matrix matched standards for grain and straw and minor modifications for parent. Parent compound was analysed directly in the initial acetonitrile/water extract. This modification (A) was shown to have no impact on the validity of the analysis. All other crop samples were analysed for parent, SYN546039 and SYN546206 using HPLC-MS-MS method GRM042.08A with matrix matched standards. Methods GRM042.03A and GRM042.08A are considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix and control samples had residues < 0.01 mg/kg, showing adequate performance at the time of analysis of the samples.

No residues of parent, SYN546039 or SYN546206 were found at or above the LOQ of 0.01 mg/kg in any of the treated samples.

Study 2

Two field crop rotation trials were carried out in Southern Europe during 2010 and 2011 [Ellis, 2012c, A17056B_10005]. Plots (24–90 m^2) were treated with a single application to bare soil at a nominal rate of 0.20 kg ai/ha using an EC formulation (A17056B). Plots were lightly cultivated (plot 01) or cultivated with a motor hoe to a depth of 20 cm (plot 02) 1–8 days prior to drilling rotational crops. Rotational crops were planted into the treated soil at 28–32, 60–69 or 357–366 days after application. The 357–366 day rotation crops were planted on the same plot as the 28–32 day plots, after the soil was cultivated to a depth of 20 cm, including the remains of the first rotation crops. Experimental conditions for trials S10-00262-01 and S10-00262-02 are indicated in Table 22. Crops sampled at immature stages were spinach (leaves) at BBCH 41–43 (DAT 79/66, 108/97, 416/416) and spring wheat (whole plant) at BBCH 39 (DAT 69/75, 111/97, 416/423) for trial 01/02. Mature samples were taken for spinach at BBCH 49 (DAT 86/75, 118/104, 429/423), for spring wheat (grain and straw) at BBCH 89 (DAT 118/125, 192/152, 471/480) and for carrot (root and tops with leaves) at BBCH 49

(DAT 154/152, 169/159, 483/483) for trial 01/02. Spinach leaves were cut above ground using a knife. Wheat forage whole plants were cut above ground using a knife, scissors or secateurs. Wheat grain and straw samples were threshed by using a plot or portable combine. Carrot plants were collected by hand and roots and tops were separated in the field using a knife or a spade. Sample sizes were generally in accordance with appendix V of the FAO manual: 1.0–2.6 kg spinach leaves from at least 12 plants, 1.1–3.5 kg wheat forage from at least 12 plants, 1.0–1.5 kg wheat grain and 0.57–1.7 kg wheat straw from at least 12 plants, 2.0–4.0 kg carrot roots and 1.0–2.0 kg carrot tops from at least 12 plants. Sample size for mature spinach leaves from the 366 day plant back interval in trial 01 is rather small (0.30 kg), but since this sample was retrieved from at least 50 plants this is considered acceptable. Samples were stored frozen within 1–5 hrs after harvest at –18 °C for a maximum period of 10 months until analysis. The storage period of 10 months is covered by the storage stability studies on parent, SYN546039 and SYN546206 in commodities with high water content, high starch content and wheat straw. During shipment, temperatures were out of range for short periods of less than 6 hrs. Since the samples remained frozen, this is considered to have no impact on the study.

Soil samples have not been analysed for parent or its soil metabolites (confirmed, Syngenta, 2014b). Wheat forage, grain and straw from the 30 and 60 day plant back intervals were analysed for parent and SYN546039 by HPLC-MS-MS method GRM042.03A with matrix matched standards and minor modifications for parent. Parent compound was analysed directly in the initial acetonitrile/water extract. This modification (A) was shown to have no impact on the validity of the analysis. All other crop samples were analysed for parent, SYN546039 and SYN546206 using HPLC-MS-MS method GRM042.08A with matrix matched standards. Methods GRM042.03A and GRM042.08A are considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix and control samples had residues < 0.01 mg/kg, showing adequate performance at the time of analysis of the samples.

No residues of parent, SYN546039 or SYN546206 were detected at or above the LOQ of 0.01 mg/kg in any of the treated samples, except in treated wheat straw:

- In trial S10-00262-02 for wheat planted 28 days after application, parent was found at 0.014 mg/kg in wheat straw (DAT 125)
- In trial S10-00262-02 for wheat planted 60 days after application, parent was found at 0.012 mg/kg in wheat straw (DAT 152).

Study 3

Four field crop rotation trials were carried out in the USA during 2009, 2010 and 2011 [Mäyer, 2012, A17056B_50013]. Plots with target crops of peanuts or soya beans were treated with three foliar applications at 14 day intervals at a nominal rate of 0.100 kg ai/ha using an EC formulation. All applications incorporated a crop-oil concentrate or non-ionic surfactant adjuvant in the spray. The peanut and soya bean target crops were harvested and removed from the field according to agronomical practices. Experimental conditions are indicated Table 22. In trial E10-9681 (Seven Springs, NC), spinach, radish, and wheat were planted into the treated soil at 30 days after the last application. Wheat grain and straw were harvested at DAT 228, wheat hay at DAT 182, wheat forage at DAT 91, roots/tops at DAT 157 and leafy crops at DAT 158. In trials E11-9678 (Elko, SC), C18-9679 (Richland, IA) and E13-9680 (Madil, TX), lettuce or spinach, radish or turnip and wheat were planted into the treated soil at 180 days after the last application. For trials E11-9678, C18-9679, E13-9680 wheat grain and straw were harvested at DALT 289/275/278, wheat forage and hay at DAT 231/233/231, roots/tops at DAT 231/231/278 and leafy vegetables at DAT 231/231/300. Wheat forage and wheat hay were collected from 12 separate areas of the plot. Wheat grain, wheat straw, radish top, radish root and leafy vegetable samples were collected from at least 12 plants and/or 12 separate areas of the plot at normal crop maturity. Samples were at least 2 kg (roots) or at least 1 kg (tuber tops, leafy vegetables, wheat forage and wheat grains) or at least 0.5 kg (wheat straw and wheat hay). Exceptions are 0.6–1.0 kg for tuber tops and 0.26–0.28 kg for wheat forage in trial E10-9681 (30 day PBI), 1.1–1.5 kg for 40 tubers in trial E11-9678 (180 day PBI), 0.8–0.9 kg for tuber tops in trial (E13-

9680) [Syngenta, 2014b]. In trial E10-9681, plant growth was retarded because of abnormally cold weather and therefore it was not possible to collect at least 1 kg wheat forage from the plot. Two replicate field samples were taken from each plot. Adhering soil was removed from radish roots by light brushing. Wheat hay was harvested on the same date as wheat forage and was dried for 2 days in a shelter (E10-9681), 2 days in the field (E11-9678), 6 days in the field (E13-9680) or 10 days in a shed (C18-9679) [Syngenta, 2014b].

Samples were stored at –20 ºC for a maximum period of 24 months until analysis [Syngenta, 2014b]. The storage period of 24 months is covered by the storage stability studies on parent, SYN546039 and SYN546206 in commodities with high water content, high starch content and wheat straw.

Soil samples have not been analysed for parent or its soil metabolites (confirmed, Syngenta, 2014b). Crop samples from the 180 day plant-back interval plots were analysed for parent and SYN546039 by HPLC-MS-MS method GRM042.03A. Parent compound was cleaned-up together with the SYN546039 hydrolysate as in the original method. Method GRM042.03A is considered valid for the purpose of this study (commodity type and concentration level of the analytes). Samples were re-analysed for SYN546206 by modification A of HPLC-MS-MS method GRM042.08A. Crop samples from 30 day plant-back interval plots were analysed for parent, SYN546039 and SYN546206 using modification A of HPLC-MS-MS method GRM042.08A. Modification A of method GRM042.08A is considered insufficiently validated for the determination of parent, SYN546039 and SYN546206 in radish roots and tops (30 day PBI samples) and in addition insufficiently validated for the determination of SYN546206 in lettuce, radish roots & tops and turnip roots & tops (180 day PBI samples) (see analytical section). Average concurrent recoveries for a limited number of samples were within 70–120% for each analyte and matrix and control samples had residues < 0.01 mg/kg, showing adequate performance at the time of analysis of the samples

No residues of parent, SYN546039 or SYN546206 were detected at or above the LOQ of 0.01 mg/kg in any of the treated samples, except in wheat:

- In trial E10-9681 with soya bean as primary crop and wheat planted 30 days after application, parent and SYN546039 were found at an average concentration of 0.022 mg/kg (range 0.012–0.033 mg/kg) and 0.021 mg/kg (range 0.015–0.028 mg/kg), respectively, in wheat forage (DALT 91). Two field samples were taken and each sample was extracted twice.
- In trial E10-9681 with soya bean as primary crop and wheat planted 30 days after application, SYN546039 was found at an average concentration of 0.016 mg/kg (range 0.016–0.017 mg/kg) in wheat straw (DAT 228). Two replicate field samples were taken and each sample was extracted twice.

Note: Since residues were found at the 30 day plant back interval, the 30 day PBI trial is considered the most critical trial (spinach, radish and wheat). The wheat forage samples of the 30 day PBI trial are considered not representative, because wheat growth is retarded because of abnormally cold weather and consequently it was not possible to collect a sufficient (representative) amount of sample from the plot. In addition, modification A of method GRM042.08A is considered insufficiently validated for the determination of parent, SYN546039 and SYN546206 in radish roots and tops (30 day PBI samples).

Table 22 Experimental conditions for field rotational crop studies

Trial & Location	Application; last appl date	Soil type	pH	% om	CEC (meq/100 g)	PBI	Rotational crops
S10-00261-01; Dollern, Germany	1× 0.20 kg ai/ha to bare soil 29 Mar 2010	sandy loam	6.5	1.4	ns	30, 60, 365 [b]	Spinach (var Tornado) Short-cycle carrots (var Laguna F1) Spring wheat (var Chasin)
S10-00261-02; Banbury,	1× 0.20 kg ai/ha to bare soil	clay loam	6.7	5.1	ns	28, 61,	Spinach (var Renegade)

Trial & Location	Application; last appl date	Soil type	pH	% om	CEC (meq/100 g)	PBI	Rotational crops
Oxfordshire, UK	17 Mar 2010					355 [b]	Short-cycle carrots (var Napoli) Spring wheat (var Belvoir)
S10-00262-01; Barry d'Islemade, Tarn et Garonne, France	1× 0.20 kg ai/ha to bare soil 20 Apr 2010	sandy clay loam	8.4	8	ns	32, 69, 366 [b]	Spinach (var Lagos F1 or Island) Short-cycle carrots (var Napoli F1 or Mokum F1) Spring wheat (var Printidor)
S10-00262-02; Castagnito, Piedmont, Italy	1× 0.20 kg ai/ha to bare soil 19 March 2010	sandy loam	7.9	1.4	ns	28, 60, 357 [b]	Spinach (var America or Viridis Olter) Short-cycle carrots (var Nantese di Chioggia 2 or Nantes 2) Spring wheat (var Valbona)
E10-9681; Seven Springs, NC, USA	3× 0.10 kg ai/ha to soya beans [a] at BBCH 75–89; 15 Oct 2010	Loamy Sand	6.4	0.7	6.5	30	Spinach (var Baker) Radish (var Cherriette) Wheat (var Coker 9804)
E11-9678; Elko, SC, USA	3× 0.10 kg ai/ha to peanuts at BBCH 73–83 [c]; 24 Aug 2009	Loamy Sand	6.8	1.1	3.1	180	Lettuce (var Butter Crunch) Radish (var Early Scarlet Globe) Wheat (var Steele)
C18-9679; Richland, IA, USA	3× 0.10 kg ai/ha to soya beans [a] at BBCH 79 [c]; 29 Sept 2009	Silt loam	6.7	4.5	18	180	Spinach (var Bloomsdale) Radish (var Champion) Spring wheat (var Briggs)
E13-9680; Madill, TX, USA	3× 0.10 kg ai/ha to peanuts at BBCH 71–85 [c]; 2 Oct 2009	Sandy Loam	7.9	0.8	18	180	Spinach (var Spargo F1) Turnip (Scarlet Queen) Spring wheat (var Glenn)

[a] Syngenta, 2014b: soya bean is the target crop

[b] The 355–365 day rotation crops were planted on the same plot as the 28–32 day plots, after the first rotation crops were removed

[c] Primary crops were harvested and removed before rotational crops were planted

Environmental fate in water/sediment systems

No data submitted.

RESIDUE ANALYSIS

The Meeting received information on enforcement/monitoring methods for the determination of benzovindiflupyr and its metabolites in plant and animal commodities. In addition the Meeting received information on analytical methods for the determination of benzovindiflupyr and its metabolites as used in the various study reports (supervised residue trials, storage stability studies, processing studies, feeding studies). The analytical methods have been evaluated according to the guidance provided by OECD (Series on Pesticides number 39) as indicated on page 25 of the FAO manual 2009.

Validation results are required for every commodity submitted for MRL-setting: at least one full validation for a commodity within the five defined crop groups (high acid content, high water content, high oil content, high protein content, high starch content) and a reduced validation for every other commodity within a certain crop group. Where validation results do not meet the criteria given below, this is indicated.

When the analytical method is validated according to a full validation scheme, it means that:

- at least five recovery experiments per level were conducted on at least two levels (LOQ and 10× LOQ) and average recovery per level was shown to be between 70–120% and the relative standard deviation (RSD_r or CV) per level was shown to be < 20%
- at least two control samples were analysed and were shown to be below 0.3× LOQ
- the calibration was conducted with at least five single points or at least three duplicate points and was shown to be linear (either standards in solvent or matrix matched standards).

When the analytical method is validated according to a reduced validation scheme, it means that:

- a full validation is available for a crop in the same crop group (high acid content, high water content, high oil content, high protein content, high starch content)
- at least three recovery experiments per level were conducted on at least two levels (LOQ and 10× LOQ) and the average recovery per level was shown to be between 70–120% and the relative standard deviation (RSD_r or CV) per level was < 20%
- at least two control samples were analysed and shown to be below 0.3× LOQ
- the calibration was conducted with at least five single points or at least three duplicate points and was shown to be linear (only relevant for matrix matched standards; standards in solvent are already covered by full validation).

Analytical methods for enforcement in plant and animal commodities

FDA PAM Multi-Residue Method

The suitability of the FDA Pesticide Analytical Manual (PAM-I) Multi-Residue Method (MRM) to analyse for benzovindiflupyr and its three metabolites (SYN546039, SYN545720 and SYN546206) in non-fatty and fatty foods was tested using the FDA MRM Testing Protocol [Barker and Ballard, 2012, SYN546039_50003]. The FDA MRM Testing Protocol [FDA, 1999] consists of several protocols: one protocol for determining if the compounds are naturally fluorescent (protocol A); one protocol for methylation of acidic analytes, in case the native volatility is insufficient for GC determination (protocol B); two protocols for determining GC characteristics of chemicals (protocol C and G); three protocols by which chemicals can be evaluated through the FDA multi-residue testing program (protocols D, E and F); Based on its molecular structure, benzovindiflupyr and its metabolites, were taken through protocols A, B, C, D and F.

Protocol A (PAM I, Section 401, module DL2) demonstrated that benzovindiflupyr, SYN546039 or SYN546206 had no detector response in a HPLC system with fluorescence detection, even with 10,000 ng on column. Signals were reported for SYN545720 but did not provide enough response to warrant further investigation (greater than 10,000 ng required 50% FSD). Further work on this protocol was terminated.

Protocol B showed that compound SYN545720 could be methylated. However, 150 ng of SYN545720-methyl ester could not be detected with GC-ECD (module DG1). Further work on this protocol was terminated due to poor sensitivity of the methyl ester.

Protocol C experiments demonstrated that benzovindiflupyr SYN546039 and SYN546206 were found to be suitable for detection by GC-ECD using module DG10. None of the modules tested

(GC-ECD modules DG1, DG7, DG10 or GC-NPD module DG17) gave a suitable response for SYN545720. For compounds which can only be determined by ECD, the extract must be cleaned up by Florisil chromatography before determination.

Protocol D (for non-ionic chemicals in non-fatty foods) showed that benzovindiflupyr and its metabolites SYN546039 and SYN546206 could not be recovered through the section 301 C1 Florisil clean-up elution procedure. Further testing through Protocol D was terminated.

Protocol F (for moderately non-polar chemicals in fatty foods) showed that only benzovindiflupyr was recovered through either the C1 or C2 Florisil clean-up elution procedure. Recovery was 60% (50% ethyl ether eluent) for C1 and 106% (eluent 3) for C2 clean-up procedures. When corn oil was fortified with parent at 0.05 and 0.50 mg/kg and extracted using Section 304 E3 conditions, recovery was poor. Recoveries through the Section 304 E3 + C1 procedure averaged 32% and 57% for the 0.05 and 0.50 mg/kg fortified samples, respectively. Recoveries through the Section 304 E3 + C2 procedure averaged 56% and 58% for the 0.05 and 0.50 mg/kg fortified sample, respectively.

Conclusion: Data generated from testing of benzovindiflupyr and its metabolites SYN546039, SYN545720 and SYN546206 through Protocols A, B, C, D and F indicate that none of the compounds of interest are successful candidates for enforcement by the FDA PAM I multi-residue methods for non-fatty or fatty foods.

QuEChERS method

In the published multi-residue QuEChERS method [ECS, 2009] homogenised samples (5.0 g) were mixed with acetonitrile/water (1:1, v/v, plant matrices) or acetonitrile (animal matrices). Fat samples were heated to approximately 40 °C before mixing with acetonitrile. Thereafter, magnesium sulfate (to bind water), sodium chloride (to salt out the analyte), trisodium citrate dihydrate and disodium hydrogen citrate sesquihydrate (slightly acidic buffer) were added. After mixing, the sample was centrifuged. The upper acetonitrile phase was cleaned-up by a dispersive solid phase extraction purification step using PSA (primary and secondary amine exchange material) and magnesium sulfate. The mixture was centrifuged and the upper clear acetonitrile phase was concentrated using a stream of air. The final extract was diluted with water/acetonitrile (9/1, v/v, or ¼, v/v). Samples were analysed by HPLC-MS-MS at their appropriate mass transitions.

The determination of parent using QuEChERS method was validated according to the full validation scheme in oranges (whole fruit), wheat (grain), lettuce (head), oilseed rape (seed) and coffee bean (roasted) [Winter and Armann, 2012, SYN545192_10193]. HPLC-MS-MS mass transitions m/z 396 → 368 and m/z 396 → 91 were used for quantification or confirmation; no preference could be seen in the results. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of determination $R^2 > 0.99$) was observed in the range of 0.25–50 ng/mL standards using five or six matrix-matched standards. This range covers at least 0.3–12LOQ in the samples. Significant matrix effects (> 20%) were found for all matrices and therefore matrix-matched standards were used for quantification for all matrices. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

The determination of parent by the QuEChERS method was independently validated according to the full validation scheme on lettuce (head), oilseed rape (seed) and coffee bean (roasted) [Watson, 2012a, SYN545192_10172]. The validation was performed for both MS-MS transitions. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of determination $R^2 > 0.999$) was observed in the range of 0.25–50 ng/mL standards using six matrix-matched standards. This range is equivalent to 0.25–25LOQ in the samples. Significant matrix effects (> 20%) were observed for oilseed rape seed and coffee bean matrix for both transitions and therefore matrix-matched standards were used for quantification for all matrices. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

The determination of parent using the published multi-residue QuEChERS method (EN 15662:2009) was validated according to the full validation scheme in bovine meat, liver, kidney, fat, milk and chicken eggs [Class and Göcer, 2011, SYN545192_10150]. HPLC-MS-MS mass transition m/z 396 → 91 was used for quantification; m/z 396 → 368 was used for confirmation. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent were within 70–120% limits and RSDs were within 20%. Linearity of detector response (correlation coefficient r > 0.99 for 1/× weighted regression) was observed in the range of 0.05–5 ng/mL (fat) or 0.10–10 ng/mL (other matrices) using seven standards in solvent. This range is equivalent to 0.20–20LOQ in the samples [Syngenta, 2014c]. Significant matrix effects (> 20%) were not observed and standards in solvent were used for calibration. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

The determination of parent using the published multi-residue QuEChERS method (EN 15662:2009) was independently validated according to the full validation scheme in bovine liver, fat, milk and chicken eggs [Watson, 2012b, SYN545192_10169]. HPLC-MS-MS mass transition m/z 396 → 91 was used for quantification; m/z 396 → 368 was used for confirmation. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of determination $R^2 > 0.99$ for 1/× weighted regression) was observed in the range of 0.05–5 ng/mL (fat) or 0.10–10 ng/mL (other matrices) using seven standards in solvent. This is equivalent to 0.2–20LOQ in the samples. Significant matrix effects (> 20%) were not observed and standards in solvent were used for calibration. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Conclusion: The multi-residue QuEChERS method is considered valid for use as enforcement method in plant commodities with high acid content (oranges), high water content (lettuce), high starch content (wheat grain), high oil content (oilseed rape, coffee beans) and animal commodities (meat, liver, kidney, fat, milk and eggs) with an LOQ of 0.01 mg/kg for benzovindiflupyr (parent).

Analytical methods used in study reports in plant commodities

HPLC-MS-MS method GRM042.03A

HPLC-MS-MS method GRM042.03A, version 4 August 2011, [Braid and Lin, 2011b, SYN545192_10118] is intended for use as pre-registration method for the determination of parent, and SYN546039 (free and conjugates) in plant commodities. The original method or its modification was used in field rotational crop studies and storage stability studies.

High water content commodities (10 g) were extracted by homogenisation in 80/20 (v/v) acetonitrile/water. Dry matrices (5 g, e.g. grains, hay, straw) were pre-soaked with 25 mL water for 30 min prior to homogenisation. An aliquot of the extracts was evaporated at 50 °C under a stream of air or nitrogen to remove the acetonitrile and to concentrate the sample. The remaining aqueous extract was acidified with 1 M HCl and partitioned with iso-hexane to separate parent off. The iso-hexane fraction was evaporated to dryness and taken up in 50/50 v/v acetonitrile/water for direct determination of parent by HPLC-MS-MS. Alternatively the parent fraction (in iso-hexane) may be stored refrigerated and then combined with the post-hydrolysis extract for sample clean-up if required. The remaining aqueous acidic fraction was diluted to 0.5 M HCl and heated at 100 °C for 6 hours to hydrolyse conjugates of SYN546039. If necessary, the parent fraction in iso-hexane was diluted with methanol and evaporated to remove the iso-hexane and to concentrate the sample. The hydrolysate (together with the parent fraction if necessary) was cleaned-up using an SPE cartridge. SYN546039 (and parent if included at this stage) was eluted with 50/50 (v/v) acetonitrile/water. Following dilution, samples were analysed by HPLC-MS/MS. The primary transition monitored for parent was m/z 396→91 with confirmatory transition m/z 396→ 368. For SYN546039 the primary transition monitored was m/z 412→ 91 with confirmatory transition m/z 412 → 340. The reported LOQ is 0.01 mg/kg for both parent and SYN546039. Samples with expected residues > 0.1 mg/kg need to be diluted before analysis.

