5.2 BENZOVINDIFLUPYR (261)

RESIDUE AND ANALYTICAL ASPECTS

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 0.1 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.

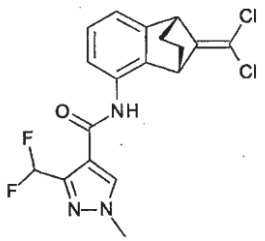
Benzovindiflupyr is a broad-spectrum fungicide belonging to the chemical class of pyrazole carboxamides. Benzovindiflupyr acts as an inhibitor of the fungal complex II respiratory chain, where it inhibits the succinate dehydrogenase enzyme (succinate dehydrogenase inhibitor, SDHI) by blocking the ubiquinone-binding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

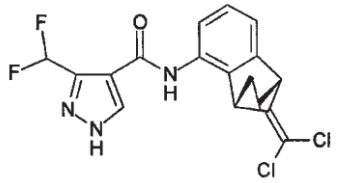
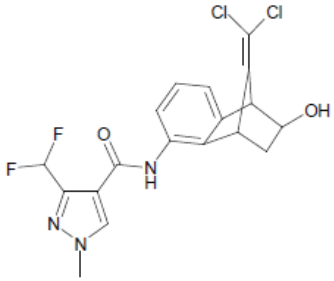
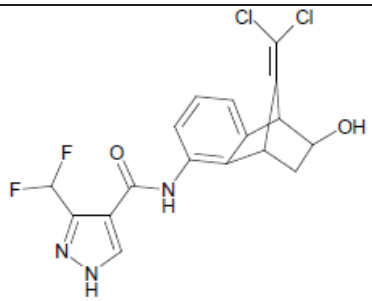
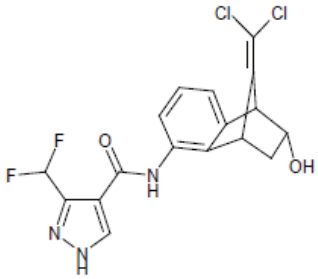
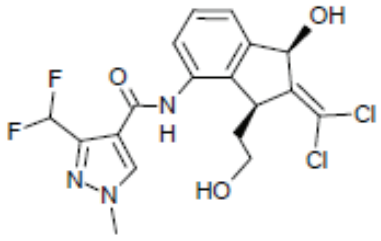
Benzovindiflupyr: N-[(1RS,4SR)-9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide (IUPAC).

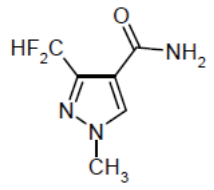
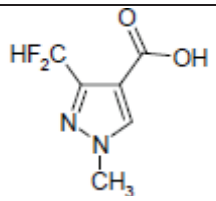
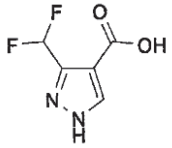
Structural formula:



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	 3-difluoromethyl-1-methyl-1*H*-pyrazole-4-carboxylic acid
SYN545720	 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 eq./kg) and kidney (0.28/0.18 mg eq./kg), followed by fat (0.098/0.070 mg eq./kg) and muscle (0.070/0.032 mg eq./kg).

Total radioactive residues in milk reached a plateau concentration of approximately 0.046 mg eq./kg following 96 hours dosing for the phenyl label and 0.035 mg eq./kg 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 eq./kg), followed by fat (0.033/0.045 mg eq./kg) and muscle (0.025/0.036. mg eq./kg). Total radioactive residues in egg yolks achieved a plateau concentration of 0.17-0.18 mg eq./kg after 168–240 hrs of dosing. Total radioactive residues in egg whites achieved a plateau concentration of 0.03–0.04 mg eq./kg after 120–168 hours 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 eq./kg for the phenyl label and 0.18 and 0.15 mg eq./kg 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 eq./kg for the phenyl label and 2.1, 6.4, 9.0, 0.092 mg eq./kg 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 eq./kg for the phenyl label and 4.1, 13 and 0.10 mg eq./kg 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 eq./kg) in soya forage, 0.039 mg eq./kg in soya hay or 0.0012 mg eq./kg 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 or 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 DT_{50} 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 eq./kg at 30 day PBI, 0.002–0.34 mg eq./kg at 90 day PBI and 0.007–0.29 mg eq./kg at 300 day PBI. Total radioactivity levels above 0.05 mg eq./kg were found in wheat forage, hay and straw at all plant back intervals.

Wheat grain had very low residues (< 0.01–0.014 mg eq./kg) 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 3× 0.1 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 (1× 0.2 kg ai/ha or 3×0.1 kg ai/ha) are higher than those used in the actual supervised residue trials submitted (3× 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 hours 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 (incl 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 hours 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 -18 ºC 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), at -20 ºC, were stable 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 eq./kg) in milk and goat tissues, 12–22% TRR(0.008–0.022 mg eq./kg) 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 eq./kg) in milk and goat kidney and 1.5–8.9% TRR (0.001-0.056 mg eq./kg) 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 eq./kg) and 6.6–12% TRR (0.002-0.020 mg eq./kg) 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 K_{ow} 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 eq./kg eq) including conjugates). A significant metabolite in soya forage and hay was SYN546039 (9.2–12% TRR (0.38–1.6 mg eq./kg) 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 eq./kg) and 24–26% TRR (0.023–0.026 mg eq./kg) 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 eq./kg, 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 (3× 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 to the 2014 Report. 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]
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 hours 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.

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 to the 2014 Report.

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 to the 2014 Report.

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.