The analysis conditions as specified in the method description need to be followed carefully. Since parent compound degrades under the hydrolysis conditions used to hydrolyse conjugates of SYN546039 into their free form, parent is separated from the sample before hydrolysis. Change in hydrolysis conditions may result in either incomplete deconjugation or degradation of SYN546039 (free). Parent compound has been observed to adsorb to glass surfaces in solutions with < 50% organic solvent. These considerations have been dealt with in the described analytical method.

HPLC-MS-MS Method GRM042.03A was validated [Lin, 2012, SYN545192_50049] according to the full validation scheme for apples, grapes, wheat commodities (forage, grain, hay and flour), spinach, peanuts, coffee beans, carrot roots, orange juice and sugarcane. The validation was performed for the quantification transition ions as well as for the confirmation transition ions. Fortifications of parent compound were performed before extraction and parent compound was quantified after the SPE clean-up stage for all commodities. Fortifications of SYN546039 were performed after the acid hydrolysis step. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent or SYN546039 (free) were within 70–120% limits and RSDs were within 20%. For wheat forage and wheat hay recoveries at 0.01 mg/kg and 0.5 mg/kg were also within these ranges. Linearity of detector response (coefficient of correlation r > 0.99) was observed in the range 0.02–0.5 ng/mL using 5–6 single standards. Equivalent concentrations in the samples were not stated. The magnitude of the matrix effect was considered not to be significant (< 20% suppression or enhancement) with the exception of coffee beans. Matrix matched standards were used for coffee beans; standards in solvent were used for all other matrices. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Reduced validation data were available for the determination of parent and SYN546039 (free) in wheat (forage, hay, grain, straw), lettuce, spinach, turnips (roots, tops) and radish (roots, tops) [Mäyer, 2012, A17056B_50013]. Parent was analysed after SPE clean-up. Fortifications of both parent and SYN546039 were performed prior to extraction. Average recoveries at 0.01 mg/kg (n=3) and 0.1 mg/kg (n=1) were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of correlation r > 0.99 for 1/× weighted regression) was observed in the range 0.1–2 ng/mL or 0.02–1 ng/mL using five single matrix matched standards. No interfering peaks > 0.005 mg/kg) were detected in any of the control extracts.

Reduced validation data were available for the determination of parent and SYN546039 (free) in wheat straw and carrot (roots, tops) [Ellis, 2012b/c, A17056B_10004, A17056B_10005]. Parent was analysed directly in the initial acetonitrile/water extract. Average recoveries at 0.01 mg/kg (n=3), 0.05 mg/kg (n=2) and 0.1 mg/kg (n=1) were within 70–120% limits and RSDs were within 20% at 0.01 mg/kg. Linearity of detector response (coefficient of correlation r > 0.999) was observed in the range 0.02–4 ng/mL using five single matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Modification A of HPLC-MS-MS method GRM042.03A was used for quantification of parent and SYN546039 in storage stability study 1 [Watson, 2013, SYN545192_10137]. Parent and SYN546039 were determined directly in the acetonitrile/water (80/20) extract without further clean-up. The hydrolysis step was not included for the purpose of this study. Validation data were available for spinach, orange, wheat grain, wheat straw and potato at the fortification level used in the storage stability study. Parent or SYN546039 (free) were added before extraction as a single analyte per sample. Average recoveries at 0.2 mg/kg of parent or SYN546039 (free) were within 70–120% limits and RSDs were within 20% (n=12). Linearity of detector response (coefficient of correlation r > 0.99) was observed in the range 0.02–10 ng/mL using 5–6 single matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Modification B of HPLC-MS-MS method GRM042.03A was used for quantification of parent and SYN546039 in storage stability study 3 [Hagan and Bertrand, 2012, SYN545192_50177]. Samples were extracted with acetonitrile/water (80:20, v/v). An aliquot of the extracts was evaporated at 50 °C under a stream of air or nitrogen to remove the acetonitrile and to concentrate the sample. The remaining aqueous extract was acidified with 1 M HCl and cleaned-up using an SPE cartridge. Parent and SYN546039 were eluted with acetonitrile/water (60:40, v/v). The hydrolysis step was not

included for the purpose of this study. Validation data were available for maize flour and maize meal, raisins, dried apple and apple juice at the fortification level used in the storage stability study. Parent or SYN546039 (free) were added before extraction as a single analyte per sample. Average recoveries at 0.2 mg/kg of parent or SYN546039 (free) were within 70–120% limits and RSDs were within 20% (n=8). Linearity of detector response (coefficient of correlation r > 0.99, 1/× weighted regression) was observed in the range 0.02–1 ng/mL using seven single standards in solvent. No interfering peaks (> 0.02 ng/mL standard signal) were detected in any of the control extracts.

Conclusion:

- HPLC-MS-MS method GRM042.03A is considered valid for the determination of parent and SYN546039 (free) in the range 0.01–0.1 mg/kg for apples, grapes, wheat (forage, grain, hay, straw, flour), spinach, lettuce, peanuts, coffee beans, carrot (roots and leaves), turnip (roots and leaves), radish (roots and leaves), orange juice and sugarcane. The valid range is extended to 0.5 mg/kg for wheat forage and wheat hay.
- The original method indicates to fortify the sample with the free form of SYN546039 after the acid hydrolysis procedure, and thereby only validates the SPE clean-up part. However, reduced validation data are available, where SYN546039 was added before extraction and therefore, the full analytical procedure including extraction, liquid-liquid partition and hydrolysis was validated for the free form of SYN546039.
- Efficiency of extraction and hydrolysis for SYN546039 including its conjugates has been verified in a radio-validation study using method GRM042.04A, where the extraction and hydrolysis procedures are identical to those in method GRM042.03A. Based on this study, HPLC-MS-MS method GRM042.03A is considered sufficiently validated for the determination of the free and conjugated forms of SYN546039.
- Modification A of HPLC-MS-MS method GRM042.03A is considered valid for the determination of parent and SYN546039 (free) at the level of 0.2 mg/kg for spinach, orange, wheat grain, wheat straw and potato for the purpose of storage stability studies only.
- Modification B of HPLC-MS-MS method GRM042.03A is considered valid for the determination of parent and SYN546039 (free) at the level of 0.2 mg/kg for spinach, orange, maize flour and maize meal, raisins, dried apple and apple juice for the purpose of storage stability studies only.

HPLC-MS-MS methods GRM042.04A and POPIT MET.134 rev 01

HPLC-MS-MS Method GRM042.04A, version 4 August 2011, [Braid and Lin, 2011c, SYN545192_10119] is intended for use as pre-registration method for the determination of parent, SYN546039 (free and conjugates) and SYN545720 (free and conjugates) in soya bean commodities. The original method or its modification was used in supervised residue trials on soya beans, processing studies on soya beans and storage stability studies.

In the original method, soya bean forage (10 g) was extracted by homogenisation in 80/20 v/v acetonitrile/water. Soya bean hay (5 g) was pre-soaked with 25 mL water for 30 min prior to homogenisation. Soya bean seeds (10 g) were extracted by homogenisation with 50/50 v/v acetonitrile/water. Soya bean oil (10 g) was dissolved in 50 mL n-hexane and residues were extracted by partition into 80/20 v/v acetonitrile/water. At this stage, an aliquot of the extract may be diluted with 50/50 acetonitrile/water for direct determination of parent by HPLC-MS-MS (soya bean oil). For further clean-up, an aliquot of the extract was evaporated at 50 °C under a stream of air or nitrogen to remove the acetonitrile and to concentrate the sample. The remaining aqueous extract was acidified with 1 M HCl and partitioned with iso-hexane to separate parent off. The iso-hexane fraction was evaporated to dryness and taken up in 50/50 v/v acetonitrile/water for direct determination of parent by HPLC-MS-MS (soya bean forage, hay and seed). Alternatively the parent fraction (in iso-hexane) may be stored refrigerated and then combined with the post-hydrolysis extract for sample clean-up if required. The remaining aqueous acidic fraction was diluted to 0.5 M HCl and heated at 100 °C for 6

hours to hydrolyse SYN546039 conjugates in all crop commodities and SYN545720 conjugates in soya bean seed. If necessary, the parent fraction in iso-hexane was diluted with methanol and evaporated to remove the iso-hexane and to concentrate the sample. The hydrolysate (together with the parent fraction if necessary) was cleaned-up on a SPE cartridge. SYN545720 was eluted with 50/50 v/v methanol/water and SYN546039 (and parent if included at this stage) were eluted with 50/50 v/v acetonitrile/water. Following dilution, samples were analysed separately by HPLC-MS/MS. The primary transition monitored for parent was m/z 396→91 with confirmatory transition m/z 396→ 368. For SYN546039 the primary transition monitored was m/z 412→ 91 with confirmatory transition m/z 412→340. The primary transition monitored for SYN545720 is m/z 161→141 with confirmatory transition m/z 161→66. Samples with expected residues > 0.1 mg/kg need to be diluted before analysis.

The analysis conditions as specified in the method description need to be followed carefully. Since parent compound degrades under the hydrolysis conditions used to hydrolyse conjugates of SYN546039 and SYN545720 into their free form, parent is separated from the sample before hydrolysis. Change in hydrolysis conditions may result in either incomplete deconjugation or degradation of SYN546039 (free). Parent compound has been observed to adsorb to glass surfaces in solutions with $< 50\%$ organic solvent. These considerations have been dealt with in the described analytical method.

The original HPLC-MS-MS Method GRM042.04A was validated [Lin, 2012, SYN545192_50049] according to the full validation scheme for soya bean commodities (forage, oil, seed and milk). For soy milk, the extraction scheme as for forage was used [Syngenta, 2014b]. The validation was performed for the quantification transition ions as well as for the confirmation transition ions. Fortifications of parent compound were performed before extraction and parent compound was quantified after the acetonitrile/water extraction stage for soya bean oil, after the iso-hexane liquid-liquid partition stage for soya bean seeds, and after the SPE clean-up stage for soya bean forage and soya bean milk. Fortifications of SYN546039 and SYN545720 were performed after the acid hydrolysis step. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent or SYN546039 (free) or SYN545720 (free) were within 70–120% limits and RSDs were within 20%. For soya bean forage recoveries at 0.01 mg/kg and 0.5 mg/kg were also within these ranges. Linearity of detector response (coefficient of correlation $r > 0.99$, no weighing) was observed in the range 0.02–0.5 ng/mL for parent and SYN546039 using six standards. Linearity of detector response (coefficient of correlation $r > 0.99$, no weighing) was observed in the range 0.05–0.5 ng/mL for SYN545720 using five standards. Equivalent concentrations in the samples were not stated. The magnitude of the matrix effect was considered not to be significant ($< 20\%$ suppression or enhancement), with the exception of SYN546039 in soya bean seed and SYN545720 in soya bean forage. Standards in solvent were used for soya bean oil and soya bean milk; matrix matched standards were used for soya bean seeds and soya bean forage. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

HPLC-MS-MS Method POPIT MET.134, Rev01, version 10 January 2012, [Volpi, 2012, SYN545192_10195] is a modification of HPLC-MS-MS method GRM042.04A and is intended for analysis of soya bean seeds only. Rev 01 is identical to the original version of 23 May 2011, except for some textual changes. The method was used in the Brazilian supervised residue trials on soya bean seeds. Extraction was conducted as described for GRM042.04A, except that 5 g soya bean seeds were taken instead of 10 g and extraction volumes were adapted accordingly. Parent was directly analysed after iso-hexane liquid-liquid partition without further clean-up. The hydrolysate, containing SYN545720 and SYN546039, was cleaned-up on a SPE cartridge. SYN545720 and SYN546039 were eluted with acetonitrile instead of 50/50 v/v methanol/water or 50/50 v/v acetonitrile/water, respectively. Following dilution, samples were analysed by HPLC-MS/MS using different transition ions. The primary transition monitored for parent was m/z 396→91 with confirmatory transition m/z 396→131. For SYN546039 the primary transition monitored was m/z 412→340 with confirmatory transition m/z 412→91. The primary transition monitored for SYN545720 is m/z 161→141 with confirmatory transition m/z 161→ 66.

HPLC-MS-MS Method POPIT MET.134 rev01 was validated [Volpi, 2012, SYN545192_10195] according to the full validation scheme for soya bean seeds. The validation was performed only for the quantification transition ions. Fortifications of parent compound were performed before extraction and parent compound was quantified after the iso-hexane liquid-liquid partition stage. Fortifications of SYN546039 and SYN545720 were performed after the acid hydrolysis step. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent or SYN546039 (free) or SYN545720 (free) were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of determination $R^2 > 0.99$) was observed in the range of 0.5–16 ng/mL for parent and 0.25–8.0 ng/mL for SYN545039 and SYN545720 using six duplicate standards. This is equivalent to 0.5–16LOQ in the samples. Standards for parent were prepared in solvent; standards for SYN545039 and SYN545720 were prepared in matrix. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Modification A of HPLC-MS-MS method GRM042.04A was used for quantification of parent and SYN546039 in storage stability study 1 [Watson, 2013, SYN545192_10137]. Dry broad bean seeds (5 g) were pre-soaked in water prior to extraction. Parent and SYN546039 were determined directly in the acetonitrile/water (80/20) extract from dry broad beans without further clean-up. Dry soya bean extracts required further clean-up. Parent was determined in dry soya bean extracts after the iso-hexane partition step, while SYN546039 was determined after SPE clean-up. The hydrolysis step to free the conjugated residues of SYN546039 was not included for the purpose of the study. Parent or SYN546039 (free) were added before extraction as a single analyte per sample. Additional validation data were available for dry soya beans and dry broad beans at the fortification level used in the storage stability study. Average recoveries at 0.2 mg/kg of parent or SYN546039 (free) were within 70–120% limits and RSDs were within 20% (n=12). Linearity of detector response (coefficient of correlation r > 0.999) was observed in the range 0.02–10 ng/mL using 5–6 single matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Modification B of HPLC-MS-MS method GRM042.04A was used for quantification of parent and SYN546039 in storage stability Study 3 [Hagan and Bertrand, 2012, SYN545192_50177]. Soya bean flour and soya bean milk were extracted with 50/50 v/v acetonitrile/water. Soya bean oil and maize oil were dissolved in 50 mL n-hexane and residues were extracted by partition into 80/20 v/v acetonitrile/water. For further clean-up, an aliquot of the extract was evaporated at 50 °C under a stream of air or nitrogen to remove the acetonitrile and to concentrate the sample. The remaining aqueous extract was acidified with 1 M HCl and cleaned up by SPE. SYN545720 was eluted with 50/50 v/v methanol/ water and parent and SYN546039 were eluted with 50/50 v/v acetonitrile/water. The hydrolysis step to free the conjugated residues of SYN546039 was not included for the purpose of the study. Parent or SYN546039 (free) or SYN545720 were added before extraction as a single analyte per sample. SYN546720 was only added to soya bean matrices. Validation data were available for maize oil, soya bean flour, soya bean milk and soya bean oil at the fortification level used in the storage stability study. Average recoveries at 0.2 mg/kg of parent or SYN546039 (free) or SYN545720 were within 70–120% limits and RSDs were within 20% (n=8). Linearity of detector response (coefficient of correlation r > 0.99, 1/× weighted regression) was observed in the range 0.02–1 ng/mL using seven single standards in solvent. No interfering peaks (> 0.02 ng/mL standard signal) were detected in any of the control extracts.

Modification C of HPLC-MS-MS method GRM042.04A was used for quantification of parent, SYN546039 and SYN545720 in a soya bean processing study [Ellis, 2012a, A17056D_10002]. Due to small sample sizes, volumes and weights for extraction were reduced. Soya bean oil was dissolved in n-hexane and partitioned into acetonitrile/water (80/20, v/v), as described in the original method. The extract was diluted with acetonitrile/water (25/75, v/v) prior to quantification of parent (soya bean oil). All other soya bean commodities were extracted with 50/50 acetonitrile/water as described in the original method for seeds. In these commodities, parent was analysed after iso-hexane liquid-liquid partition without SPE clean-up step. As in the original method validation, the free forms of SYN546039 and SYN545720 were added after the hydrolysis step to

verify the recovery. Matrix matched standards were used for quantification of all three analytes for all matrices. For soya bean meal, fat soy flour, pollard, refined oil, crude oil and aspirated grain fractions the secondary transition m/z 396 to 368 was used for quantification of parent, since the primary transition gave interferences. For pollard and fat soy flour the secondary transition of m/z 412 to 340 was used for quantification of SYN546039, since the primary transition gave interferences.

Modification C of HPLC-MS-MS method GRM042.04A was validated with very limited data [Ellis, 2012a, A17056D_10002]. Limited validation data were available in the range 0.01–1.0 mg/kg for soya bean seeds and aspirated grain fractions, 0.01–0.5 mg/kg for soya bean hulls, soy oil, soya bean meal, fat soy flour, pollard, okara, soy milk, tofu, soy sauce and miso. Fortifications of parent compound were performed before extraction and parent compound was quantified after the acetonitrile/water extraction stage for soya bean oil and after the iso-hexane liquid-liquid partition stage for all other soya bean commodities. Individual recoveries were between 72–116% for each matrix and analyte (range 0.01–1.0 mg/kg, n=1–2 per level, 3–4 levels per matrix). These limited method recoveries confirm the validity of the method under the modified conditions and for the processed soya bean commodities. Linearity of detector response (coefficient of correlation r > 0.99) was observed in the range 0.2–4 ng/mL using five single matrix matched standards. No interfering peaks (> 0.01 mg/kg) were detected in any of the control extracts.

The extraction and hydrolysis procedure used in method GRM042.04A was radio-validated using samples of soya bean hay and soya bean seed from the pyrazole labelled metabolism study [Oddy, 2012, SYN545192_10102]. An aliquot of the homogenised soya bean hay sample (5 g) was soaked in 25 mL water for 30 min and subsequently extracted with acetonitrile/water (80:20 v/v). An aliquot of the homogenised soya bean seed sample (10 g) was extracted twice with acetonitrile/water (1:1 v/v). Following centrifugation aliquots of the supernatants of each sample were analysed by LSC to determine the efficiency of extraction. A separate aliquot of the supernatants was evaporated to remove the acetonitrile and to concentrate the extract, acidified and partitioned (3×) with iso-hexane. The aqueous phase was adjusted to 0.5 M HCl and heated at 100 °C for 6 hrs. Aliquots of the hexane phase and the hydrolysate were analysed by LSC and TLC to determine levels of parent and the metabolites SYN546039 (free and conjugates) and SYN545720 (free and conjugates).

Results are shown in Table 23. Analysis of soya bean hay and soya bean seed extracts by LSC showed that at least 94% of the total radioactive residue could be extracted by method GRM042.04A. Good agreement was obtained between the levels of parent, SYN546039 (free including conjugates) and SYN546720 (free including conjugates) found after extraction (and hydrolysis) as performed in the metabolism study and as performed in method GRM042.04A. The slightly higher levels of parent and SYN546720 in soya bean hay and seeds obtained by method GRM042.04A could be the result of a different extraction and hydrolysis scheme used in the metabolism study.

Table 23 Radiovalidation of extraction procedures used in analytical method GRM042.04A

Commodity	Components	Metabolism Study		Method GRM042.04A		Trueness
		%TRR	Residue mg/kg eq	%TRR	Residue mg/kg eq	%TRR method: metabolism
Soya bean hay	TRR extracted	98 [a]	12	100 [b]	13	103%
	parent	67	8.4	68 [b]	8.5	99%
	SYN546039 incl conjugates	12 [c]	1.5	14 [e]	1.7	112%
Soya bean seed	TRR extracted	98 [a]	0.099	94 [b]	0.095	96%
	parent	15	0.015	18 [b]	0.018	124%
	SYN545720 incl conjugates	47 [d]	0.048	57 [e]	0.058	121%

[a] In the metabolism study primary extraction of soya bean hay and seeds was by hexane (3×), diethyl ether (2×), acetonitrile (2×), acetonitrile/water 4:1 v/v (2×–7×), acetonitrile/water 3:7 v/v (1×–2×), water (1×), and acetonitrile (1×).
[b] In method GRM042.04A primary extraction was by acetonitrile/water 4:1 vv (1×) for hay and acetonitrile/water 1:1 v/v (2×) for seeds. After concentration and acidification of the extract, parent was partitioned into iso-hexane.
[c] In the metabolism study for hay only the acetonitrile/water extracts (26.0% TRR) were subjected to 3 hydrolysis procedures, of which bovine rumen fluid (pH 7, 24 hrs at 39 °C) was the most effective.
[d] In the metabolism study for seeds only the acetonitrile/water extracts (85.0% TRR) were subjected to hydrolysis by

bovine rumen fluid (pH 7, 24 hrs at 39 °C). The resulting hydrolysate was partitioned between diethyl ether and water. The resulting aqueous phase (29.8% TRR) was subjected to hydrolysis by 0.5 M HCl for 6 hrs at 100 °C.
[e] In GRM042.04A the aqueous phase after liquid-liquid partition was subjected to 0.5 M HCl for 6 hrs at 100 °C.

Conclusion:

- Analytical method GRM042.04A and its modifications have three possible moments to analyse parent: just after acetonitrile/water extraction, just after iso-hexane partition or after SPE clean-up. Validation of the method therefore needs to mention the conditions for the parent analysis.
- Analytical method GRM042.04A and its modifications indicate that fortifications of SYN546039 and SYN545720 need to be conducted after the acid hydrolysis procedure. In this way, the full analytical procedure is not validated for the free forms of SYN546039 and SYN545720, because loss or degradation during extraction, liquid-liquid partition and hydrolysis remains unnoticed. It is recommended to include a validation for the whole procedure in case method compliance needs to be shown during actual sample analysis.
- Analytical method GRM042.04A and its modifications use a hydrolysis procedure to cleave the SYN546039 and SYN545720 conjugates. The extraction efficiency of the free forms and the conjugates, the efficacy of the hydrolysis procedure for the conjugates and the stability of the free forms during hydrolysis is validated by a separate radio-validation experiment in soya bean seeds and soya bean hay. The recovery of any SYN546039 or SYN545720 released after hydrolysis is validated by the fortifications of SYN546039 or SYN546720 after the acid hydrolysis procedure.
- The original HPLC-MS-MS method GRM042.04A is considered valid for the determination of parent, SYN546039 (free and conjugated) and SYN545720 (free and conjugated) in the range 0.01–0.1 mg/kg for soya bean seeds, soya bean oil, soya bean milk and 0.01–0.5 mg/kg for soya bean forage. The validity for the parent compound extents only to quantification after the acetonitrile/water extraction stage for soya bean oil, after iso-hexane partition for soya bean seeds and after SPE clean-up for soya bean milk and soya bean forage.
- HPLC-MS-MS method POPIT MET134 Rev 01 is considered valid for the determination of parent, SYN546039 (free and conjugated) and SYN545720 (free and conjugated) in the range 0.01–0.1 mg/kg for soya bean seed. The validity for the parent compound extents only to quantification after iso-hexane partition for soya bean seeds.
- Modification A of HPLC-MS-MS method GRM042.04A is considered valid for the determination of parent and SYN546039 (free) at the level of 0.2 mg/kg for dry soya beans and dry broad beans for the purpose of storage stability studies only.
- Modification B of HPLC-MS-MS method GRM042.04A is considered valid for the determination of parent and SYN546039 (free) at the level of 0.2 mg/kg for maize oil, soya bean flour, soya bean milk and soya bean oil for the purpose of storage stability studies only.
- Modification C of HPLC-MS-MS method GRM042.04A is considered valid for the determination of parent, SYN546039 (free and conjugated) and SYN545720 (free and conjugated) in the range 0.01–1.0 mg/kg for soya bean seeds and aspirated grain fractions, 0.01–0.5 mg/kg for soya bean hulls, soy oil, soya bean meal, fat soy flour, pollard, okara, soy milk, tofu, soy sauce and miso. The validity for the parent compound extents only to quantification after the acetonitrile/water extraction stage for soya bean oil and after the iso-hexane liquid-liquid partition stage for all other soya bean commodities.

HPLC-MS-MS method GRM042.08A

HPLC-MS-MS Method GRM042.08A, version 26 June 2012, [Braid, 2012, SYN545192_10216] is intended for use as pre-registration method for the determination of parent (free), SYN546039 (free

and conjugates) and SYN546206 (free) in plant commodities. The original method or its modification was used in field rotational crop studies and storage stability studies.

High water content crops (10 g) were extracted by homogenisation in 80/20 (v/v) acetonitrile/water. Dry sample matrices (5 g, e.g. grains, straw, hay) were pre-soaked with water (25 mL) for 30 min prior to homogenisation. For the determination of parent and SYN546206 (free), diluted aliquots of the filtered extracts were analysed directly by HPLC-MS-MS. For the determination of SYN546039, aliquots of the extracts were evaporated under a stream of air or nitrogen to remove the acetonitrile, acidified with 1 M HCl and partitioned with 75/25 (v/v) iso-hexane/diethyl ether to separate parent and SYN546206 off. The iso-hexane/diethyl ether fraction was back-partitioned with water to recover the small amount of conjugated SYN546039. The water fraction was then combined with the aqueous acidic fraction. The aqueous acidic fraction was heated at 100 °C for 6 hrs to hydrolyse conjugates of SYN546039. The hydrolysate was cleaned up by SPE and SYN546039 was eluted with 65/35 (v/v) acetonitrile/ water. Following dilution, samples were analysed by HPLC-MS-MS. The primary transition monitored for parent was m/z 396→91 with confirmatory transition m/z 396→368. For SYN546039 the primary transition monitored was m/z 412→91 with confirmatory transition m/z 412→340. The primary transition monitored for SYN546206 is m/z 382→342 with confirmatory transition m/z 382→362. Samples with expected residues > 0.1 mg/kg need to be diluted before analysis.

The analysis conditions as specified in the method description need to be followed carefully. Since parent compound degrades under the hydrolysis conditions used to hydrolyse conjugates of SYN546039 into their free form, parent is separated from the sample before hydrolysis. This step will also remove residues of SYN546206 as these are not conjugated. The metabolite SYN546039 is mainly conjugated in rotational crops and to release the free metabolite, an acid hydrolysis step is required. Change in hydrolysis conditions may result in either incomplete deconjugation or degradation of SYN546039 (free). Parent compound has been observed to adsorb to glass surfaces in solutions with < 50% organic solvent and therefore storage conditions need to be chosen carefully. These considerations have been dealt with in the described analytical method.

HPLC-MS-MS Method GRM042.08A was validated [Watson, 2012c, SYN545192_10203] according to the full validation scheme for spinach, carrot roots and wheat commodities (grain, straw, forage). The validation was performed for the quantification transition ions as well as for the confirmation transition ions. Samples were fortified with a mixture of SYN545192, SYN546039 and SYN546206 standard before extraction. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent or SYN546039 (free) or SYN546206 (free) were within 70–120% limits and RSDs were within 20%. For wheat straw and wheat forage recoveries at 0.01 mg/kg and 0.5 mg/kg were also within these ranges. Linearity of detector response (coefficient of determination $R^2 > 0.99$) was observed in the range 0.1–4 ng/mL using six single standards. This range is equivalent to 0.25–10× LOQ in the samples. Samples which were fortified at 10× LOQ were diluted by a factor of two and samples which were fortified at 50× LOQ were diluted by a factor of 10 to fall within the linear range. The magnitude of the matrix effect was considered to be significant (≥ 20% suppression or enhancement). Matrix matched standards were used for each matrix/analyte combination. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Additional validation data for the determination of SYN546206 were available for spinach, wheat grain, wheat straw and potato at the fortification level used in the storage stability study [Watson, 2013, SYN545192_10137]. SYN546206 was added before extraction as a single analyte per sample. Average recoveries at 0.2 mg/kg of SYN546206 were within 70–120% limits and RSDs were within 20% (n=12). Linearity of detector response (coefficient of correlation r > 0.999) was observed in the range 0.02–10 ng/mL using six single matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Reduced validation data were available for the determination of parent, SYN546206 (free) and SYN546039 (free) in carrot (roots and tops) [Ellis, 2012b/c, A17056B_10004, A17056B_10005]. Average recoveries at 0.01 mg/kg (n=3), 0.05 mg/kg (n=2) and 0.1 mg/kg (n=1) were within 70–

120% limits and RSDs were within 20% at 0.01 mg/kg. Linearity of detector response (coefficient of correlation r > 0.999) was observed in the range 0.02–4 ng/mL using five single matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

Modification A of HPLC-MS-MS method GRM042.08A was used in the US field rotational crop studies [Mäyer, 2012, A17056B_50013]. Parent and SYN546206 were analysed after liquid-liquid partition and SPE clean-up. An aliquot of the acetonitrile/water extracts was evaporated under a stream of nitrogen to remove the acetonitrile. The remaining aqueous extract was acidified with 1 M HCl and partitioned with 75:25 iso-hexane/diethyl ether to separate parent and SYN546206 (organic phase) from SYN546039 (acidic aqueous phase). The iso-hexane/diethyl ether fraction was back-partitioned with water. The organic fraction was evaporated to dryness and taken up in 50/50 v/v acetonitrile/water. The aqueous fractions were combined, diluted to 0.5 M HCl, and heated at 100 °C for 6 hours to hydrolyse conjugates of SYN546039. The post-hydrolysis extract containing the SYN546039 residues was combined with the fraction containing the parent and SYN546206 residues and cleaned-up on SPE using acetonitrile/water (50/50, v/v) as eluent. The eluent was analysed by HPLC-MS-MS.

Only limited validation data were available for modification A for the determination of parent, SYN546206 (free) and SYN546039 (free) in lettuce, spinach, wheat commodities (forage, hay, straw and grain), turnip (roots, tops), radish (roots and tops) [Mäyer, 2012, A17056B_50013]. The original method determines parent and SYN546206 directly in the initial extract, while the modification determines parent and SYN546206 after liquid-liquid partition and an SPE clean-up step, which may introduce losses of both analytes. Individual recoveries at 0.01 mg/kg (n=1) and 0.1 mg/kg (n=1) for each of the commodities were within 70–120% limits. Linearity of detector response (coefficient of correlation r > 0.999) was observed in the range 0.02–4 ng/mL using five single matrix matched standards. No interfering peaks > 0.005 mg/kg) were detected in any of the control extracts.

The extraction procedure used in method GRM042.08A was radio-validated using samples of wheat hay and straw from the pyrazole labelled confined rotational crop study [Roohi and Adams, 2012, SYN545192_10246]. An aliquot of the homogenised wheat hay and straw sample (5 g) was soaked in 25 mL water for 30 min and subsequently extracted with acetonitrile/water (80:20 v/v). Following centrifugation aliquots of the supernatants of each sample were analysed by LSC and TLC

Results are shown in Table 24. Analysis of wheat hay and wheat straw extracts showed that the efficiency of extraction for the residue analytical method was at least 71% for parent and 81% for SYN546206 (free). Efficiency of extraction of SYN546039 from wheat hay and wheat straw was not verified and efficiency of hydrolysis to extract the SYN546049 conjugates from wheat hay and wheat straw was not verified either.

Table 24 Radiovalidation of primary extraction procedures used in analytical method GRM042.08A

Commodity	Components	Confined rotational crop study [a] 90 day PBI		Method GRM042.08A [b]		Efficiency %TRR method: confined study
		%TRR	Residue mg/kg eq	%TRR	Residue mg/kg eq	
Wheat hay	TRR [c] extract	100 [c] 88	0.34 [c] 0.30	100 [c] 68	0.34 [c] 0.23	– 77%
	parent	25	0.085	18	0.061	71%
	SYN546039	1.9—total 1.9—free ND—conj	0.006—total 0.006—free ND—conj	not verified	not verified	–
	SYN546206	14—total 11—free 2.6—conj	0.048—total 0.039—free 0.009—conj	– 9.2—free –	– 0.031—free –	– 81–free –
Wheat straw	TRR [c] extract	100 [c] 87	0.33 [c] 0.29	100 [c] 66	0.33 [c] 0.22	– 76%
	parent	18	0.059	14	0.047	81%

Commodity	Components	Confined rotational crop study [a] 90 day PBI		Method GRM042.08A [b]		Efficiency %TRR method: confined study
		%TRR	Residue mg/kg eq	%TRR	Residue mg/kg eq	
	SYN546039	5.8—total 1.9—free 3.9—conj	0.019—total 0.006—free 0.013—conj	not verified	not verified	
	SYN546206	15—total 14—free 1.3—conj	0.050—total 0.046—free 0.004—conj	– 14—free –	 0.045—free –	– 99–free –

[a] In the confined rotational crop study primary extraction of wheat hay and straw was by acetonitrile (1×), acetonitrile/water (4:1, v/v) (2–3×), acetonitrile/water (3:7, v/v) (1×), water (1×), acetonitrile (1×). Extracts were combined, concentrated, re-suspended in pH 2 water and partitioned with hexane/diethyl ether (1:3 v/v). The organic fraction contained the free metabolites. The aqueous fraction was subjected to hydrolysis by bovine rumen fluid (pH 7, 24 hrs at 39 ℃, under nitrogen). This fraction contained the conjugates.
[b] In method GRM042.08A primary extraction was by acetonitrile/water 4:1 vv (1×).

Conclusion:

- Efficiency of extraction is at least 71% for parent and 81% for SYN546206 (free) as shown by a radio-validation study in wheat hay and wheat straw.
- Efficiency of extraction and hydrolysis for SYN546039 conjugates has been confirmed in a radio-validation study using method GRM042.04A, where the extraction and hydrolysis procedures are identical to those in method GRM042.08A.
- HPLC-MS-MS method GRM042.08A is considered valid for the determination of parent, SYN546039 (free/conjugated) and SYN546206 (free) in the range 0.01–0.1 mg/kg for spinach, carrot (roots, leaves) and wheat grain and 0.01–0.5 mg/kg for wheat straw and wheat forage.
- HPLC-MS-MS method GRM042.08A is considered valid for the determination of SYN546206 (free) at a single level of 0.2 mg/kg in spinach, wheat grain, wheat straw and potato tuber for the purpose of a storage stability study.
- Modification A of HPLC-MS-MS method GRM042.08A is considered valid for the determination of parent, SYN546206 (free) and SYN546039 (free/conjugated) in the range 0.01–0.1 mg/kg for spinach and wheat commodities (forage, hay, straw and grain), since it could be shown that extension of the original method with liquid-liquid partition and an SPE clean-up step did not introduce losses compared to the original method.
- Modification A of HPLC-MS-MS method GRM042.08A (or the original method) is considered insufficiently validated for the determination of parent, SYN546206 (free) and SYN546039 (free/conjugated) in lettuce, turnip (roots, tops) and radish (roots, tops) since only one recovery per level (0.01 and 0.1 mg/kg) is available for each commodity.

HPLC-MS-MS method GRM023.03A

HPLC-MS-MS method GRM023.03A, version 11 February 2010, [Hargreaves, 2010, SYN508210_10287] is intended for use as pre-registration method for the determination of SYN508272 (CSCC210616, free and conjugated) and SYN545720 (CSCD465008, free only) and seven other related chemicals in plant commodities. The original method or its modification was used in a storage stability study.

High water samples and grains (10 g) were extracted by homogenisation in 80/20 (v/v) acetonitrile/water. Straw and hay samples (5 g) were pre-soaked with 25 mL water for 30 min prior to homogenisation. Soya bean hay was extracted twice with 80/20 acetonitrile/water and extracts were combined. For the determination of SYN508272 (CSCC210616), separate aliquots of the initial extracts (equivalent to 0.2 g sample) were evaporated to remove the acetonitrile. The aliquots were

buffered to pH 5 with 0.2 M sodium acetate buffer and hydrolysed using driselase® at 37 °C (6–20 hrs) and cleaned-up through an SPE procedure using Oasis TM HLB cartridges. SYN508272 was eluted with acetonitrile. The acetonitrile was evaporated to dryness and the residue re-dissolved in water. For the determination of SYN545720 (CSCD465008), separate aliquots of the initial extracts (equivalent to 0.2 g) were evaporated to remove the acetonitrile and partitioned with iso-hexane. The aqueous phase was then acidified to pH 1 with 1 M HCl to ensure that SYN545720 is retained on the SPE cartridge and taken through an SPE clean-up procedure, using Oasis TM HLB cartridges. SYN545720 was eluted with 50/50 v/v water/acetonitrile. The eluate was evaporated to remove the acetonitrile and then diluted with water. SYN508272 and SYN545720 were analysed separately by HPLC-MS-MS using different solvent and ionisation systems. The primary transition monitored for SYN508272 was m/z 176→136 with confirmatory transition m/z 176→ 156. For SYN545720 the primary transition monitored was m/z 161→ 141 with confirmatory transition m/z 161 → 66. Samples with expected residues > 0.5 mg/kg need to be diluted before analysis.

The analysis conditions as specified in the method description need to be followed carefully. It is important that all the acetonitrile is removed from the sample prior to enzyme hydrolysis, since any remaining acetonitrile may adversely affect the efficiency of the hydrolysis. Change in enzyme hydrolysis conditions may result in either incomplete deconjugation or degradation of free metabolites. These considerations have been dealt with in the described analytical method.

HPLC-MS-MS Method GRM023.03A was validated [Klimmek and Gizler, 2009, SYN524464_11238] according to the full validation scheme for SYN508272 (CSCC210616) in wheat (grain, straw, whole plant), carrot roots, spinach, potatoes, whole oranges, oilseed rape seeds, dry lentils, tomatoes and maize kernels, using the driselase hydrolysis step as indicated the analytical method. Further the method was validated according to the full validation scheme for SYN545720 (CSCD465008) in whole oranges and dry soya bean seeds without addition of a pectinase hydrolysis step and for SYN545720 (CSCD465008) in potatoes, whole oranges, oil seed rape seeds, lentils, tomatoes, maize kernels and dry soya bean seeds with addition of a pectinase hydrolysis step (see note below). The validation was performed for the quantification transition ions as well as for the confirmation transition ions. Samples were fortified with a mixture of SYN508272 and SYN545720 (and seven other related chemicals) before extraction. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of SYN508272 and SYN545720 were within 70–120% limits and RSDs were within 20%. Linearity of detector response (determination coefficient $R^2 > 0.99$) was observed in the range 0.05–20 ng/mL using 6–8 single standards. This range is equivalent to 0.25–12× LOQ in the samples. Significant enhancement or suppression (> ± 10%) of detector response was observed for some of the matrices tested. Matrix matched standards were used to compensate for these effects observed in method validation. For the determination of SYN508272 (CSCC210616), matrix matched standards were used for wheat grain, wheat straw, orange, oilseed rape seed, lentils, tomatoes, maize kernels and soya bean seeds. For the determination of SYN545720 (CSCD465008), matrix matched standards were used in orange, oilseed rape seed, lentils, tomatoes, maize kernels and soya bean seeds. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts. The viability of individual batches of driselase® to cleave sugar conjugates was investigated by hydrolysing 2-Nitrophenyl β-D-glucopyranoside into 2-nitrophenol, causing a colour change from colourless to yellow/green.

Note: SYN545720 (CSCD465008) residues in crops may be present as conjugates and therefore a pectinase hydrolysis step was included during validation of the analytical method. For the analysis of SYN545720 (free + conjugated) aliquots (equivalent to 0.2 g) were evaporated to remove acetonitrile then buffered to pH 5 with sodium acetate buffer then hydrolysed using pectinase at 37 °C (16-20 hrs). Thereafter, the solution was partitioned with hexane and the aqueous phase was acidified to pH 1 with 1 M HCl and taken through a SPE clean-up procedure using Oasis TM HLB cartridges. SYN545720 was eluted with 50:50 v/v water:acetonitrile. The column eluates were evaporated to remove the acetonitrile and then diluted with water. During this method validation it was demonstrated in oranges and soya bean seed matrix, that the removal/addition of the pectinase hydrolysis step had no adverse effect on the recovery of free SYN545720. The analytical method as used in the storage stability studies did not include the pectinase step.

Additional validation data are available for the determination of SYN508272 and SYN545720 in whole oranges, dry soya bean seeds and dry broad beans [Klimmek *et al,* 2011, SYN524464_11446]. The analytical method included an enzyme hydrolysis step (driselase for SYN508272 and pectinase for SYN465008) as described in the validation report [Klimmek and Gizler, 2009, SYN524464_11238]. Samples were fortified separately with SYN508272 or SYN545720 before extraction. Average recoveries at 0.2 mg/kg of SYN508272 and SYN545720 were within 70–120% limits and RSDs were within 20%.

Conclusion:

- HPLC-MS-MS method GRM023.03A is considered valid for the determination of SYN508272 (free) in the range 0.01–0.1 mg/kg for wheat (grain, straw, whole plant), carrot roots, spinach, potatoes, whole oranges, oilseed rape seeds, dry lentils, tomatoes, maize kernels for the full analytical method including the driselase hydrolysis step.
- HPLC-MS-MS method GRM023.03A is considered valid for the determination of SYN545720 (free) in the range 0.01–0.1 mg/kg in potatoes, whole oranges, oil seed rape seeds, lentils, tomatoes, maize kernels and dry soya bean seeds. For whole oranges and dry soya bean seeds it has been shown that inclusion of a pectinase step does not have an effect on the recovery of the free SYN545720.
- HPLC-MS-MS method GRM023.03A is considered valid for the determination of SYN508272 (free) or SYN545720 (free) at the level of 0.2 mg/kg for whole oranges, dry soya beans and dry broad beans for the purpose of storage stability studies only.
- HPLC-MS-MS method GRM023.03A is considered not valid for the determination of conjugated SYN545720 and conjugated SYN508272, since the efficacy of the hydrolysis procedure has not been verified. However, since the method was only used in a storage stability study (free metabolites), this will have no impact on the results.

Analytical methods used in study reports in animal commodities

HPLC-MS-MS method GRM042.06A and Method 1887 version 2.0

HPLC-MS-MS Method GRM042.06A, version 27 January 2012, [Harris and Braid, 2012, SYN545192_10182] is identical to Method 1887 rev 2.0, version 22 September 2011, [Ward and Harris, 2011, SYN545192_10157]. The method is intended for use as pre-registration method for the determination of parent (free), SYN546039 (free and conjugates) and SYN546422 (free and conjugates) in animal commodities. A modification of the method was used in a cow feeding study.

Meat, liver, kidney, milk, and eggs (10 g) were homogenised with acetonitrile:water (80:20, v/v). Fat (10 g) was dissolved into n-hexane before liquid-liquid partitioning into acetonitrile:water (80:20, v/v). For the determination of parent, diluted aliquots of the extract were analysed directly by HPLC-MS-MS. For determination of SYN546039 and SYN546422, an aliquot of the extract was diluted with water prior to removal of the acetonitrile by evaporation. The remaining extract was then buffered with 0.4 M sodium acetate and conjugates of SYN546039 and SYN546422 were hydrolysed with β-glucuronidase by incubation at 37 °C and pH 5 for a minimum of 6 hours. After hydrolysis, the sample was diluted with acetonitrile:water (50:50, v/v), centrifuged and analysed by HPLC-MS-MS. The primary transition monitored for parent was m/z 396→91 with confirmatory transition m/z 396→ 368. For SYN546039 the primary transition monitored was m/z 412→ 91 with confirmatory transition m/z 412 → 340. The primary transition monitored for SYN546422 is m/z 430→364 with confirmatory transition m/z 430→ 394. Samples with expected residues > 0.1 mg/kg need to be diluted before analysis.

The analysis conditions as specified in the method description need to be followed carefully. To release the free metabolites of SYN546039 and SYN546422 a hydrolysis step is required. It is important that hydrolysis conditions are not changed to ensure efficient hydrolysis. The viability of the β-glucuronidase enzyme needs to be checked prior to use by hydrolysing phenolphthalein

glucuronide. Parent compound has been observed to adsorb to glass surfaces in solutions with < 50% organic solvent. These considerations have been dealt with in the described analytical method.

HPLC-MS-MS Analytical Method 1887 version 2.0 [Ward and Harris, 2011, SYN545192_10157] including the hydrolysis step was validated according to the full validation scheme for bovine meat, liver, kidney, fat, milk, and chicken eggs. The validation was performed for the quantification transition ions as well as for the confirmation transition ions. Samples were fortified with a mixture of SYN545192, SYN546039 and SYN546422 standard before extraction. The reported LOQ was 0.01 mg/kg for each commodity and each analyte. Average recoveries at 0.01 mg/kg and 0.1 mg/kg of parent or SYN546039 (free) or SYN546422 (free) were within 70–120% limits and RSDs were within 20%. Linearity of detector response (coefficient of determination $R^2 > 0.99$ for 1/× weighted regression) was observed in the range of 0.15–10 ng/mL for parent and 0.06–4 ng/mL for SYN546039 and SYN546422 using six single standards. This range is equivalent to 0.3–20× LOQ in the samples. The magnitude of the matrix effect was considered to be significant (≥ 20% suppression or enhancement) for parent, SYN546039 or SYN546422 in meat, liver, kidney or eggs. Matrix-matched calibration standards were used for all analytes in meat, liver and kidney and for SYN546039 and SYN546422 in eggs. Solvent standards were used for all analytes in fat and milk and for SYN545192 in eggs. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts. The viability of the β-glucuronidase enzyme was checked visually before use by observing a colour change from colourless to pink after hydrolysis of phenolphthalein glucuronide to phenolphthalein.

Modification A of HPLC-MS-MS method 1887 Version 2.0 was used in the cow feeding study and the storage stability study in animal commodities [Ward and Vance, 2012, SYN545192_10188]. During analysis of bovine tissues, a chromatographic peak was found that interfered with the analysis of SYN546039. The extraction and work-up procedure were unchanged; modifications were made to the HPLC-MS-MS system only. Samples were fortified with a mixture of parent, SYN546039 and SYN546422 standard before extraction. Additional validation data were available for eggs, whole milk, skimmed milk, cream, bovine muscle, liver, kidney and fat. Average recoveries at 0.01–0.2 mg/kg (milk, muscle and liver) or 0.01–0.1 mg/kg (skimmed milk, cream, kidney and fat) or 0.2 mg/kg (eggs) were within 70–120% limits and RSDs were within 20% (n=2–16 per level). Linearity of detector response (coefficient of correlation r > 0.99, weighting factor 1/×) was observed in the range 0.15–10 ng/mL (parent) or 0.06–4.0 ng/mL (metabolites) using seven single solvent standards or matrix matched standards. No interfering peaks > 0.3LOQ (i.e. 0.003 mg/kg) were detected in any of the control extracts.

The extraction and hydrolysis procedure used in method GRM042.06A was radio-validated using samples of milk, liver and muscle from the pyrazole labelled metabolism study in lactating goats [Green, 2012, SYN545192_10184] and samples of egg yolk from the pyrazole labelled metabolism study in laying hens [Lowrie and Kadow, 2012, SYN545192_10170]. An aliquot of milk (50 g), liver (25 g), muscle (25 g) and egg yolk (25 g) was extracted once with acetonitrile/water (80:20 v/v). An aliquot of the liver extract was evaporated to remove the acetonitrile. The remaining extract was then buffered with 0.4 M sodium acetate and conjugates were hydrolysed with β-glucuronidase by incubation at 37 °C and pH 5 for a minimum of 6 hours. After hydrolysis, the enzyme was denatured by addition of acetonitrile. Aliquots of the milk, liver, muscle and egg extracts and the liver hydrolysate were analysed by LSC and 2D-TLC.

Results are shown in Table 25. Good agreement was obtained between the levels of parent, SYN546039 (free including conjugates) and SYN546422 (free including conjugates) found after extraction (and hydrolysis) as performed in the metabolism study and as performed in method GRM042.06A.

Table 25 Radiovalidation of extraction procedures used in analytical method GRM042.06A

Commodity	Components	Metabolism Study [a]		Method GRM042.06A [b]		Trueness
		%TRR	Residue mg/kg eq	%TRR	Residue mg/kg eq	%TRR method: metabolism
Milk	TRR extract PES	100 100 NA	0.034 0.034 NA	100 96 4.1	0.038 0.036 0.002	– 96% –
	parent	7.3	0.002	10	0.004	142%
	SYN546039	22	0.008	25	0.010	112%
	SYN546422	20	0.007	21	0.008	106%
Liver	TRR extract (hydrolysate) PES	100 78 22	0.70 0.54 0.16	100 73 (71) 27	0.71 0.52 (0.51) 0.19	– 94% –
	parent	10	0.071	11	0.079	109%
	SYN546039 total free conjugates	38 3.3 35	0.26 0.023 0.24	38 3.6 35	0.27 0.026 0.25	101% – –
	SYN546422 total free conjugates	6.0 0.4 5.6	0.042 0.003 0.039	4.0 ND 4.0	0.029 ND 0.029	67% – –
Muscle	TRR extract PES	100 96 3.7	0.032 0.031 0.001	100 95 4.9	0.034 0.032 0.002	– 99% –
	parent	25	0.008	28	0.009	113%
	SYN546039	39	0.012	42	0.014	107%
	SYN546422	8.9	0.003	8.8	0.003	99%
Egg yolk	TRR extract PES	100 83 17	0.18 0.15 0.030	100 75 33	0.18 0.13 0.058	– 90% –
	parent	14	0.024	15	0.026	110%
	SYN546039	12	0.021	13	0.023	110%
	SYN546422	not verified	not verified	not verified	not verified	–

[a] In the metabolism study liver and muscle were primary extracted sequentially with acetonitrile (1×), acetonitrile/water 4:1 v/v (1–4×), acetonitrile/water 3:7 v/v (1–2×), water (1×) and acetonitrile (1×). Liver extracts were subject to β-glucuronidase (pH 5, 37 °C, 18 hrs) and subsequently partitioned with ethyl acetate. Milk was primary extracted with dichloromethane (3×), ethyl acetate (2×) and acetone (1×). Egg yolk was primary extracted with dichloromethane (1×), acetonitrile (1×), acetonitrile/water 4:1 v/v (2–3×), acetonitrile/water 3:7 v/v (1–4×), water (1×) and acetonitrile (1×).
[b] In method GRM042.06A primary extraction was by acetonitrile/water 4:1 vv (1×). Extracts were then subjected to β-glucuronidase (pH 5, 37 °C, 6 hrs). In this experiment only liver extracts were subjected to β-glucuronidase; in the actual method, all matrices were β-glucuronidase.

Conclusion:

- The efficacy of the extraction procedure of parent and SYN546039 (free) is confirmed by a separate radio-validation experiment in muscle, liver, milk and eggs, while the efficacy of the extraction procedure of SYN546422 (free) is confirmed in muscle, liver and milk.
- The efficacy of the extraction and hydrolysis procedure of SYN546039 conjugates or SYN546422 conjugates is confirmed by a separate radio-validation experiment in bovine liver.
- HPLC-MS-MS method GRM042.06A or Analytical Method 1887 version 2.0 is considered valid for the determination of parent (free), SYN546039 (free/conjugated) and SYN546422 (free/conjugated) in the range 0.01–0.1 mg/kg for meat, liver, kidney, fat, milk and eggs.
- Modification A of HPLC-MS-MS method GRM042.06A is considered valid for the determination of parent (free), SYN546039 (free/conjugated) and SYN546422 (free/conjugated) in the range 0.01–0.2 mg/kg (milk, muscle, liver) or 0.01–0.1 mg/kg (skimmed milk, cream, kidney and fat) or 0.2 mg/kg (eggs).

Analytical methods used in study reports in soil and water

The Meeting received analytical methods descriptions and validations for the determination of parent and its metabolites in soil and water. Since soil or water samples were not analysed in the field rotational crop studies, analytical methods for soil and water were not summarized.

Stability of pesticide residues in stored analytical samples

The Meeting received information on storage stability of parent, SYN546039, SYN546206 and SYN545720 in raw and processed plant commodities and of parent, SYN546039 and SYN546422 in animal commodities.

Study 1

Storage stability was investigated by spiking various homogenised plant commodities with 0.20 mg/kg parent or SYN546039 or SYN546206 (one analyte per sample) [Watson, 2013, SYN545192_10137]. Samples were stored for 22–24 months at –18 ºC and were analysed in duplicate at various intervals. Modification A of HPLC-MS-MS method GRM042.03A was used for quantification of parent and SYN546039 in spinach, orange, wheat grain, wheat straw and potato. Modification A of HPLC-MS-MS method GRM042.04A was used for quantification of parent and SYN546039 in dry soya bean seeds and dry broad bean seeds. HPLC-MS-MS method GRM042.08A was used for quantification of SYN546206 in spinach, wheat grain, wheat straw and potato. Methods GRM042.03A, GRM04.04A and GRM042.08A are considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix and control samples had residues < 0.3LOQ (i.e. < 0.003 mg/kg), showing adequate performance at the time of analysis of the samples.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries are shown in Table 26 and 27. Residues of parent and SYN546039 (free) are stable for at least 24 months in crop commodities representative of the high water (spinach), high acid (orange), high starch (wheat grain and potato), high protein (dry broad bean seed), high oil (soya bean seed) commodity groups as well as in wheat straw when stored at or below –18 °C. Residues of SYN546206 are stable for at least 22 months in crop commodities representative of the high water (spinach) and high starch (wheat grain, potato) commodity groups as well as in wheat straw when stored at or below -18 °C.

Table 26 Storage stability of 0.20 mg/kg parent or SYN546039 in crop commodities stored at -18 °C

Matrix	Storage period (days)	parent mg/kg	parent Mean % remaining	parent Mean concurrent recovery (%)	SYN546039 mg/kg	SYN546039 Mean % remaining	SYN546039 Mean concurrent recovery (%)
spinach	0	0.20	100 [a]	109	0.20	100 [a]	102
	91	0.20	101	98	0.22	111	107
	186	0.18	94	101	0.19	98	94
	368	0.20	101	97	0.20	99	98
	550	0.20	102	104	0.19	97	104
	734	0.22	110	98	0.21	105	97
orange	0	0.21	100 [a]	101	0.19	100 [a]	100
	91	0.20	96	97	0.20	102	91
	186	0.19	94	101	0.19	99	98
	368	0.19	94	102	0.19	100	99
	550	0.20	98	103	0.21	106	105
	734	0.20	99	100	0.21	106	99
wheat grain	0	0.20	100 [a]	104	0.21	100 [a]	107
	91	0.19	94	103	0.16	77	78
	186	0.20	98	101	0.20	99	105
	368	0.20	100	103	0.20	98	103
	549	0.20	99	106	0.20	96	106
	733	0.20	101	106	0.22	106	103

Matrix	Storage period (days)	parent mg/kg	parent Mean % remaining	parent Mean concurrent recovery (%)	SYN546039 mg/kg	SYN546039 Mean % remaining	SYN546039 Mean concurrent recovery (%)
wheat straw	0	0.19	100 [a]	93	0.19	100 [a]	96
	91	0.19	103	92	0.15	80	74
	186	0.20	106	95	0.18	95	95
	368	0.22	115	98	0.20	103	100
	549	0.22	115	101	0.20	104	98
	733	0.23	120	105	0.21	112	100
potato	0	0.19	100 [a]	106	0.20	100 [a]	99
	91	0.19	100	99	0.19	94	99
	186	0.18	96	100	0.19	98	104
	368	0.19	99	99	0.19	95	97
	550	0.20	103	102	0.20	100	105
	734	0.20	107	102	0.21	104	103
dry soya bean seed	0	0.19	100 [a]	100	0.20	100 [a]	108
	91	0.19	100	101	0.19	97	95
	184	0.15	81	83	0.17	87	93
	366	0.18	96	84	0.21	106	103
	548	0.20	105	106	0.20	102	105
	730	0.17	89	82	0.18	90	97
dry broad bean seed	0	0.20	100 [a]	100	0.19	100 [a]	99
	91	0.20	101	107	0.20	106	99
	184	0.21	105	107	0.20	105	97
	366	0.21	105	99	0.19	102	98
	548	0.21	105	102	0.21	108	102
	730	0.22	108	104	0.21	110	102

[a] The% remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.

Table 27 Storage stability of 0.20 mg/kg SYN546206 in crop commodities stored at -18 °C

Matrix	Nominal storage period (months)	SYN546206 mg/kg	SYN546206 Mean % remaining	SYN546206 Mean concurrent recovery (%)
spinach	0	0.18	100 [a]	94
	93	0.19	110	100
	183	0.20	113	101
	366	0.18	103	93
	554	0.18	102	99
	672	0.21	120	106
wheat grain	0	0.19	100 [a]	97
	93	0.19	98	101
	183	0.18	93	100
	366	0.18	92	101
	554	0.18	90	99
	672	0.20	102	107
wheat straw	0	0.19	100 [a]	107
	92	0.21	107	107
	182	0.20	102	95
	365	0.18	93	100
	553	0.18	94	95
	671	0.21	110	107
potato	0	0.19	100 [a]	98
	92	0.19	101	98
	182	0.19	98	99
	365	0.19	96	99

Matrix	Nominal storage period (months)	SYN546206 mg/kg	SYN546206 Mean % remaining	SYN546206 Mean concurrent recovery (%)
	553	0.19	97	102
	671	0.21	110	106

[a] The % remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.

Study 2

Storage stability was investigated by spiking various homogenised plant commodities with 0.20 mg/kg SYN508272 and SYN545720 [Klimmek *et al,* 2011, SYN524464_11446]. Samples were stored for 24 months at –18 °C and were analysed in duplicate at various intervals using HPLC-MS-MS method GRM023.03A. The analytical method included an enzyme hydrolysis step (driselase for SYN508272 and pectinase for SYN465008) as described in the validation report of the method [Klimmek and Gizler, 2009, SYN524464_11238]. Method GRM023.03A is considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix and control samples had residues < 0.3LOQ (i.e. < 0.003 mg/kg), showing adequate performance at the time of analysis of the samples.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries are shown in Table 28. Residues of SYN508272 (free) and SYN545720 (free) are stable for at least 24 months in crop commodities representative of the high oil (soya bean seeds), high acid (orange) and high protein (dry broad bean seeds) commodity groups when stored at or below -18 °C.

Note: The study report describes storage stability for a set of seven related chemicals. SYN508272 (CSCC210616) and SYN545720 (CSCD465008) were added as a single compound to separate samples. The other related chemicals were added to other samples. Addition of these chemicals did therefore not affect the storage stability results of SYN508272 or SYN545720.

Table 28 Storage stability of 0.20 mg/kg SYN545720 or SYN508272 in crop commodities stored frozen at -18 °C

Matrix	Storage period (days)	SYN545720 mg/kg	mean % remaining	Mean procedural recovery (%)	SYN508272 mg/kg	mean % remaining	Mean procedural recovery (%)
orange	0–1	0.21	100 [a]		0.15	100 [a]	
	90	0.20	95	96	0.17	113	81
	181	0.19	91	85	0.18	118	98
	365	0.20	96	100	0.20	132	96
	540–559	0.19	92	74	0.17	113	90
	735	0.18	87	90	0.21	135	102
dry soya bean seed	0–4	0.17	100 [a]		0.21	100 [a]	
	90	0.16	93	94	0.17	81	84
	183	0.20	113	92	0.20	95	102
	369	0.21	122	103	0.19	90	102
	540	0.14	80	78	0.18	85	81
	743	0.17	98	86	0.18	89	86
dry broad bean seed	0–5	0.16	100 [a]		0.18	100 [a]	
	89	0.20	124	95	0.17	95	88
	180	0.17	106	94	0.21	114	102
	198	–	–	–	0.24	132	118
	364	0.20	123	92	0.20	109	105

Matrix	Storage period (days)	SYN545720 mg/kg	mean % remaining	Mean procedural recovery (%)	SYN508272 mg/kg	mean % remaining	Mean procedural recovery (%)
	539	0.16	101	101	0.18	97	86
	734	0.18	112	88	0.21	115	109

[a] The % remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.

Study 3

Storage stability was investigated by spiking homogenised processed plant commodities with 0.20 mg/kg parent or SYN546039 or SYN545720 (one analyte per sample) [Hagan and Bertrand, 2012/2013, SYN545192_50177, SYN545192_50544]. The SYN545720 compound was only added to processed soya bean commodities. Samples were stored for 24 months at –10 ºC and were analysed in duplicate at various intervals. Modification B of HPLC-MS-MS method GRM042.03A was used for the quantification of parent and SYN546039 in maize flour and maize meal, raisins, dried apple and apple juice. Modification B of HPLC-MS-MS method GRM042.04A was used for the quantification of parent, SYN546039 and SYN545720 in maize oil, soya bean flour, soya bean milk and soya bean oil. Methods GRM042.03 and GRM042.04A are considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix. Levels in control samples were < 0.04 mg/kg, but since the fortification levels are 0.2 mg/kg, this is considered to have no impact on the study results. Therefore, performance is considered adequate at the time of analysis of the samples.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries are shown in Tables 29 and 30. Residues of parent, SYN546039 (free) and SYN545720 (free) are stable for at least 24 months in crop commodities various processed crop commodities (flour, meal, oil, soymilk, dried fruit, fruit juice) when stored at or below –10 °C.

Table 29 Storage stability of 0.20 mg/kg parent or SYN546039 in processed crop commodities stored at -10 °C

Matrix	Storage period (days)	parent mg/kg	parent Mean % remaining	parent Mean concurrent recovery (%)	SYN546039 mg/kg	SYN546039 Mean % remaining	SYN546039 Mean concurrent recovery (%)
Maize flour	0	0.23	100 [a]	103	0.22	100 [a]	104
	93	0.23	100	119	0.21	93	110
	179	0.20	85	101	0.20	89	104
	274	0.17	72	98	0.17	77	91
	375	0.22	93	110	0.15	66	83
	554	0.21	89	102	0.19	84	92
	731	0.23	98	113	0.21	93	103
Maize meal	0	0.22	100 [a]	109	0.21	100 [a]	101
	93	0.23	105	114	0.20	96	109
	179	0.19	86	99	0.18	89	93
	274	0.16	76	94	0.16	76	88
	362	0.18	81	104	0.15	71	80
	555	0.21	96	102	0.18	87	101
	731	0.17	79	103	0.20	97	101
Maize refined oil	0	0.23	100 [a]	113	0.22	100 [a]	111
	93	0.18	79	100	0.19	86	104
	181	0.19	81	101	0.17	76	92
	275	0.16	67	78	0.16	69	81
	366	0.21	89	93	0.15	68	77
	554	0.20	88	116	0.22	96	99
	734	0.17	72	79	0.18	81	99

Matrix	Storage period (days)	parent mg/kg	parent Mean % remaining	parent Mean concurrent recovery (%)	SYN546039 mg/kg	SYN546039 Mean % remaining	SYN546039 Mean concurrent recovery (%)
Soya bean flour	0	0.21	100 [a]	102	0.21	100 [a]	91
	94	0.22	107	93	0.19	90	84
	181	0.16	76	80	0.17	81	81
	277	0.18	83	81	0.17	83	75
	371	0.21	98	100	0.18	88	86
	554	0.21	100	99	0.22	105	107
	732	0.19	89	86	0.16	77	79
Soya bean milk	0	0.20	100 [a]	92	0.20	100 [a]	110
	92	0.19	96	95	0.19	93	95
	180	0.18	92	98	0.18	86	102
	273	0.17	86	84	0.16	79	97
	364	0.22	109	104	0.17	85	80
	558	0.18	93	83	0.20	95	102
	730	0.20	101	97	0.19	94	99
Soya bean crude oil	0	0.19	100 [a]	89	0.17	100 [a]	92
	95	0.20	106	98	0.16	96	87
	181	0.17	90	97	0.17	100	90
	277	0.19	97	104	0.17	102	86
	372	0.22	114	112	0.16	96	82
	558	0.18	96	92	0.16	97	101
	731	0.22	114	101	0.19	111	99
Grape raisins	0	0.21	100 [a]	116	0.23	100 [a]	118
	93	0.22	104	99	0.22	94	119
	181	0.21	98	113	0.21	92	112
	273	0.20	93	116	0.20	85	108
	361	0.24	115	128	0.21	88	119
	560	0.23	107	115	0.21	90	111
	734	0.24	112	127	0.23	100	118
Apple dried	0	0.22	100 [a]	107	0.23	100 [a]	111
	97	0.23	102	114	0.23	101	120
	180	0.22	96	105	0.23	98	116
	272	0.19	85	114	0.18	80	107
	365	0.23	102	125	0.17	73	90
	564	0.21	92	114	0.22	93	120
	739	0.23	102	119	0.23	99	114
Apple juice	0 [b]	–	–	–	–	–	–
	97	0.22	110	120	0.23	117	113
	180	0.19	96	103	0.20	100	100
	272	0.15	76	88	0.16	80	99
	365	0.18	90	112	0.14	71	86
	557	0.20	98	105	0.19	93	103
	734	0.22	112	111	0.21	103	107

[a] The % remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.
[b] Zero day sample is missing,% remaining based on nominal fortification level of 0.20 mg/kg

Table 30 Storage stability of 0.20 mg/kg SYN545720 in crop commodities stored at -10 °C

Matrix	Storage period (days)	SYN545720 mg/kg	SYN545720 mean % remaining	Mean concurrent recovery (%)
soya bean flour	0	0.18	100 [a]	89
	94	0.22	122	95
	181	0.15	84	86

Matrix	Storage period (days)	SYN545720 mg/kg	SYN545720 mean % remaining	Mean concurrent recovery (%)
	277	0.17	93	85
	371	0.18	100	87
	554	0.18	100	86
	732	0.16	89	81
soya bean milk	0	0.15	100 [a]	78
	92	0.19	127	96
	180	0.18	122	94
	273	0.16	104	73
	364	0.18	120	101
	558	0.16	103	102
	730	0.20	129	93
soya bean crude oil	0	0.19	100 [a]	104
	95	0.17	89	92
	181	0.16	85	95
	277	0.16	84	80
	372	0.19	100	109
	558	0.20	106	104
	731	0.20	106	89

[a] The % remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.

Study 4

Storage stability was investigated by spiking various animal commodities with 0.20 mg/kg parent, SYN546039 or SYN546422 (one analyte per sample) [Ward and Vance, 2012, SYN545192_10188]. Samples for concurrent recoveries were spiked with a mixture of these three analytes. Samples were stored for 56–78 days at –20 ºC and were analysed in duplicate using modification A of HPLC-MS-MS method 1887 (see method GRM042.06). The modification of Method 1887 is considered valid for the purpose of this study (commodity type and concentration level of the analytes). Average concurrent recoveries were within 70–120% for each analyte and matrix and control samples had residues < 0.3LOQ (i.e. < 0.003 mg/kg), showing adequate performance at the time of analysis of the samples.

Storage stability results (not corrected for concurrent recovery) and concurrent recoveries are shown in Table 31. Residues of SYN545192, SYN546039 and SYN546422 are stable in milk stored frozen for at least 62 days, in eggs for at least 56 days, in liver for at least 78 days and in muscle for at least 76 days. According to OECD Guideline 506 storage stability in kidney and fat may be extrapolated from the other animal tissues.

Table 31 Storage stability of 0.20 mg/kg parent, SYN546039 and SYN546422 in animal commodities stored at -20 °C

Analyte	Matrix	Storage period (days)	Mean residue (mg/kg)	Mean % remaining	Mean concurrent recovery (%)
parent	milk	0	0.18	100 [a]	89
		62	0.21	119	94
SYN546039	milk	0	0.22	100 [a]	111
		62	0.19	86	88
SYN546422	milk	0	0.24	100 [a]	119
		62	0.19	77	83
parent	egg	0	0.20	100 [a]	100
		56	0.22	108	109
SYN546039	egg	0	0.19	100 [a]	94
		56	0.22	115	104
SYN546422	egg	0	0.18	100 [a]	90
		56	0.19	103	98
parent	muscle	0	0.20	100 [a]	100
		76	0.21	105	109

Analyte	Matrix	Storage period (days)	Mean residue (mg/kg)	Mean % remaining	Mean concurrent recovery (%)
SYN546039	muscle	0	0.19	100 [a]	96
		76	0.17	88	86
SYN546422	muscle	0	0.19	100 [a]	93
		76	0.16	83	85
parent	liver	0	0.17	100 [a]	87
		56	0.21	118	100
		78	0.21	118	110
SYN546039	liver	0	0.21	100 [a]	106
		56	0.19	87	102
		78	0.19	87	93
SYN546422	liver	0	0.19	100 [a]	97
		56	0.17	85	98
		78	0.16	80	94

[a] The % remaining was set at 100% at time zero. If 0.16 mg/kg was found at time zero, instead of the expected 0.20 mg/kg, the 0.16 mg/kg was set at 100%, to see the development of this residue level in time.

USE PATTERN

Benzovindiflupyr is a broad-spectrum fungicide belonging to the chemical class of pyrazole carboxamides (like bixafen, fluxapyroxad, furametpyr, isopyrazam, penflufen, penthiopyrad, sedaxane). Benzovindiflupyr acts as an inhibitor of the fungal complex II respiratory chain, where it inhibits the succinate dehydrogenase enzyme (succinate dehydrogenase inhibitor, SDHI fungicide) by blocking the ubiquinone-binding sites in the mitochondrial complex. Benzovindiflupyr is a broad-spectrum fungicide, especially used for its effect against rust. [Label information and Mäyer, 2012, A17056B_50013].

Benzovindiflupyr is a registered fungicide in soya beans only and only in Paraguay (Table 32). The original registered labels were submitted in the original language as well as in its English translation.

Benzovindiflupyr registrations are intended in the USA in 2014 for pome fruit, blueberries, grapes, fruiting vegetables (including cucurbits and sweet corn), root and tuber vegetables, pulses (dry beans, dry peas and soya beans), cereals (barley, maize and wheat), oilseeds (cottonseed; peanuts and rapeseed), sugarcane and coffee beans. At the time of this evaluation, the respective labels were not authorised and consequently the residue trials were not submitted.

Table 32 Registered pre-harvest uses of benzovindiflupyr

Crop	Country	Form (g ai/kg)	Application				PHI, days
			Method	Rate kg ai/ha	Spray volume	Number (interval in days)	
Soya bean	Paraguay	WG 150 [a, b]	Foliar spray	3× 0.045	100–200 L/ha	3 (14)	21

[a] water dispersible granule containing 150 g/kg benzovindiflupyr and 300 g/kg azoxystrobin.
[b] The product must be used with a paraffinic mineral oil, used as an adjuvant. The recommendation is to add the adjuvant to the water volume together with the formulated product for benzovindiflupyr [Syngenta, 2014b].

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

The Meeting received information on supervised residue trials of benzovindiflupyr for soya beans only. Application rates and spray concentrations have been rounded to two significant figures; residues at around the LOQ or 0.01 mg/kg have been rounded to one figure. Residue data are recorded unadjusted for percentage recoveries or for residue values in control samples unless otherwise stated. Unquantifiable residues are shown as below the reported LOQ (e.g. < 0.01 mg/kg). Where multiple samples were taken from a single plot or where multiple analyses were conducted on a single sample, the average value is reported. Where results from separate plots with distinguishing characteristics such as different formulations, crop varieties or treatment schedules were reported, results are listed

separately for each plot, but only one value is selected for MRL derivation. Residues from the trials conducted according to critical GAP have been used for the estimation of maximum residue levels, STMR and HR values. Those results are underlined.

Soya beans

Supervised residue field trials were conducted on soya beans in the 2010–2011 season in Brazil [Casallanova, 2011a/b/c, A17961A_10022; A17056F_10022; A18126B_10032]. Soy plants were treated with an EC formulation containing 150 g ai/L benzovindiflupyr alone (report M11063) or 50 g ai/L benzovindiflupyr in combination with 100 g ai/L azoxystrobin (report M11075) or with a WG formulation containing 150 g ai/kg benzovindiflupyr in combination with 300 g ai/kg azoxystrobin (report M11086). Small plots of soya beans (60–90 m^2) were treated as indicated in Table 33 using boom sprayers with a spray volume of 200 L/ha. All treatments included Nimbus adjuvant at 600 mL/ha.

Soya bean plants were harvested at maturity at three time points after the final application with growth stages BBCH 85–97, 86–97 and 87–97. Soya bean plants were threshed immediately to separate the seeds and the rest of plant. Samples were collected from at least 12 different points along the plot reaching at least 2 kg of soya bean seeds after threshing. Sample sizes were in accordance with appendix V of the FAO manual. All samples were stored frozen for 15–73 days at –20 ºC. This storage period is covered by the storage stability studies on parent, SYN546039 and SYN545720 in commodities with high oil content.

Samples were analysed for parent and its metabolites SYN546039 and SYN545720 using HPLC-MS-MS method POPIT MET.134 (see GRM042.04A). The analytical method is considered valid in the range 0.01–0.1 mg/kg for the determination of parent, free and/or conjugated SYN546039 and free and/or conjugated SYN545720 in dry soya bean seed. Average concurrent recoveries were within 70–120% for each analyte and control samples had residues < LOQ (i.e. < 0.01 mg/kg), showing adequate performance at the time of analysis of the samples.

Residues of benzovindiflupyr and its metabolites SYN546039 and SYN545720 from the trials are summarised in Table 33.

Table 33 Residues in soya bean seeds after foliar spray applications in the field

SOYA BEAN SEEDS Location, year, (Variety) Soil type	Form	No (Interval days)	kg ai/ha	kg ai/hL	GS, date (last appl)	DAT	parent mg/kg	SYN 546039 mg/kg	SYN 545720 mg/kg	Report; Trial; (remarks)
Holambra, SP, Brazil, 2010–2011, (CD 214 RR) Soil: Sa/Si/Cl= 52.0/12.5/35.5	EC [a]	3 (19,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 76–78, 30-Mar	21 28 35	< 0.01 0.02 0.02	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-DMO1 I
Holambra, SP, Brazil, 2010–2011, (CD 214 RR) Soil: Sa/Si/Cl= 52.0/12.5/35.5	EC [a]	3 (19,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 76–78; 30-Mar	21 28 35	< 0.01 < 0.01 0.02	< 0.01 < 0.01 < 0.01	< 0.01 0.01 0.01	M11063; M11063-DMO1 [c]
Holambra, SP, Brazil, 2010–2011, (CD214 RR) Soil: Sa/Si/Cl= 52.0/12.5/35.5	EC [a, b]	3 (19,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 76–78, 30-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-DMO3 [c]
Holambra, SP, Brazil, 2010–2011, (CD214 RR)	EC [a, b]	3 (19,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 76–78, 30-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-DMO3

SOYA BEAN SEEDS Location, year, (Variety) Soil type	Form	No (Interval days)	kg ai/ha	kg ai/hL	GS, date (last appl)	DAT	parent mg/kg	SYN 546039 mg/kg	SYN 545720 mg/kg	Report; Trial; (remarks)
Soil: Sa/Si/Cl= 52.0/12.5/35.5										[c]
Holambra, SP, Brazil, 2010–2011, (CD214 RR) Soil: Sa/Si/Cl= 52.0/12.5/35.5	WG [a, b]	3 (19,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 76–78, 30-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-DMO1 [c]
Holambra, SP, Brazil, 2010–2011, (CD214 RR) Soil: Sa/Si/Cl= 52.0/12.5/35.5	WG [a, b]	3 (19,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 76–78, 30-Mar	21 28 35	0.03 < 0.01 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-DMO1 [c]
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	EC [a]	3 (22,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-DMO2
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	EC [a]	3 (22,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-DMO2
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	EC [a, b]	3 (22,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-DMO2
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	EC [a, b]	3 (22,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-DMO2
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	WG [a, b]	3 (22,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-DMO2
Bandeirantes, PR, Brazil, 2010–2011, (BMX Potencia RR) Soil: Sa/Si/Cl= 2.0/18.0/80.0	WG [a, b]	3 (22,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 79, 1-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-DMO2
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR)	EC [a]	3 (22,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-JJB1

SOYA BEAN SEEDS Location, year, (Variety) Soil type	Form	No (Interval days)	kg ai/ha	kg ai/hL	GS, date (last appl)	DAT	parent mg/kg	SYN 546039 mg/kg	SYN 545720 mg/kg	Report; Trial; (remarks)
Soil: Sa/Si/Cl= 15.1/15.5/69.3										
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR) Soil: Sa/Si/Cl= 15.1/15.5/69.3	EC [a]	3 (22,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-JJB1
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR) Soil: Sa/Si/Cl= 15.1/15.5/69.3	EC [a, b]	3 (20,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB1
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR) Soil: Sa/Si/Cl= 15.1/15.5/69.3	EC [a, b]	3 (20,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB1
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR) Soil: Sa/Si/Cl= 15.1/15.5/69.3	WG [a, b]	3 (22,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB1
Uberlândia, MG, Brazil, 2010–2011, (SYN 9070 RR) Soil: Sa/Si/Cl= 15.1/15.5/69.3	WG [a, b]	3 (22,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 80, 9-Mar	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB1
Engenheiro Coelho, SP, Brazil, 2010–2011, (Vallosa) Soil: Sa/Si/Cl= 58.0/8.5/33.5	EC [a]	3 (59,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 73–74, 25-Apr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-AMA
Engenheiro Coelho, SP, Brazil, 2010–2011 (Valosa) Soil: Sa/Si/Cl= 58.0/8.5/33.5	EC [a]	3 (59,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 73–74, 25-Apr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11063; M11063-AMA
Engenheiro Coelho, SP, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 44.0/13.8/42.2	EC [a, b]	3 (59,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 73–74, 25-Apr	14 21 28	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-AMA
Engenheiro Coelho, SP, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 44.0/13.8/42.2	EC [a, b]	3 (59,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 73–74, 25-Apr	14 21 28	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-AMA

SOYA BEAN SEEDS Location, year, (Variety) Soil type	Form	No (Interval days)	kg ai/ha	kg ai/hL	GS, date (last appl)	DAT	parent mg/kg	SYN 546039 mg/kg	SYN 545720 mg/kg	Report; Trial; (remarks)
Engenheiro Coelho, SP, Brazil, 2010–2011 (Valiosa) Soil: Sa/Si/Cl= 44.0/13.8/42.2	WG [a, b]	3 (59,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 73–74, 25-Apr	14 21 28	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-AMA
Engenheiro Coelho, SP, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 44.0/13.8/42.2	WG [a, b]	3 (59,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 73–74, 25-Apr	14 21 28	< 0.01 < 0.01 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-AMA
Rio Verde, GO, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 42.5/8.8/48.7	EC [a, b]	3 (23,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB2 [c]
Rio Verde, GO, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 42.5/8.8/48.7	EC [a, b]	3 (23,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB2 [c]
Rio Verde, GO, Brazil, 2010–2011, (Valiosa) Soil: Sa/Si/Cl= 42.5/8.8/48.7	WG [a, b]	3 (23,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB2 [c]
Rio Verde, GO, Brazil 2010–2011 (Valiosa) Soil: Sa/Si/Cl= 42.5/8.8/48.7	WG [a, b]	3 (23,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB2 [c]
Rondonópolis, MT, Brazil, 2010–2011, (TMG 132) Soil: Sa/Si/Cl= 61.0/0.1/39.0	EC [a, b]	3 (23,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB3 [c]
Rondonópolis MT, Brazil, 2010–2011, (TMG 132) Soil: Sa/Si/Cl= 61.0/0.1/39.0	EC [a, b]	3 (23,14)	0.050 0.050 0.050	0.025 0.025 0.025	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11075; M11075-JJB3
Rondonópolis, MT, Brazil, 2010–2011 (TMG 132) Soil: Sa/Si/Cl= 61.0/0.1/39.0	WG [a, b]	3 (23,14)	0.030 0.030 0.030	0.015 0.015 0.015	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB3 [c]
Rondonópolis, MT, Brazil, 2010–2011, (TMG 132)	WG [a, b]	3 (23,14)	0.045 0.045 0.045	0.022 0.022 0.022	BBCH 79, 24-Febr	21 28 35	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	< 0.01 < 0.01 < 0.01	M11086; M11086-JJB3

SOYA BEAN SEEDS Location, year, (Variety) Soil type	Form	No (Interval days)	kg ai/ha	kg ai/hL	GS, date (last appl)	DAT	parent mg/kg	SYN 546039 mg/kg	SYN 545720 mg/kg	Report; Trial; (remarks)
Soil: Sa/Si/Cl= 61.0/0.1/39.0										[c]

Brazilian States: SP Sao Paulo, GO Goiás, MG Minas Gerais, PA Parana
[a] 600 mL/ha of Nimbus adjuvant added to the spray solution. Nimbus is a paraffinic mineral oil, used as an adjuvant [Syngenta, 2014b]
[b] The formulation also contained azoxystrobin. The treatment of 0.030/0.045/0.050 kg ai/ha for benzovindiflupyr resulted in 0.060/0.090/0.100 kg ai/ha for azoxystrobin. All benzovindiflupyr treatments were preceded by a single treatment with azoxystrobin at 0.050 kg ai/ha.
[c] Rainfall within 24 hrs after the last application: 16 mm in in Holambra, 10 mm in Rio Verde and 0.4 mm in Rondonopolis. Given the high residues found in Holambra, trials can be selected for MRL derivation.

FATE OF RESIDUES IN STORAGE AND PROCESSING

In storage

No data submitted.

In processing

The Meeting received information on the nature of residues under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation. In addition the Meeting received processing studies on soya beans.

Study 1

The behaviour of benzovindiflupyr was studied under conditions simulating pasteurisation, baking/brewing/boiling and sterilisation [Simmonds, 2011, SYN545192_10162]. Duplicate solutions of 1.00–1.01 mg/L [pyrazole-5-^{14}C]-benzovindiflupyr were prepared in 0.1 M sterile citrate buffers at pH 4, 5 and 6 in glass tubes. The buffer solutions were incubated in the dark for 20 minutes at 88–90 °C (pH 4), 60 minutes at 98–100 °C (pH 5) or 20 minutes at 120–121 °C (pH 6) and 0.95–1.00 bar. Control samples from each pH group were incubated in the dark at room temperature. The buffer solution was then removed from each tube and the tubes rinsed with acetonitrile to recover the radioactivity adsorbed to the glassware. The buffer and rinse solutions were combined and analysed by LSC on the day of incubation. All samples were analysed by HPLC with radio-detection and representative samples were also analysed by TLC with radio-detection to confirm the identity of benzovindiflupyr parent. Parent was used as reference standard for both analyses.

The pH of the solutions did not change throughout the course of each experiment. The mean recovery of radioactivity applied to the test systems ranged from 98.4 to 100.7% for hydrolysed samples and from 97.7 to 101.8% for control sample. Table 34 shows radioactivity in the treated samples and control samples comprised only parent (no hydrolysis products were observed).

Table 34 Recovery and identification of radioactivity in 1 mg/L [^{14}C]benzovindiflupyr solutions under pasteurisation, baking/brewing/boiling and sterilisation simulating conditions

Conditions	post incubation (%TAR)	parent (%TRR)
pH 4 (90 °C, 20 min) (pasteurisation)	99/101	100/100
pH 5 (100 °C, 60 min) (baking/brewing/boiling)	99/100	100/100
pH 6 (120 °C, 20 min) (sterilisation)	99/98	100/100

Study 2

Two supervised residue field trials with soya beans were conducted in Greece in 2010 [Ellis, 2012a, A17056D_10002]. Small plots of soya beans (360 m^2) were treated with an EC formulation containing 150 g ai/L benzovindiflupyr alone. Four foliar applications were made, the first two at 0.074–0.075 kg ai/ha and the second two at an exaggerated rate of 0.369–0.372 kg ai/ha, each with a spray interval of 7 days. The applications were made with a backpack sprayer at a spray volume of 591–598 L/ha (no adjuvant). The last application was made at growth stage BBCH 82 (19–20 October 2010). Soya bean plants were harvested at normal commercial harvest (BBCH 89), 14 days after the last application. Plants were harvested and threshed using a combine and then separated further using an air stream to remove lighter parts and heavy parts were removed by hand. The seeds (50 kg per trial) were transported to the processing facility (Melissohori, Greece) at ambient temperature (< 5 hrs). The soya bean seeds were processed into refined oil, fat soy flour, tofu and soy sauce using standardised procedures designed to simulate relevant industrial processes. All weight fractions have been calculated for the initial weight of sub-sample processed in the relevant step for trial 01 and 02 respectively.

Refined oil processing

Soya bean seeds (26.140/25.460 kg) were cleaned by air cleaning, resulting in cleaned soya bean seeds (25.907/25.267 kg) and aspirated grain fractions (0.233/0.193 kg). The cleaned soya bean seeds were then dried until the optimal moisture content was reached. Afterwards the conditioned soya bean seeds were ground and sieved, resulting in soya bean hulls (2.393/2.300 kg) and dehulled soya bean seeds (22.054/20.626 kg). Subsequently the dehulled soya bean seeds were conditioned and flaked. The flakes were then extracted in two steps. Firstly n-hexane was added, heated up to around 60 °C and circulated for about 2 hours and then the solvent-oil mixture was distilled to separate oil and solvent. A rotary evaporator was used to remove any remaining solvent from the oil. The resulting fractions were crude oil (5.109/4.941 kg) and wet soya bean meal (17.936/17.168 kg). The wet soya bean meal was toasted at 120 °C to inactivate trypsin inhibitors. The toasted soya bean meal was conditioned to obtain an optimal moisture content of 15%. After conditioning the meal was milled, resulting in milled conditioned soya bean meal (20.033/19.216 kg). The crude oil was refined in several steps. For hydration, the crude oil was heated up to 60–70 °C and 10% of water was added. The mixture was continuously stirred for 45 min at 85–90 °C. After that the mixture was left for phase separation and the watery phase was removed. The remaining oily phase was heated up to 85 °C and 1% of phosphorus acid was added. The mixture was continuously stirred for 45 min at 85 °C. After that, 10% water was added and the mixture was left at 85 °C for phase separation. The watery phase was removed. For neutralisation the oily phase was heated up to 90 °C while continuously stirred. After that sodium hydroxide was added and the mixture was left for about 20 min. Then 10% of water was added and after 5 min the stirrer was switched off. After separation of phases the soapstock was removed. For washing, the remaining oily phase was heated up to 90 °C while stirred. After that, 10% of water was added and the mixture was left for 20 min for phase separation. The watery phase was removed. For drying, the remaining oily phase was heated up to 95 °C while stirred and citric acid was added. Drying was done using a vacuum until no more water remained in the oil. For bleaching, the oil was heated up to 95 °C while stirred. The oil was bleached for 35 min after addition of 1% of podsol. After that, the oil was filtered to remove the podsol. For deodorisation the oil was heated up and steam was transferred through the oil when it reached 160 °C. The oil was heated up further, until it reached 240 °C and then stayed at this temperature for about 20 min. The steam supply was stopped after cooling down to 160 °C. Drying of the oil was performed until a temperature ≤ 80 °C was reached. The resulting fraction is the refined oil (2.606/2.452 kg).

Fat soy flour processing

Soya bean seeds (5.000/5.000 kg) were cleaned by air cleaning, resulting in cleaned soya bean seeds (4.945/4.967 kg) and aspirated grain fractions (0.055/0.033 kg). Afterwards the soya bean seeds were milled, resulting in pollard (1.058/1.174 kg) and dehulled soya bean seeds (3.875/3.769 kg). Dehulled soya bean seeds were milled to get fat soy flour (3.440/3.385 kg).

Tofu processing

Soya bean seeds (5.000/5.000 kg) were cleaned by air cleaning, resulting in cleaned soya bean seeds (4.934/4.955 kg) and aspirated grain fractions (0.066/0.045 kg). The cleaned soya bean seeds were mixed with 2.5 fold rate of water for 12 hours swelling time. Afterwards mixture was separated to steep water and swelled soya bean seeds. The swelled soya bean seeds were mixed with 5 fold tap water and cooked for 10 min with continuous stirring. After cooking, the suspension was squeezed to get okara (9.792/9.627 kg) and soy milk (48.384/48.445 kg). The soy milk was heated up and at 85 °C calcium sulfate was added. The mixture was pressed to get raw tofu. The raw tofu was pasteurised at 82–87 °C for 5 min. After cooling down the resulting product was pasteurised tofu (3.664/3.497 kg).

Soy sauce processing

Soya bean seeds (5.000/5.000 kg) were cleaned by air cleaning, resulting in cleaned soya bean seeds (4.946/4.967 kg) and aspirated grain fractions (0.054/0.033 kg). The cleaned soya bean seeds were mixed with 6.25 fold water for 12 hours swelling time. Afterwards soya bean seeds were cooked for 85 min until they were soft. The cooked soya bean seeds were mixed with wheat flour (type 405), wheat pollards, *Aspergillus oryzae* and water. All the ingredients were kneaded to a loaf and matured for 5 days to get koji. Mixed salt and water (brine) was added to koji and a mash (moromi) was created. After one day a pH value of 4.5–5.0 was reached with lactic acid. One day later *Saccharomyces rouxii* was added to the mash. The moromi underwent a fermentation process which took around 3 months at 33 °C. After the maturing process, the moromi was pressed, resulting in raw soy sauce (12.730/12.030 kg) and miso (9.355/10.636 kg). The raw soy sauce was filtered and pasteurised to get pasteurized soy sauce (12.730/12.030 kg).

Soya bean seeds and processed fractions were stored at –18 °C for a maximum of 12 months prior to analysis. The storage period of 12 months is covered by the storage stability studies on parent, SYN546039 and SYN545720 in commodities with high oil content, soya bean flour, soya bean milk and soya bean crude oil.

Samples were analysed for parent, SYN546039 and SYN545720 using modification C of HPLC-MS-MS method GRM042.04A using matrix matched standards. Modification C of HPLC-MS-MS method GRM042.04A is considered valid for the determination of parent, free and/or conjugated SYN546039 and free and/or conjugated SYN545720 in the range 0.01–1.0 mg/kg for soya bean seeds and aspirated grain fractions, 0.01–0.5 mg/kg for soya bean hulls, soy oil, soya bean meal, fat soy flour, pollard, okara, soy milk, tofu, soy sauce and miso. Soya bean seeds were analysed immediately before processing. Results were not corrected for control levels (< 0.01 mg/kg for each analyte and matrix). Results were not corrected for individual concurrent method recoveries (72–116% for each analyte and matrix). Residue results and processing factors are shown in Table 35.

Table 35 Residues and processing factors in soya bean seeds and processed soy commodities

Trial, Location, year, (variety), dose rate, interval, DALT	Soya bean commodities	parent, mg/kg	SYN546039	SYN545720	PF parent
S10-02879-01, Kolchiko, Thessaloniki, Greece, 2010, (variety M10), 0.074+0.074+0.371+0.372 kg ai/ha, interval 7 days, DALT=14 Soil: sandy loam	Soya bean seeds (RAC)	0.025	< 0.01	< 0.01	–
	aspirated grain fractions	0.29	0.017	< 0.01	11
	soya bean hulls	0.26	< 0.01	< 0.01	10
	soy oil, crude	0.024	< 0.01	< 0.01	0.96
	soy oil, refined	0.017	< 0.01	< 0.01	0.68
	milled soya bean meal (15% moisture)	< 0.01	< 0.01	< 0.01	< 0.40
	Soya bean seeds (RAC)	0.022	< 0.01	< 0.01	–
	aspirated grain fractions	0.17	0.017	0.010	7.7
	fat soy flour	< 0.01	< 0.01	< 0.01	< 0.44
	pollard	0.11	< 0.01	< 0.01	4.8
	Soya bean seeds (RAC)	0.022	< 0.01	< 0.01	–
	aspirated grain fractions	0.32	0.022	< 0.01	14
	okara	< 0.01	< 0.01	< 0.01	< 0.44
	soy milk	< 0.01	< 0.01	< 0.01	< 0.44

Trial, Location, year, (variety), dose rate, interval, DALT	Soya bean commodities	parent, mg/kg	SYN546039	SYN545720	PF parent
	tofu, pasteurised	0.013	< 0.01	< 0.01	0.58
	Soya bean seeds (RAC)	0.028	< 0.01	< 0.01	–
	aspirated grain fractions	0.21	0.022	< 0.01	7.6
	soy sauce, pasteurised	< 0.01	< 0.01	< 0.01	< 0.36
	miso pasteurized	< 0.01	< 0.01	< 0.01	< 0.36
S10-02879-02, Galarinos, Chalkidiki, Greece, 2007, (variety M10), 0.075+0.075+0.370+0.369 kg ai/ha, interval 7 days, DALT=14 Soil: clay loam	Soya bean seeds (RAC)	0.026	< 0.01	< 0.01	–
	aspirated grain fractions	0.22	0.022	< 0.01	8.3
	soya bean hulls	0.28	0.010	< 0.01	11
	soy oil, crude	0.020	< 0.01	< 0.01	0.77
	soy oil, refined	0.017	< 0.01	< 0.01	0.65
	milled soya bean meal (15% moisture)	< 0.01	< 0.01	< 0.01	< 0.38
	Soya bean seeds (RAC)	0.029	< 0.01	< 0.01	–
	aspirated grain fractions	0.23	0.016	< 0.01	7.9
	fat soy flour	< 0.01	< 0.01	< 0.01	< 0.34
	pollard	0.10	< 0.01	< 0.01	3.6
	Soya bean seeds (RAC)	0.031	< 0.01	< 0.01	–
	aspirated grain fractions	0.23	0.012	< 0.01	7.4
	okara	< 0.01	< 0.01	< 0.01	< 0.32
	soy milk	< 0.01	< 0.01	< 0.01	< 0.32
	tofu, pasteurised	0.016	< 0.01	< 0.01	0.52
	Soya bean seeds (RAC)	0.030	< 0.01	< 0.01	–
	aspirated grain fractions	0.28	0.019	< 0.01	9.6
	soy sauce, pasteurised	< 0.01	< 0.01	< 0.01	< 0.34
	miso, pasteurized	< 0.01	< 0.01	< 0.01	< 0.34

Processing studies summary

Calculated processing factors for soya beans are summarized in Table 36.

Table 36 Overview of processing factors

Soya bean commodities	PF parent	PF median or best estimate
Soya bean seeds (RAC)	–	
aspirated grain fractions	7.4, 7.6, 7.7, 7.9, 8.3, 9.6, 11, 14	8.1
soya bean hulls	10, 11	10
soy oil, crude	0.77, 0.96	0.86
soy oil, refined	0.65, 0.68	0.66
soya bean meal, dried	< 0.38, < 0.40	< 0.4
soy fat flour	< 0.34, < 0.44	< 0.4
pollard	3.6, 4.8	4.2
okara	< 0.32, < 0.44	< 0.4
soy milk	< 0.32, < 0.44	< 0.4
tofu, pasteurised	0.52, 0.58	0.55
soy sauce, pasteurised	< 0.34, < 0.36	< 0.4
miso, pasteurised	< 0.34, < 0.36	< 0.4

Residues in the edible portion of food commodities

No data submitted.

Residues in animal commodities

Direct animal treatments

Not applicable.

Farm animal feeding studies

The Meeting received information on feeding studies in dairy cows.

A residue feeding study in lactating Holstein/Friesian dairy cows was conducted in the UK in 2011 [Ward and Vance, 2012, SYN545192_10188]. Benzovindiflupyr was administered orally in gelatine capsules once daily to three groups of three cows for 28 consecutive days. Two control cows received gelatine dosing capsules containing no active substance. Experimental conditions are indicated in Table 37. Milk samples were collected twice daily throughout acclimation and feeding periods (days –1, 1, 2, 3, 5, 7, 10, 14, 17, 21, 24 and 28) and PM and AM milk was combined to produce a 24 hr bulk milk sample. Average daily milk yields ranged from 7.5–16 kg/animal. Skimmed milk and cream were produced from milk collected at day 21 and 28 using a centrifugal cream separator. Animal 4 (1× dose group) was killed on welfare grounds on day 14 due to an underlying health condition unrelated to on-study procedures. All remaining treated cows and one cow from the control group were killed approximately 22–24 hours following the final dose. Samples of liver, kidney, muscle (diaphragm, loin and hind leg) and fat (perirenal, mesenterial and subcutaneous) were collected from all animals. Milk and tissue samples were stored at –20 °C until analysed. The maximum frozen storage intervals prior to analysis were 18 days for milk, 15 days for skimmed milk, 24 days for cream, 20 days for liver, 23 days for kidney, 34 days for muscle and 57 days for fat.

Samples were analysed for parent, SYN546039 (including conjugates) and SYN546422 (including conjugates) using modification A of HPLC-MS-MS method 1887 (see method GRM042.06) and using matrix-matched standards. Method 1886 version 2.0 is considered valid for the purpose of this study (commodity type and concentration level of the analytes). Residues of parent, SYN546039 or SYN546422 were not found in any of the control samples (each < 0.01 mg/kg). Mean concurrent recoveries for all analytes and all tissues were within 70–120%, showing adequate performance at the time of sample analysis.

Analytical results in tissue samples are shown in Table 38. Samples from the 1× and 5× dose groups were only analysed when residues in one or more samples from the next highest dose rate contained residues ≥ 0.01 mg/kg (LOQ). Residues of parent and SYN546039 (free plus conjugated) were highest in liver. Residues in other tissues decreased in the order kidney > fat > muscle for both parent and SYN546039. Residues of SYN546422 (free plus conjugated) were not found (< 0.01 mg/kg) in any of the tissues.

No residues (< 0.01 mg/kg, each analyte) were found in whole milk at any of the treatment days (10× dose rate samples analysed only). No residues (< 0.01 mg/kg, each analyte) were found in skimmed milk in the day 21 and day 28 samples (10× dose rate samples analysed only). Residues in cream for the 21 and 28 day samples are shown in Table 39.

Table 37 Experimental conditions of the cow feeding study

Cow number	Mean individual and group bodyweight over the feeding period (kg)	Mean daily dry feed intake (kg feed/animal)	Actual mean dose (ppm ai in feed)	Actual mean dose (mg ai/animal/day)	Actual mean dose (mg/kg bw/day)
1, 2	676, 648 (group 662)	14	0 0× dose group	0	0
3, 4, 5	734, 596, 613 (group 648)	15	3.5 1× dose group	53	0.082
6, 7, 8	623, 679, 674 (group 658)	17	16 5× dose group	281	0.43
9, 10, 11	610, 694, 679 (group 661)	16	32 10× dose group	529	0.80

Table 38 Benzovindiflupyr related residues in tissues for 1×, 5× and 10× dose groups

Sample	Dose rate (ppm feed)	Cow	parent (mg/kg)	SYN546039 (mg/kg)	SYN546422 (mg/kg)	Parent Mean (mg/kg)	Parent Max (mg/kg)
Hind Leg Muscle	3.5 (1× dose)	3	NA	NA	NA	NA	NA
		4	NS	NS	NS		
		5	NA	NA	NA		
	16 (5× dose	6	< 0.01	< 0.01	NA	< 0.01	< 0.01
		7	< 0.01	< 0.01	NA		
		8	< 0.01	< 0.01	NA		
	32 (10× dose)	9	0.01	0.01	< 0.01	0.010	0.01
		10	< 0.01	< 0.01	< 0.01		
		11	< 0.01	< 0.01	< 0.01		
Loin muscle	3.5 (1× dose)	3	NA	NA	NA	NA	NA
		4	NS	NS	NS		
		5	NA	NA	NA		
	16 (5× dose	6	NA	< 0.01	NA	NA	NA
		7	NA	< 0.01	NA		
		8	NA	< 0.01	NA		
	32 (10× dose)	9	< 0.01	0.01	< 0.01	< 0.01	< 0.01
		10	< 0.01	< 0.01	< 0.01		
		11	< 0.01	< 0.01	< 0.01		
Diaphragm muscle	3.5 (1× dose)	3	NA	< 0.01	NA	NA	NA
		4	NS	NS	NS		
		5	NA	< 0.01	NA		
	16 (5× dose	6	< 0.01	< 0.01	NA	< 0.01	< 0.01
		7	< 0.01	< 0.01	NA		
		8	< 0.01	< 0.01	NA		
	32 (10× dose)	9	0.02	0.02	< 0.01	0.017	0.02
		10	0.02	< 0.01	< 0.01		
		11	< 0.01	< 0.01	< 0.01		
Liver	3.5 (1× dose)	3	< 0.01	< 0.01	NA	< 0.01	< 0.01
		4	NS	NS	NS		
		5	< 0.01	< 0.01	NA		
	16 (5× dose	6	0.07	0.04	NA	0.037	0.07
		7	0.02	0.03	NA		
		8	0.02	0.04	NA		
	32 (10× dose)	9	0.07	0.21	< 0.01	0.047	0.07
		10	0.04	0.08	< 0.01		
		11	0.03	0.05	< 0.01		
kidney	3.5 (1× dose)	3	< 0.01	< 0.01	NA	< 0.01	< 0.01
		4	NS	NS	NS		
		5	< 0.01	< 0.01	NA		
	16 (5× dose	6	< 0.01	< 0.01	NA	0.010	0.01
		7	< 0.01	< 0.01	NA		
		8	0.01	< 0.01	NA		
	32 (10× dose)	9	0.02	0.03	< 0.01	0.013	0.02
		10	0.01	0.02	< 0.01		
		11	< 0.01	0.01	< 0.01		
subcutaneous fat	3.5 (1× dose)	3	< 0.01	< 0.01	NA	< 0.01	< 0.01
		4	NS	NS	NS		
		5	< 0.01	< 0.01	NA		
	16 (5× dose	6	< 0.01	< 0.01	NA	< 0.01	< 0.01
		7	< 0.01	0.01	NA		
		8	< 0.01	< 0.01	NA		
	32 (10X dose)	9	0.02	0.04	< 0.01	0.017	0.02
		10	0.02	0.01	< 0.01		
		11	< 0.01	< 0.01	< 0.01		
perirenal fat	3.5 (1× dose)	3	< 0.01	< 0.01	NA	< 0.01	< 0.01
		4	NS	NS	NS		
		5	< 0.01	< 0.01	NA		
	16	6	< 0.01	0.02	NA	0.013	0.02

Sample	Dose rate (ppm feed)	Cow	parent (mg/kg)	SYN546039 (mg/kg)	SYN546422 (mg/kg)	Parent Mean (mg/kg)	Parent Max (mg/kg)
	(5× dose	7	0.02	0.02	NA		
		8	< 0.01	< 0.01	NA		
	32 (10× dose)	9	0.03	0.04	< 0.01	0.023	0.03
		10	0.03	0.02	< 0.01		
		11	< 0.01	< 0.01	< 0.01		
mesenterial fat	3.5 (1× dose)	3	< 0.01	< 0.01	NA	< 0.01	< 0.01
		4	NS	NS	NS		
		5	< 0.01	< 0.01	NA		
	16 (5× dose	6	< 0.01	0.01	NA	0.010	0.01
		7	0.01	0.01	NA		
		8	< 0.01	< 0.01	NA		
	32 (10× dose)	9	0.03	0.03	< 0.01	0.023	0.03
		10	0.03	0.02	< 0.01		
		11	< 0.01	< 0.01	< 0.01		

NA—not analysed, no residue > LOQ in next highest dose group
NS—no sample (animal killed on welfare grounds on day 14)

Table 39 Benzovindiflupyr related residues in cream for 1×, 5× and 10× dose groups

Dose rate ppm feed	Sampling	Cow	parent mg/kg	SYN546039 (including conjugates) mg/kg	SYN546422 (including conjugates) mg/kg
3.5 (1× dose)	Day 21	3 4 5	< 0.01 NS < 0.01	< 0.01 NS < 0.01	NA NS NA
	Day 28	3 4 5	< 0.01 NS < 0.01	< 0.01 NS < 0.01	NA NS NA
	Average		< 0.01	< 0.01	NA
	Highest residue		< 0.01	< 0.01	NA
16 (5× dose)	Day 21	6 7 8	0.01 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA NA NA
	Day 28	6 7 8	0.01 0.01 < 0.01	< 0.01 < 0.01 < 0.01	NA NA NA
	average		0.01	< 0.01	NA
	highest residue		0.01	< 0.01	NA
32 (10× dose)	Day 21	9 10 11	0.03 0.02 0.01	0.02 0.01 < 0.01	< 0.01 < 0.01 < 0.01
	Day 28	9 10 11	0.03 0.03 0.01	0.02 0.01 < 0.01	< 0.01 < 0.01 < 0.01
	average		0.02	0.013	< 0.01
	highest residue		0.03	0.02	< 0.01

NA = not analysed, no residue > LOQ in next highest dose group
NS = no sample (animal killed on welfare grounds on day 14)

Residues in food in commerce or at consumption

Data no longer required.

National Residue Definition

No information available.

APPRAISAL

Benzovindiflupyr was scheduled for residue evaluation as a new compound by the 2014 JMPR at the 45th session of the CCPR (2013). The toxicological review was conducted in 2013, which established an ADI of 0–0.05 mg/kg bw and an ARfD of 0.1 mg/kg bw. Additional toxicological data were provided for the metabolites SYN546039 and SYN545720. Benzovindiflupyr was defined by the WHO panel as the only toxicologically significant compound in animals, plants and the environment.

Benzovindiflupyr is a broad-spectrum fungicide belonging to the chemical class of pyrazole carboxamides. Benzovindiflupyr acts as an inhibitor of the fungal complex II respiratory chain, where it inhibits the succinate dehydrogenase enzyme (succinate dehydrogenase inhibitor, SDHI) by blocking the ubiquinone-binding sites in the mitochondrial complex.

The Meeting received information from the manufacturer on identity, metabolism, storage stability, residue analysis, use pattern, residues resulting from supervised trials on soya beans, fate of residue during processing, and livestock feeding studies.

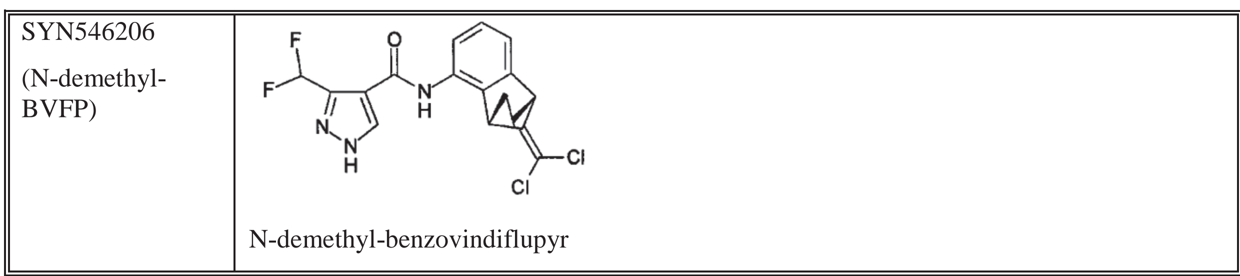
Chemical name:

Benzovindiflupyr: N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide (IUPAC).

Structural formula:

Benzovindiflupyr contains chiral centres at both the bridgehead carbon atoms potentially resulting in four stereoisomeric forms. However, the bicyclic ring is a rigid structure, and therefore only two stereoisomers exist (an enantiomeric pair). Technical benzovindiflupyr consists of a racemic mixture of two enantiomers SYN546526 and SYN546527, at a ratio of 50:50. SYN546526 represents N-[(1R,4S)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. SYN546527 represents N-[(1S,4R)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide. The enantiomers were unresolved using the chromatographic solvent systems in the reports. Both enantiomers were measured within the same single chromatographic peak and they were collectively reported as one concentration. Both enantiomers are fungicidally active. No toxicological studies were performed on the individual enantiomers.

Metabolites referred to in the appraisal by codes:

SYN546206 (N-demethyl-BVFP)	 N-demethyl-benzovindiflupyr

SYN546039 (BVFP-OH)	hydroxy-benzovindiflupyr
SYN546041 (N-demethyl-BVFP-OH)	N-demethyl-hydroxy-benzovindiflupyr
SYN546042 (N-demethyl-BVFP-OH)	N-demethyl-hydroxy-benzovindiflupyr
SYN546422	N- [(1SR,3RS)-2-(dichloromethylene)-1-hydroxy-3-(2-hydroxy-ethyl)-indan-4-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide
SYN508272	3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid amide

NOA449410	HF_2C, OH, O, N, N, CH_3 3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid
SYN545720	F, F, O, OH, N, N, H 3-difluoromethyl-1*H*-pyrazole-4-carboxylic acid

Animal metabolism

The Meeting received results of metabolism studies in laboratory animals, lactating goats and laying hens.

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2013. In absorption, distribution, metabolism and excretion (ADME) studies, overnight-fasted animals showed clinical signs at doses that were non-toxic to fed animals. Therefore, most of the ADME studies were performed in fed animals. Absorption of benzovindiflupyr was approximately 80% at the low dose (1 mg/kg bw) and showed saturation at the higher dose (approximately 60% absorption at 40 mg/kg bw). In low-dose bile duct cannulated animals, 4% of the administered dose was found in urine, 17% in faeces and 69–76% in bile; in high-dose animals, 9% of the administered dose was found in urine, 32% in faeces and 47–57% in bile. At both dose levels, 86–97% of the administered dose was excreted within 48 hours after administration. The major route of excretion was by bile. For tissues, the elimination half-lives were in the range of 40–316 hours. Highest residues were identified in the liver, kidney, adrenals, thyroid and heart. After repeated daily dosing, levels of radioactivity in tissues appeared to have reached steady-state concentrations after 14 days. The predominant metabolic pathway for benzovindiflupyr is N-demethylation, phenyl and/or bicyclo hydroxylation and opening of the bicyclo system. Additionally, subsequent formation of glucuronic acid or sulfate conjugates was observed. The amide bond of benzovindiflupyr is preserved.

One lactating goat per radiolabel was dosed orally once daily for 7 consecutive days with a gelatin capsule containing [^{14}C-phenyl]-benzovindiflupyr or [^{14}C-pyrazole]-benzovindiflupyr. The equivalent actual mean daily doses in the dry feed were 41 or 32 ppm for the phenyl or pyrazole label, respectively. Goats were sacrificed 12 hours after the last dose. Total recovered radioactivity amounted to 90% and 86% of the administered dose for the phenyl and pyrazole radiolabelled forms, respectively. The majority of the radioactivity was recovered in faeces (79%/73%, phenyl/pyrazole). The remainder of the dose was recovered in urine (4.5%/5.2%, phenyl/pyrazole) and GI tract contents (6.8%/7.2%), while only low levels were found in milk and tissues (<0.5% in total).

The highest radioactivity concentrations were found in liver (1.3/0.70 mg/kg eq) and kidney (0.28/0.18 mg/kg eq), followed by fat (0.098/0.070 mg/kg eq) and muscle (0.070/0.032 mg/kg eq). Total radioactive residues in milk reached a plateau concentration of approximately 0.046 mg/kg eq following 96 hours dosing for the phenyl label and 0.035 mg/kg eq following 72 hours dosing for the pyrazole label..

Following solvent extraction, residue extractabilities were 78–89% TRR for liver and ≥ 94% TRR for milk and all other tissues. Liver and kidney extracts were treated with β-glucuronidase to cleave glucuronide and sulphate conjugates.

Parent was identified in milk and all goat tissues at levels of 5.5–13% TRR in milk, kidney and liver, 24–25% in muscle and 41–44% TRR in fat. Conjugated metabolites formed a significant

part of the extracted residue in liver and kidney. The most significant metabolites (including conjugates) identified in all tissues and milk were the mono-hydroxylated metabolite SYN546039 (22–50% TRR) and metabolite SYN546422 (16–25% TRR in milk and kidney, 1.5–8.9% TRR in other tissues). Levels of SYN546039 in milk and tissues (except fat) or levels of SYN546422 in milk and kidney were higher than those of the parent compound. Other metabolites (including conjugates) were found at levels below 10% TRR. Post-extraction solids from liver (11–22% TRR) were shown to be associated with protein. Protease treatment of the post-extraction solids resulted in a mixture of highly polar metabolites.

Five laying hens per radiolabel were dosed orally once daily for 14 consecutive days with a gelatin capsule containing [^{14}C-phenyl]-benzovindiflupyr or [^{14}C-pyrazole]-benzovindiflupyr. The equivalent actual mean daily doses in the dry feed were 16–20 or 17–20 ppm for the phenyl or pyrazole label, respectively. Hens were sacrificed 12 hours after the last dose. Total recovered radioactivity amounted to 88% and 93% of the administered dose for the phenyl and pyrazole radiolabelled forms, respectively. The majority of the radioactivity was recovered in excreta (88%/92%, phenyl/pyrazole), while only low levels were found in eggs and tissues (<0.2% in total).

The highest radioactivity concentrations were found in liver (0.19/0.25 mg/kg eq), followed by fat (0.033/0.045 mg/kg eq) and muscle (0.025/0.036. mg/kg eq). Total radioactive residues in egg yolks achieved a plateau concentration of 0.17–0.18 mg/kg eq after 168–240 hrs of dosing. Total radioactive residues in egg whites achieved a plateau concentration of 0.03–0.04 mg/kg eq after 120–168 hrs of dosing.

Following solvent extraction, residue extractabilities were ≥83% TRR for egg yolk and egg white, 68–73% TRR for skin with fat, 48–49% TRR for liver and 24–37% TRR for muscle. Liver extracts were treated with β-glucuronidase to cleave glucuronide and sulphate conjugates.

Parent was identified at levels of 0.2–3.3% TRR in liver and muscle, 11–14% in eggs and 38–42% TRR in skin with fat. Benzovindiflupyr was extensively metabolised, resulting in low levels of various metabolites in tissues (<6.0% TRR each). The most significant metabolites in eggs were the mono-hydroxylated metabolites SYN546039 (12–22% TRR in eggs, 1.3–5.1% TRR in tissues) and the mono-hydroxylated demethylated metabolites SYN546041 (10.6–12.5% TRR) and SYN546042 (6.6–12.2% TRR). Levels of SYN546039, SYN546041 and SYN546042 in eggs were in the same order of magnitude as those of the parent compound. Post-extraction solids from liver (51–52% TRR) and muscle (63–76% TRR) were shown to be associated with protein. Protease treatment of the post-extraction solids resulted in a mixture of highly polar metabolites.

In summary, metabolism observed in lactating goats and laying hens arose via hydroxylation on the alicyclic ring to form SYN546039 (all tissues, milk, eggs). In hens metabolism proceeded through N-demethylation to form SYN546041 and SYN546042 isomers (mainly in eggs), while in ruminants metabolism proceeded through an alternative pathway consisting of oxidative opening of the alicyclic ring to form SYN546422 (mainly in milk, kidney). Several other minor metabolites arose in livestock by cleavage between the pyrazole and phenyl rings and/or conjugation as glucuronide or sulphate compounds. Further metabolism involves association with proteins.

The major compounds identified in goat, hen tissues, milk or eggs are: parent, SYN546039, SYN546422, SYN546041 and SYN546042 and their conjugates. Parent and SYN546039 and its conjugates comprise a significant part of residue in tissues, milk and eggs. Significant additional contributions are found for metabolites SYN546041 and SYN546042 in eggs and SYN546422 and its conjugates in milk and goat kidney.

In general, metabolism between goat, hen and rat is similar, with a few exceptions. Formation of SYN546422 is only found in goat. Opening of the bicyclo system as such is found in rat and metabolite SYN546422 is postulated as a plausible rat intermediate between the open bicyclo rat metabolites SYN546634 and SYN546707 (the demethylated form of SYN546422). Cleavage between the pyrazole and phenyl rings to form SYN508272 is found in the pyrazole labelled studies in hens and goats, while in the rat studies this bond is preserved.

Plant metabolism

The Meeting received plant metabolism studies for benzovindiflupyr after foliar application on fruits (tomatoes), cereals (wheat) and pulses/oilseeds (soya beans).

The metabolism of ^{14}C-phenyl-benzovindiflupyr or ^{14}C-pyrazole-benzovindflupyr in indoor grown tomatoes was studied following four foliar applications at 0.13–0.14 kg ai/ha at weekly intervals. Total radioactive residues (TRR) in mature tomato fruits at DAT = 1 and 14 were 0.047 and 0.092 mg/kg eq for the phenyl label and 0.18 and 0.15 mg/kg eq for the pyrazole label, respectively. A high proportion of the residue remained on the surface of the fruit (65–79% TRR). The residues in or on the fruit could be extracted by acetonitrile/water (> 99% TRR). The principal component of the residue was the parent compound (91–95% TRR). A number of metabolites were detected, none reaching >0.5% TRR.

The metabolism of ^{14}C-phenyl-benzovindiflupyr or ^{14}C-pyrazole-benzovindflupyr in indoor grown wheat was studied following two foliar applications at 0.14 kg ai/ha at a 35 day interval. Residue levels in wheat forage harvested 9 days after the first application and residue levels in wheat hay, wheat straw and wheat grains harvested 10, 40 and 41 days after the second application were 3.0, 4.9, 8.1, 0.12 mg/kg eq for the phenyl label and 2.1, 6.4, 9.0, 0.092 mg/kg eq for the pyrazole label, respectively. The major part of the residues (> 97% TRR) could be extracted with acetonitrile/water. The principal component of the residue was the parent compound: 84–87% TRR in grain, 81–84% TRR in straw and 89–103% in wheat forage and hay. A number of metabolites were detected, none reaching >5.0% TRR. Several of these metabolites were present in the free and conjugated form.

The metabolism of ^{14}C-phenyl-benzovindiflypyr or ^{14}C-pyrazole-benzovindflupyr in indoor grown soya beans was studied following two foliar applications at 0.12–0.13 kg ai/ha at a 22 day interval. Residue levels in soya bean forage harvested 11 days after the first application and soya bean hay and soya bean seeds harvested at 13 and 30 days after the second application were 3.4, 14 and 0.029 mg/kg eq for the phenyl label and 4.1, 13 and 0.10 mg/kg eq for the pyrazole label, respectively. The major part of the residues (> 89% TRR) could be extracted with solvents. Parent was identified at levels of 15–31% TRR in soya bean seeds, 67–72% TRR in soya bean hay and 83–85% TRR in soya bean forage. The most significant metabolite in soya bean seeds was the cleavage product SYN545720 (47% TRR). The major part of the SYN545720 metabolite was present in the conjugated form (30% TRR), particularly as an aspartic acid conjugate or monosaccharide conjugate. The most significant metabolite in soya bean forage and hay was the mono-hydroxylated metabolite SYN546039 (9.2–12% TRR). The major part of the SYN546039 metabolite was present in the conjugated form (8.5–12% TRR), particularly as a malonyl glycoside or glycoside conjugate. A number of other metabolites were detected, none reaching >14% TRR. One of these metabolites was NOA449410, a plant specific metabolite, which was not detected in rat. NOA449410 was present at very low levels: < 1.2% TRR or 0.012 mg/kg eq) in soya forage, 0.039 mg/kg eq in soya hay or 0.0012 mg/kg eq in soya seeds.

In summary, the degree of metabolism in crops after foliar application varied. In fruits and cereals parent compound represented the principal part of the residue in tomato fruits, wheat grain, wheat straw and wheat forage and hay. Metabolism proceeded further in pulses/oilseeds with parent representing 15–31% TRR in soya bean seeds, 67–72% TRR in soya bean hay and 83–85% TRR in soya bean forage. Metabolism observed in pulses/oilseeds arose via hydroxylation on the alicyclic ring to form SYN546039). Metabolism proceeded through N-demethylation and cleavage between the pyrazole and phenyl rings to form SYN545720.

In general, metabolism between plants and rat is similar, except for the cleavage between the pyrazole and phenyl rings. In rat this bond is preserved. The cleavage product SYN508272 is found in the pyrazole labelled studies in hens and goats. Cleavage products NOA449410 and SYN545720 are not found in rat nor in livestock.

Environmental fate in soil

The Meeting received information on photolysis in water and on soil and information on rotational crops.

All plant metabolism studies have been conducted indoors. Since the interval between the first application and harvest for the investigated commodities is long (29–111 days) and the residue is a surface residue, photolysis may form a major route of degradation. Since indoor grown plants are not subjected to the full spectrum of sunlight, degradation on field grown plants may show a different behaviour. Photolysis studies in water or on soil show a DT50 of 44 or 144–244 days, respectively, confirming the potential for photolysis. Photolysis in water after 15 days and on soil after 30 days demonstrated the formation of low levels of SYN546039, SYN508272, NOA449410, and SYN545720 (0.6–8.5% TAR), indicating the absence of an alternative degradation pathway under outdoor conditions. In addition, preliminary experimental work on photolysis on leaf surfaces grown under greenhouse, under artificial sunlight and outdoor conditions for 7 days, demonstrated very minor degradation in all three test conditions while the resulting minor degradates were qualitatively similar. The Meeting concluded that the indoor plant metabolism studies are considered acceptable for deriving a residue definition for plant commodities.

Metabolism of ^{14}C-phenyl-benzovindiflypyr or ^{14}C-pyrazole-benzovindflupyr was investigated in confined rotational crops following a single bare soil treatment. A sandy loam soil was treated at a rate of 0.53–0.54 kg ai/ha under indoor conditions. Rotational crops (lettuce, wheat and turnip) were sown at 30, 90 and 300 day plant back intervals (PBI). Total radioactivity in rotational crops ranged from 0.003–0.77 mg/kg eq at 30 day PBI, 0.002–0.34 mg/kg eq at 90 day PBI and 0.007–0.29 mg/kg eq at 300 day PBI. Total radioactivity levels above 0.05 mg/kg eq were found in wheat forage, hay and straw at all plant back intervals.

Wheat grain had very low residues (< 0.01–0.014 mg/kg eq) and most extracts were not amenable to chromatographic examination. In turnip roots, parent was the principal component and ranged from 81–90% TRR at the 30 day plant back interval, 69–72% TRR at the 90 day plant back interval and 64–71% at the 300 day plant back interval. In the leafy parts of crops (lettuce, turnip leaves, wheat forage, hay, straw) parent compound was the principal component of the residue at the 30 day PBI. Parent ranged from 14–37% TRR at the 30 day PBI, 13–35% TRR at the 90 day PBI and 6.5–29% TRR at the 300 day PBI. At the 90 and 300 day PBI, significant metabolites were the cleavage products NOA449410 and SYN545720, together accounting for 53–73% TRR, 34–46% TRR and 24–26% TRR in immature lettuce, mature lettuce and wheat forage respectively. NOA449410 was mainly present in the conjugated form whereas SYN545720 was present in the free and conjugated form. Other metabolites were generally present at low levels (each < 10% TRR). In wheat forage, wheat hay and wheat straw, SYN546206 and SYN546039 were more significant, frequently occurring at > 10% TRR and occasionally approaching 20% TRR each (including conjugates). SYN546206 was mainly present in the free form; SYN546039 was mainly present in the conjugated form.

From these data the Meeting concluded that benzovindiflupyr can be taken up from the soil under confined conditions even after long plant back intervals (300–366 days). Metabolites found in confined rotational crops (SYN546206, SYN546039, NOA449410 and SYN545720) are identical to those observed in primary crops and may have arisen from photolysis, degradation in and uptake from soil as well as from metabolism within the crop itself.

In two field rotational crop studies at four different locations in the EU benzovindiflupyr was applied onto bare soil at a single application of 0.20 kg ai/ha. Rotational crops (spinach, wheat, carrots) were sown 28–32, 60–69 or 355–366 days after application.

In another field rotational crop study at four different locations in the USA benzovindiflupyr was applied as foliar application to soya beans or peanuts at 3x 0.10 kg ai/ha with 14 day intervals. The last application was at BBCH 71–89 of the target crops. The soya bean and peanut target crops were harvested and removed from the field. Rotational crops (spinach or lettuce, radish or turnip, wheat) were sown 30 or 180 days after application.

No residues > 0.01 mg/kg of parent benzovindiflupyr or metabolites SYN546206 or SYN546039 (including conjugates) were found in any of the harvested commodities at any of the rotations, except in wheat forage and wheat straw from the 28–30 or 60 day plant back intervals. Parent and SYN546039 (including conjugates) were found in wheat forage and wheat straw at levels up to 0.012–0.022 mg/kg. Metabolites NOA449410 and SYN545720 were not analysed.

The dose rates as used in the field rotational crop studies (1x0.20 kg ai/ha or 3x0.10 kg ai/ha) are higher than those used in the actual supervised residue trials submitted (3x 0.045 kg ai/ha for soya beans). Based on the current uses, the Meeting concluded that no residues are expected in rotational crops. Should additional uses be developed in future, rotational crop studies may need to be re-evaluated.

Methods of Analysis

The Meeting received description and validation data for analytical methods for the determination of benzovindiflupyr related residues in plant and animal commodities.

The existing multi-residue method QuEChERS was submitted as enforcement/monitoring method for the determination of parent compound in plant and animal commodities. Plant commodities were extracted with acetonitrile/water (1:1, v/v), Samples were cleaned-up by SPE prior to quantification by HPLC-MS/MS. The Meeting considers validation sufficient for plant commodities with high acid content, high water content, high starch content, high oil content and all animal commodities (meat, liver, kidney, fat, milk and eggs). The LOQ was 0.01 mg/kg for parent compound in each matrix.

Several other HPLC-MS/MS methods were submitted for the determination of parent and its metabolites SYN546206 (free), SYN545720 (including conjugates) and/or SYN546039 (including conjugates) in plant material. Crop commodities were extracted with acetonitrile/water (80/20). Parent and SYN546206 were separated off by liquid-liquid partition and the remaining extract was treated with acid at pH 2 for 6 hrs at 100 ºC to cleave the SYN545720 and/or SYN546039 conjugates. Extraction efficiency for acetonitrile/water was at least 71% for parent and 81% for SYN546206 (free) as shown by a radio-validation study in wheat hay and wheat straw. Efficiency of extraction and hydrolysis was >100% for SYN546039 (including conjugates) and SYN545720 (including conjugates) as shown by a radio-validation study in soya bean hay and soya bean seed. Most analytical methods were considered fit for purpose with LOQs of 0.01 mg/kg for individual analytes.

Another HPLC-MS/MS method was submitted for the determination of parent and its metabolites SYN546039 (including conjugates) and SYN546422 (including conjugates) in milk, eggs or animal tissues. Animal commodities were extracted with acetonitrile/water (80/20). Parent was determined directly in the primary extract, while the remaining extract was treated with beta-glucuronidase at pH 5 for 6 hrs at 37 ºC to cleave the SYN546039 and/or SYN546422 conjugates. Extraction efficiency for acetonitrile/water was >100 % for parent, free SYN546039 and free SYN546422 as shown by a radio-validation study in milk, muscle, and egg yolk. Efficiency of extraction and hydrolysis was >100% for SYN546039 (including conjugates) and at least 67% for SYN546422 (including conjugates) as shown by a radio-validation study in liver. The analytical method was considered fit for purpose with LOQs of 0.01 mg/kg for individual analytes.

Stability of pesticide residues in stored analytical samples

The Meeting received information on the storage stability of parent, SYN546039, SYN546206 and SYN545720 in raw and processed plant commodities and of parent, SYN546039 and SYN546422 in animal commodities.

Storage stability studies showed that benzovindiflupyr and metabolite SYN546039 (free) were stable for at least 24 months at -18ºC in crop commodities representative of the high water, high acid, high starch, high protein and high oil commodity groups as well as in wheat straw. Metabolite SYN546206 (free) was stable for at least 22 months at -18 ºC in crop commodities representative of the high water and high starch commodity groups as well as in wheat straw. Metabolites SYN545720

(free) and SYN508272 (free) were stable for at least 24 months at -18 ºC in crop commodities representative of the high oil, high acid and high protein commodity groups.

Benzovindiflupyr and metabolites SYN546039 (free) and SYN545720 (free) were stable for at least 24 months at -10 ºC in various processed commodities: flour (maize, soya), meal (maize), oil (maize, soya), soymilk, dried fruits (grape, apple) and fruit juice (apple).

Benzovindiflupyr and metabolites SYN546039 (free) and SYN546422 (free) were stable at -20 ºC for at least 56–62 days in milk and eggs and at least 76–78 days in liver and muscle. According to OECD Guideline 506 storage stability in kidney and fat may be extrapolated from the other animal tissues, so The Meeting concluded that residues in kidney and fat are likely to be stable for at least 76 days.

Definition of the residue

The major compounds identified in goat, hen tissues, milk or eggs are: parent, SYN546039, SYN546422, SYN546041 and SYN546042 and their conjugates. Parent was identified at levels of 38–44% TRR (0.012–0.040 mg/kg) in goat fat and hen skin with fat, 24–25% (0.008–0.017 mg/kg) in goat muscle and 0.2–14% TRR (<0.001–0.14 mg/kg) in milk, eggs, and all other tissues. The mono-hydroxylated metabolite SYN546039 and its conjugates was identified at levels of 22–50% TRR (0.008–0.64 mg/kg eq) in milk and goat tissues, 12–22% TRR(0.008–0.022 mg/kg eq) in eggs and <6.0% TRR in hen tissues. Metabolite SYN546422 and its conjugates was only found in goat and was identified at levels of 16–25% TRR (0.010–0.052 mg/kg eq) in milk and goat kidney and 1.5–8.9% TRR (0.001–0.056 mg/kg eq) in other goat tissues. The mono-hydroxylated demethylated metabolite isomers SYN546041 and SYN546042 were identified at levels of 11–12% TRR (0.003–0.020 mg/kg eq) and 6.6–12% TRR (0.002–0.020 mg/kg eq) in eggs, respectively, <5.0% TRR in hen tissues and <10% TRR in goat tissues and milk.

Benzovindiflupyr parent is found in every animal commodity, although the levels in milk, eggs, edible offal and hen muscle are low. To be able to detect benzovindiflupyr related residues, metabolites SYN546039, SYN546422 (goat only), SYN546041 (eggs only) and SYN546042 (eggs only) could be included in the residue definition for enforcement/monitoring. Since a significant part of these metabolites is present as conjugates, a hydrolysis procedure is required to be able to measure these metabolites. Including the metabolites in the residue definition means the residue is unlikely to be measured by a multi-residue method. Since benzovindiflupyr itself can be measured by a multi-residue method and use of a multi-residue method is encouraged, the Meeting decided to define the residue for enforcement/monitoring as parent only.

The log Kow for benzovindiflupyr is 4.3. The goat metabolism study and the goat feeding study did not show a clear partition of the parent compound into the fat tissues, although in the high dose cow feeding study, parent was found in cream and not in the corresponding whole milk. In a hen metabolism study, the partitioning of the parent compound into the fatty tissues is more pronounced: highest levels of benzovindiflupyr are found in egg yolks (0.022–0.024 mg/kg) and skin with fat (0.012–0.019 mg/kg). Since benzovindiflupyr has a preference for fat in the poultry tissues as well as in high dose milk, the Meeting considers the residue fat soluble.

Apart from benzovindiflupyr, metabolites found at significant levels in livestock commodities were: SYN546039, SYN546041, SYN546042, SYN546422 and their conjugates. The toxicity of SYN546039, SYN546041, SYN546042 and SYN546422 is considered to be covered by toxicity studies on benzovindiflupyr since each of the free metabolites was actually found or agreed to be a possible intermediate in the rat. N-demethylation is regarded as neutral for toxicological potency while hydroxylation generally lowers toxicity. The JMPR 2013 received additional toxicological data for the mono-hydroxylated metabolite SYN546039, showing that this compound is at least 10 fold less toxic than parent. For metabolites SYN546041, SYN546042 and SYN546422 a read across to SYN546039 toxicity studies seems justified based on the close structural similarity and this suggests that they are also at least 10 fold less toxic than the parent.. Therefore none of these metabolites is considered relevant for the residue definition for dietary risk assessment. The Meeting decided to define the residue for dietary risk assessment as parent only.

In primary crops, parent compound represented the principal part of the residue in most crop commodities: 91–95% TRR (0.004–0.16 mg/kg) in tomato fruits, 84–87% TRR (0.077–0.10 mg/kg) in wheat grain, 81–84% TRR (6.6–7.6 mg/kg) in wheat straw and 89–103% (2.1–5.9 mg/kg) in wheat forage and hay, 67–72% TRR (8.7–10 mg/kg) in soya bean hay and 83–85% TRR (2.8–3.5 mg/kg) in soya bean forage. Metabolism proceeded further in pulses/oilseeds with parent representing 15–31% TRR (0.009–0.015 mg/kg) in soya bean seeds. A significant metabolite in soya bean seeds was SYN545720 (47% TRR (0.047 mg/kg eq eq) including conjugates). A significant metabolite in soya forage and hay was SYN546039 (9.2–12% TRR (0.38–1.6 mg/kg eq) including conjugates).

In rotational crops parent was the principal component in root commodities (64–90% TRR, 0.008–0.023 mg/kg) and a significant component (6.5–37% TRR, 0.001–0.085 mg/kg) in the leafy parts of crops (lettuce, turnip leaves, wheat forage, hay, straw). Significant metabolites were the cleavage products NOA449410 and SYN545720, together accounting for 34–73% TRR (0.009–0.014 mg/kg eq) and 24–26% TRR (0.023–0.026 mg/kg eq) in lettuce and wheat forage, respectively. In wheat forage, wheat hay and wheat straw, SYN546206 and SYN546039 were frequently occurring at > 10% TRR each, and occasionally approaching 20% TRR each (0.006–0.12 mg/kg eq, including conjugates).

Benzovindiflupyr is found in every primary crop commodity, although the levels in soya bean seeds are low. Benzovindiflupyr is found in every rotational crop commodity, except cereal grains, although levels in leafy crop parts vary. To be able to detect benzovindiflupyr related residues metabolites SYN545720 and NOA449410 could be included in the residue definition for enforcement/monitoring. Since a significant part of these metabolites is present as conjugates, a hydrolysis procedure is required to be able to measure them. Including the metabolites in the residue definition means the residue is unlikely to be measured by a multi-residue method. Furthermore, SYN545720 and NOA449410 can also arise in plant commodities as a result of treatment with other pyrazole fungicides like bixafen, fluxapyroxad, isopyrazam and sedaxane. Since SYN545720 and NOA449410 cannot be seen as a marker for benzovindiflupyr, the Meeting decided to define the residue for enforcement/monitoring as parent only.

Apart from benzovindiflupyr, metabolites found at significant levels in plant commodities were: SYN546206, SYN546039, NOA449410, SYN545720 and their conjugates. The toxicity of SYN546206 and SYN546039 is considered to be covered by toxicity studies on benzovindiflupyr since each of the free metabolites was actually found in the rat. The cleavage products NOA449410 and SYN545720 are not found in rat. N-demethylation is regarded as neutral for toxicological potency, while hydroxylation generally lowers toxicity. The JMPR 2013 received additional toxicological data for the mono-hydroxylated metabolite SYN546039, showing that this compound is at least 10 fold less toxic than parent. Toxicity studies for NOA449410 (from sedaxane studies) and SYN545720 (from isopyrazam and sedaxane studies) showed that the toxicity of these metabolites is probably 100–1000 fold less toxic than parent.. SYN546206 is the only compound which might be relevant for the residue definition for dietary risk assessment, since the toxicological potency might be similar to the parent. Metabolite SYN546206 is only found in feed commodities (wheat forage, wheat hay, wheat straw) in the confined rotational crop studies and its presence could not be confirmed in the field rotational crops studies. The Meeting decided to define the residue for dietary risk assessment as parent only.

The Meeting recommended the following residue definition for benzovindiflupyr:

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant and animal commodities: *benzovindiflupyr.*

The Meeting considers the residue fat soluble.

Results of supervised residue trials on crops

Soya beans (dry)

Field trials involving soya beans were performed in Brazil.

Critical GAP for soya beans in Paraguay is for three foliar applications without adjuvant at 0.045 kg ai/ha at 14 day intervals with a PHI of 21 days. Trials from Brazil (3x 0.045 kg ai/ha, interval 19–59 and 14 days, PHI 21–28 days, adjuvant added) matched this GAP. For each plot location, replicate trials were conducted with two-three different formulations. Only the highest residue was selected from these trials. For trials, where residues at longer PHIs (28 – 35 days) were higher than residues at PHI 21 days, the highest residue was selected. Benzovindiflupyr residues were: <0.01, <0.01, <0.01, <0.01, 0.01, 0.03 mg/kg (n=6).

The Meeting estimated a maximum residue level of 0.05 mg/kg on soya beans (dry). The Meeting estimated an STMR of 0.01 mg/kg.

Fate of residues during processing

Information on the fate of residues during processing showed that benzovindiflupyr is stable (100% recovery) under standard conditions simulating pasteurisation, baking/brewing/boiling and sterilisation.

Processing studies were undertaken for soya beans. Processing factors based on the residue for parent only are listed in the table below. Using the $STMR_{RAC}$ obtained from benzovindiflupyr use, the Meeting estimated STMR-Ps for processed commodities to be used in the livestock dietary burden calculations and/or dietary intake calculations.

Commodity	Processing factors Residue: parent only	Processing factor (PF) (median or best estimate)	STMR-P = $STMR_{RAC}$ x PF (mg/kg)
soya aspirated grain fractions	7.4, 7.6, 7.7, 7.9, 8.3, 9.6, 11, 14	8.1	0.081
soya bean hulls	10, 11	10	0.10
soya oil, crude	0.77, 0.96	0.86	0.0086
soya oil, refined	0.65, 0.68	0.66	0.0066
soya meal, dried	<0.38, <0.40	<0.4	0.004
soya fat flour	<0.34, <0.44	<0.4	0.004
soya pollard	3.6, 4.8	4.2	0.042
soya okara	<0.32, <0.44	<0.4	0.004
soya milk	<0.32, <0.44	<0.4	0.004
soya tofu, pasteurised	0.52, 0.58	0.55	0.0055
soya sauce, pasteurised	<0.34, <0.36	<0.4	0.004
soya miso, pasteurised	<0.34, <0.36	<0.4	0.004

Residues in animal commodities

The Meeting estimated the dietary burden of benzovindiflupyr residues on the basis of the livestock diets listed in the FAO manual appendix IX (OECD feedstuff table). For bulk commodities like soya beans, calculation from STMR provides the levels in feed suitable for estimating maximum residue levels as well as STMR values for animal commodities. Commodities used in the dietary burden calculation are soya beans and soya bean processed commodities. Supervised residue trials on soya bean forage and fodder were not available, whereby the dietary burden for livestock might be underestimated.

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Annex 6. A mean and maximum dietary burden for livestock, based on benzovindiflupyr use, is shown in the table below.

Livestock dietary burden for benzovindiflupyr residues, expressed as ppm of dry matter diet

	US	EU	AU	JP	overall	
	Max and mean	Max and mean	Max and mean	Max and mean	Max and mean	
beef cattle	0.017	0.013	0.0022	0.011	0.017	[a]
dairy cattle	0.0016	0.013	0.0029	0.0069	0.013	[b]

	US	EU	AU	JP	overall	
	Max and mean	Max and mean	Max and mean	Max and mean	Max and mean	
poultry broiler	0.0033	0.015	0.0081	0.0015	0.015	c
poultry layer	0.0033	0.0081	0.0081	0.0013	0.0081	d

[a] Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for mammalian meat

[b] Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for milk

[c] Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for poultry meat

[d] Highest mean and maximum dietary burden suitable for maximum residue level and STMR estimates for eggs

The Meeting received a feeding study on lactating cows.

Three groups of three lactating Holstein cows were dosed once daily via capsules at levels of 3.5, 16 and 32 ppm parent compound in dry weight feed for 28 consecutive days. Two control cows received a placebo. Milk was collected throughout the study and tissues were collected on day 28 within 22–24 hrs after the last dose. No parent residues >º0.01 mg/kg were found in whole milk, cream, muscle, liver, kidney or fat at the 3.5 ppm dose level.

The dietary burden for beef and dairy cattle of 0.017 and 0.013 ppm, respectively, is 200 times lower than the lowest dose administered in the cow feeding study (3.5 ppm). Therefore, no parent residues > 0.01 mg/kg are expected in milk, cream and cattle tissues.

No feeding study is available for poultry. In a metabolism study laying hens were dosed at 16–20 ppm parent compound in the dry feed for 14 consecutive days. Parent residues were: 0.024 mg/kg in egg yolks, 0.0037 mg/kg in egg whites, 0.019 mg/kg in fat, in 0.00050 mg/kg in liver and 0.0012 mg/kg in muscle. The dietary burden for broiler and layer poultry of 0.015 and 0.0081 ppm, respectively, is 1000 times lower than the dose administered in the hen metabolism study (16–20 ppm). Therefore, no parent residues > 0.01 mg/kg are expected in eggs, egg yolks and hen tissues.

The Meeting estimated maximum residue levels of 0.01* mg/kg in milk, eggs and all animal commodities. The Meetings estimated an STMR and HR of 0 mg/kg in milk, eggs and all animal commodities. The residue in animal commodities is considered fat soluble.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed below are suitable for establishing maximum residue limits

Definition of the residue for compliance with the MRL and for dietary risk assessment for plant and animal commodities: *benzovindiflupyr.*

The Meeting considers the residue fat soluble.

Summary of recommendations

CCN	Commodity name	MRL mg/kg	STMR or STMR-P mg/kg	HR or HR-P mg/kg	Comment
VD 0541	soya bean (dry)	0.05	0.01	-	-
MM 0095	Meat (from mammals other than marine mammals)	0.01*	0	0	-
MO 0105	Edible offal (Mammalian)	0.01*	0	0	-
MF 0100	Mammalian fats (except milk fats)	0.01*	0	0	-
ML 0106	Milks	0.01*	0	0	-
PM 0110	Poultry meat	0.01*	0	0	-
PF 0111	Poultry fats	0.01*	0	0	-
PO 0111	Poultry, Edible offal of	0.01*	0	0	-
PE 0112	Eggs	0.01*	0	0	-

CCN	Commodity name	MRL mg/kg	STMR or STMR-P mg/kg	HR or HR-P mg/kg	Comment
-	soya aspirated grain fractions	-	0.081	-	feed stuff

CCN	Commodity name	MRL mg/kg	STMR or STMR-P mg/kg	HR or HR-P mg/kg	Comment
-	soya bean hulls	-	0.10	-	feed stuff
OC 0541	soya oil, crude	-	0.0086	-	-
OR 0541	soya oil, refined	-	0.0066	-	-
-	soya meal, dried	-	0.004	-	feed stuff
-	soya fat flour	-	0.004	-	-
-	soya pollard	-	0.042	-	feed stuff
-	soya okara	-	0.004	-	feed stuff
-	soya milk	-	0.004	-	-
-	soya tofu, pasteurised	-	0.0055	-	-
-	soya sauce, pasteurised	-	0.004	-	-
-	soya miso, pasteurised	-	0.004	-	-

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDI) for benzovindiflupyr was calculated from recommendations for STMRs for raw and processed commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 3.

The IEDI of the 17 GEMS/Food cluster diets, based on the estimated STMRs represented 0% of the maximum ADI of 0.05 mg/kg bw, expressed as benzovindiflupyr.

The Meeting concluded that the long-term intake of residues of benzovindiflupyr from uses considered by the Meeting is unlikely to present a public health concern.

Short-term intake

The International Estimated Short Term Intake (IESTI) for benzovindiflupyr was calculated from recommendations for STMRs for raw and processed commodities in combination with consumption data for corresponding food commodities. The results are shown in Annex 4.

The IESTI for the diets submitted to the JMPR represented 0% of the ARfD (0.1 mg/kg bw, expressed as benzovindiflupyr). The Meeting concluded that the short-term intake of residues of benzovindiflupyr from uses considered by the Meeting is unlikely to present a public health concern.

REFERENCES

Code	Author	Year	Title, Institute, Report reference
SYN546039_50003	Barker, W and Ballard, TD	2012	FDA PAM Multiresidue Method (MRM) Testing for SYN545192 and Three Metabolites in Plant Samples—Final report Amendment 1 EN-CAS Analytical Laboratories, Winston-Salem, USA, Report TK0060877, Study 11-0019, Task TK0060877, 15 October 2012 Syngenta, GLP, not published
SYN545192_10216	Braid, S	2012	SYN545192—Analytical Method GRM042.08A for the Determination of SYN545192 and its Metabolites SYN546039 and SYN546206 in Rotational Crops Syngenta—Jealott's Hill, Bracknell, United Kingdom, Report GRM042.08A, Task T001407-08, 26 June 2012 Syngenta, Non-GLP, not published
SYN545192_10118	Braid, S and Lin, K	2011b	SYN545192—Analytical Method GRM042.03A for the determination of SYN545192 and its metabolite SYN546039 in Crops Syngenta—Jealott's Hill, Bracknell, United Kingdom, Report GRM042.03A, Task T001407-08, 4 August 2011 Syngenta, Non-GLP, not published

Code	Author	Year	Title, Institute, Report reference
SYN545192_ 10119	Braid, S and Lin, K	2011c	SYN545192—Analytical Method GRM042.04A for the Determination of SYN545192 and its Metabolite SYN546039 in Soya bean Commodities and SYN545720 in Soya bean Seed Only Syngenta—Jealott's Hill, Bracknell, United Kingdom, Report GRM042.04A, Task T001407-08, 4 August 2011 Syngenta, Non-GLP, not published
A17961A_10 022	Casallanovo, F	2011a	A17961—Magnitude of residues of SYN545192 and its metabolites, Azoxystrobin and R230310 in soya bean grains—Brazil, 2010–11 Syngenta Proteção de Cultivos Ltda, São Paulo, Brazil, Study M11075, 30 June 2011 Syngenta, GLP, not published
A17056F_10 022	Casallanovo, F	2011b	A17056—Magnitude of residues of SYN545192 and metabolites in soya beans—Brazil, 2010–11 Syngenta Proteção de Cultivos Ltda, São Paulo, Brazil, M11063, 30 August 2011 Syngenta, GLP, not published
A18126B_10 032	Casallanovo, F	2011c	A18126—Magnitude of residues of SYN545192 and metabolites, Azoxystrobin and R230310 in soya bean grains—Brazil, 2010–11 Syngenta Proteção de Cultivos Ltda, São Paulo, Brazil, Study M11086, 30 June 2011 Syngenta, GLP, not published
SYN545192_ 10150	Class, T and Göcer, M	2011	SYN545192—Validation of the QuEChERS Method for the Determination of Residues of SYN545192 in Animal Matrices by LC-MS/MS PTRL Europe GmbH, Ulm, Germany, Report B 2392 G, Study P 2392 G, Task TK0002550, 7 October 2011 Syngenta, GLP, not published
SYN545192_ 10057	Das, R	2010a	SYN545192—Color, physical state and odor Syngenta Crop Protection, Münchwilen, Switzerland, Study 121822, 2 November 2010 Syngenta, GLP, not published
SYN545192_ 10058	Das, R	2010b	SYN545192—Color, physical state and odor Syngenta Crop Protection, Münchwilen, Switzerland, Study 121830, 2 November 2010 Syngenta, GLP, not published
-	ECS	2009	EN 15662 Foods of plant origin—Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE—QuEChERS-method. European Committee for Standardisation, Edition 2009-02.
A17056D_10 002	Ellis, C	2012a	SYN545192—Residue Study on Soya beans and Processed Products in Greece in 2010 Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S10-02879-REG, Study S10-02879, Task TK0030542, 24 Feb 2011 Syngenta, GLP, not published
A17056B_10 004	Ellis, C	2012b	SYN545192—Residue Study on Rotational Crops in Germany and the United Kingdom in 2010 and 2011 Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S10-00261, Study S10-00261, Task TK0025356, 4 September 2012 Syngenta, GLP, not published
A17056B_10 005	Ellis, C	2012c	SYN545192—Residue Study on Rotational Crops in Southern France and Italy in 2010 and 2011 Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S10-00262, Study S10-00262, Task TK0025355, 6 September 2012 Syngenta, GLP, not published
-	FDA	1999	Pesticide analytical Manual (PAM)—Volume I, appendix II: Protocol and Reporting Forms for Testing Chemicals Through PAM I Multiresidue Methods. U.S. Department of Health and Human Services, Food and Drug administration. 3rd edition, January 1994, Updated October 1999 http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm111455.htm#updates

Code	Author	Year	Title, Institute, Report reference
SYN545192_10184	Green, M	2012	SYN545192—Metabolism in the Lactating Goat— Final report Amendment 1 Charles River Laboratories, Edinburgh, United Kingdom, Report 32394, Study 214703, Task T001828-08, 23 February 2012 Syngenta, GLP, not published
SYN545192_50177	Hagan, M and Bertrand, N	2012	SYN545192—Stability of SYN545192, SYN546039 and SYN545720 (soya bean fractions only) in Processed Commodities of Soya bean, Corn and Fruiting Vegetables under Freezer Storage Conditions ALS Environmental, Edmonton, Alberta, Canada, Report 12SYN313.REP, Study TK0058644, Task TK0058644, 15 October 2012 Syngenta, GLP, not published
SYN545192_50544	Hagan, M and Bertrand, N	2013	SYN565192—Stability of SYN545192, SYN546039 and SYN545720 (soya bean fractions only) in Processed Commodities of Soya bean, Corn, Grapes and Apples under Freezer Storage Conditions ALS Environmental, Edmonton, Alberta, Canada, Report 12SYN313A.REP, Study TK0058644, Task TK0058644, 19 November 2013, addendum to SYN545192_50177 Syngenta, GLP, not published
SYN508210_10287	Hargreaves, S	2010	SYN524464—Analytical Method for the Determination of Residues of SYN508210 and SYN508211 and the Metabolites CSCD667584, CSCD658906, CSCD659089, CSCD668403, CSCD667555, CSCD465008 and CSCC210616 in Crops. Final Determination by LC-MS/MS Syngenta—Jealott's Hill, Bracknell, Berkshire, United Kingdom, Report GRM023.03A, Task T014684-05, 11 February 2010 Syngenta, Non-GLP, not published
SYN545192_10182	Harris, J and Braid, S	2012	SYN545192—Analytical Method GRM042.06A for the Determination of SYN545192 and its Metabolites SYN546039 and SYN546422 in Bovine Meat, Liver, Kidney, Fat, Milk, Blood and Chicken Eggs Charles River Laboratories, Edinburgh, United Kingdom, Report GRM042.06A, Task TK0002551, 27 January 2012 Syngenta, Non-GLP, not published
SYN524464_11238	Klimmek, S and Gizler, A	2009	SYN524464—Validation of the Residue Analytical Method GRM023.03A for the Determination of Residues of SYN524464 (SYN508210 and SYN508211 and its Metabolites) in Crops Eurofins—Dr Specht GLP GmbH, Hamburg, Germany, Study SYN-0843V, Task T00014685-05, 7 December 2009 Syngenta, GLP, not published
SYN524464_11446	Klimmek, S, Gizler, A and Goellner, C	2011	SYN524464—Storage Stability of Residues of CSCD667584, CSCD658906, CSCD659089, CSCD668403, CSCD667555, CSCD465008 and CSCC210616 in Plant Matrices Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, Report S09-00233, Study S09-00233, Task T014683-05, 2 September 2011 Syngenta, GLP, not published
SYN545192_10059	Kühne, M	2010a	SYN545192—Determination of the physicico-chemical property "boiling temperature" Harlan Laboratories Ltd., Itingen, Switzerland, Study D10197, Task number TK0002534, 1 December 2010 Syngenta, GLP, not published
SYN545192_10060	Kühne, M	2010b	SYN545192—Determination of the physicico-chemical property "melting/freezing temperature" Harlan Laboratories Ltd., Itingen, Switzerland, Study D08702, Task TK0002534, 1 December 2010 Syngenta, GLP, not published
SYN545192_10068	Kühne, M	2010d	SYN545192—Determination of the physicico-chemical property "relative density" Harlan Laboratories Ltd., Itingen, Switzerland, Study D08713, Task TK 0002534, 14 December 2010 Syngenta, GLP, not published

Code	Author	Year	Title, Institute, Report reference
SYN545192_ 10069	Kühne, M	2010e	SYN545192—Determination of the dissociation constant in water Harlan Laboratories Ltd., Itingen, Switzerland, Study D08735, Task TK0002534, 20 December 2010 Syngenta, GLP, not published
SYN545192_ 50049	Lin, K	2012	SYN545192—Validation of Analytical Methods GRM042.03A for the Determination of SYN545192 and its Metabolite SYN546039 in Crops and GRM042.04A for the Determination of SYN545192 and its Metabolite SYN546039 in Soya bean Commodities and SYN545720 in Soya bean Seed Only—Final report amendment 1 Syngenta Crop Protection, LLC, Greensboro, NC, USA, Report TK0002511, Task TK0002511, 9 February 2012 Syngenta, GLP, not published
SYN545192_ 10010	Lowrie, C	2009	SYN545192—Hydrolysis in Sterile Buffer at pH 4, 5, 7 and 9 Charles River Laboratories, Edinburgh, United Kingdom, Report 30389, Study 214939, Task T001809-08, 6 November 2009 Syngenta, GLP, not published
SYN545192_ 10170	Lowrie, C and Kadow, J	2012	SYN545192—Metabolism in the Laying Hen Charles River Laboratories, Edinburgh, United Kingdom, Report 32288, Study 215199, Task T001829-08, 19 January 2012 Syngenta, GLP, not published
A17056B_50 013	Mäyer, T	2012	SYN545192 EC (A17056B and A17056D)—Field Accumulation in Rotational Crops Syngenta Crop Protection, LLC, Greensboro, NC, USA and ALS Environmental, Edmonton, Alberta, Canada, Report T002300-09, ALS study 11SYN290.REP, Task TK0002571, 23 October 2012 Syngenta, GLP, not published
SYN545192_ 10186	McCorquodale, G and White, D	2012	SYN5451–2—Soil Surface Photolysis of [^{14}C]-Phenyl and [^{14}C] Pyrazole Labelled SYN545192 Charles River Laboratories, Edinburgh, United Kingdom, Report 32453, Study no 809207, Task number TK0002646 31 January 2012 Syngenta, GLP, not published
SYN545192_ 10444	O'Connor, BJ	2013a	Benzovindiflupyr—Determination of Density Harlan Laboratories Ltd., Shardlow, Derbyshire, UK, Report 41302902, Study 41302902, Task TK0002534, 25 September 2013 Syngenta, GLP, not published
SYN545192_ 10445	O'Connor, BJ	2013b	Benzovindiflupyr—Determination of Melting Temperature Harlan Laboratories Ltd., Shardlow, Derbyshire, UK, Report 41302904, Study 41302904, Task TK0002534, 25 September 2013 Syngenta, GLP, not published
SYN545192_ 10117	Oddy, A	2011	SYN545192—Metabolism in Tomatoes Battelle UK Ltd., Ongar, Essex, United Kingdom, Report NC/08/038, Study NC/08/038, Task T001824-08, 1 August 2011 Syngenta, GLP, not published
SYN545192_ 10102	Oddy, A	2012	SYN545192—Metabolism in Soy—Final report amendment 1 Battelle UK Ltd., Ongar, Essex, United Kingdom, Report NC/08/037, Study NC/08/37, Task T001823-08, 18 January 2012 Syngenta, GLP, not published
SYN545192_ 10087	Oggenfuss, P	2011	SYN545192—Spectra Syngenta Crop Protection, Münchwilen, Switzerland, Study 122154, 27 January 2011 Syngenta, GLP, not published
SYN545192_ 10246	Roohi, A and Adams, A	2012	SYN545192—Uptake and Metabolism in Confined Rotational Crops Battelle UK Ltd., Ongar, Essex, United Kingdom, Report NC/08/040, Study NC/08/040, Task T001826-08, 7 September 2012 Syngenta, GLP, not published

Code	Author	Year	Title, Institute, Report reference
SYN545192_ 10136	Simmonds, M and Mackenzie, E	2011	SYN545192—Metabolism in Spring Wheat Battelle UK Ltd., Ongar, Essex, United Kingdom, Report NC/08/039, Study NC/08/039, Task T001825-08, 8 September 2011 Syngenta, GLP, not published
SYN545192_ 10162	Simmonds, R	2011	SYN545192—Aqueous Hydrolysis at 90, 100 and 120 °C Battelle UK Ltd., Ongar, Essex, United Kingdom, Report NC/09/021, Study NC/09/021, Task TK0002633, 20 December 2011 Syngenta, GLP, not published
SYN545192_ 10468	Syngenta	2014a	Benzovindiflupyr JMPR Residue 2014—Response to Questions from JMPR Review, February 2014 Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Non-GLP, not published
-	Syngenta	2014b	Benzovindiflupyr JMPR Residue 2014—Response to Questions (2) from JMPR Review, July 2014 Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Non-GLP, not published
-	Syngenta	2014c	Benzovindiflupyr—Comments on Draft Codex Evaluation. July 2014. Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Non-GLP, not published
-	Syngenta	2014d	Benzovindiflupyr: Leaf photolysis—pyrazole label. Syngenta Crop Protection AG, Basel, Switzerland Syngenta, Non-GLP, not published Document submitted to the 2014 JMPR Meeting
SYN545192_ 10066	Vijayakumar, C	2010a	SYN545192—Solubility in organic solvents Syngenta Biosciences Pvt. Ltd., Corlim, Ilhas Goa, India, Study SMG10480, 16 December 2010 Syngenta, GLP, not published
SYN545192_ 10070	Vijayakumar, C	2010b	SYN545192—Octanol/water partition coefficient Syngenta Biosciences Pvt. Ltd., Corlim, Ilhas Goa, India, Study SMG10481, 31 December 2010 Syngenta, GLP, not published
SYN545192_ 10072	Vijayakumar, C	2011	SYN545192—Solubility in water Syngenta Biosciences Pvt. Ltd., Corlim, Ilhas Goa, India, Study SMG10503, 11 January 2011 Syngenta, GLP, not published
SYN545192_ 10195	Volpi, R	2012	Determination of Residues of SOLATENOL and its Metabolites SYN546039 and SYN545720 in Soya bean by LC/MS/MS Syngenta Proteção de Cultivos Ltda., Department of Chemical and Environmental Studies, Brazil Standard Operating Procedure POPIT MET. 134. Rev01, 10 January 2012 Syngenta, Non-GLP, not published
SYN545192_ 10157	Ward, C and Harris, J	2011	SYN545192—Validation of an Analytical Method for the Determination of SYN545192 and its Metabolites SYN546039 and SYN546422 in Bovine Meat, Liver, Kidney, Fat, Milk, Blood and Chicken Eggs Charles River Laboratories, Edinburgh, United Kingdom, Report 32556, Study 218870, Task TK0002551, 13 December 2011 Syngenta, GLP, not published
SYN545192_ 10188	Ward, C and Vance, C	2012	SYN545192—Magnitude of Residues in Milk and Tissues of Dairy Cows Following Multiple Oral Administrations of SYN545192 and the Storage Stability of SYN545192 and Related Metabolites in Milk, Eggs, Liver and Muscle Charles River Laboratories, Edinburgh, United Kingdom, Report 32505, Study 218823, Task TK0002553, 3 February 2012 Syngenta, GLP, not published
SYN545192_ 10132	Wardrope, L	2011	SYN545192—Photodegradation in sterile aqueous solution Charles River Laboratories, Edinburgh, United Kingdom, Report 32108, Study 808989, Task TK0002647, 2 September 2011 Syngenta, GLP, not published

Code	Author	Year	Title, Institute, Report reference
SYN545192_ 10172	Watson, G	2012a	SYN545192—Independent Laboratory Validation of the QuEChERS Method for the Determination of Residues of SYN545192 in Crops Matrices by LC-MS/MS Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S11-03524, Study S11-03524, Task TK0002509, 25 January 2012 Syngenta, GLP, not published
SYN545192_ 10169	Watson, G	2012b	SYN545192—Independent laboratory validation of the QuEChERS method for the determination of residues of SYN545192 in animal matrices by LC-MS/MS Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S11-03525-REG, Study S11-03525, Task TK0060248, 17 Jan 2012 Syngenta, GLP, not published
SYN545192_ 10203	Watson, G	2012c	SYN545192—Validation of Method GRM042.08A for the Determination of SYN545192 and its Metabolites SYN546039 and SYN546206 in Rotational Crops Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S12-00079, Study S12-00079, Task TK0025355, 11 April 2012 Syngenta, GLP, not published
SYN545192_ 10137	Watson, G	2013	SYN545192—Storage Stability of Residues of SYN545192, SYN546039 and SYN546206 in Crop Matrices Stored Frozen for up to Two Years Eurofins Agroscience Services Ltd, Wilson, Derbyshire, UK, Report S11-02333-REG, Study S11-02333, Task TK0002512, 10 July 2013 Syngenta, GLP, not published
SYN545192_ 10067	Weissenfeld, M	2010	SYN545192—Determination of the vapor pressure Harlan Laboratories Ltd., Itingen, Switzerland, Study D08724, Task Number TK0002534, 23 December 2010 Syngenta, GLP, not published
SYN545192_ 10088	Williams, C	2011	SYN545192—Thermal stability / stability in air Syngenta Technology & Projects, Huddersfield, United Kingdom, Study HT10/312, sponsor reference 121835, 21 January 2011 Syngenta, GLP, not published
SYN545192_ 10193	Winter, O and Amann, S	2012	SYN545192—Validation of the Multiple Residue Method QuEChERS for the Determination of Residue of SYN545192 in Crop Matrices Eurofins Agroscience Services Chem GmbH, Hamburg, Germany, Report S11-03082, Study S11-03082, Task TK0002510, 24 February 2012 Syngenta, GLP, not published