5.15 Mandestrobin (307)

RESIDUE AND ANALYTICAL ASPECTS

At the Forty-ninth Session of the CCPR (2017), mandestrobin was scheduled for toxicology and residue evaluation as a new compound by the 2018 JMPR. During the 2018 JMPR it appeared that soil degradation studies, field dissipation studies and additional supervised field trials were available that could aid in the definition of the residue. The residue evaluation was therefore postponed to the 2019 JMPR.

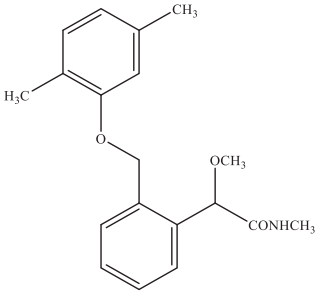
The 2018 JMPR established an ADI of 0–0.2 mg/kg bw and an ARfD of 3.0 mg/kg bw for women of childbearing age. The 2018 Meeting concluded that it was not necessary to establish an ARfD for mandestrobin for the remainder of the population.

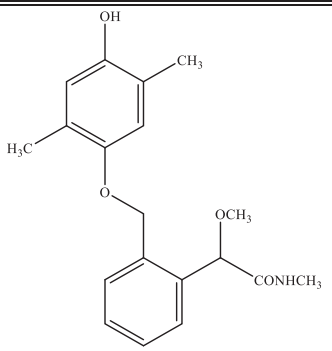
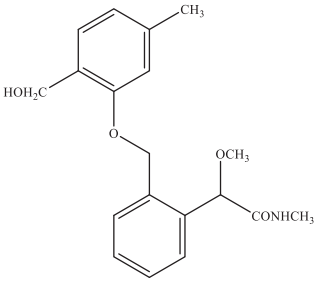
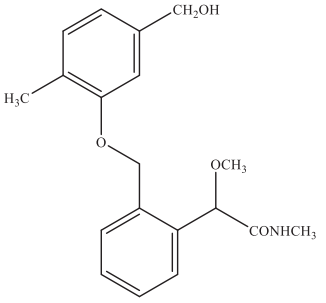
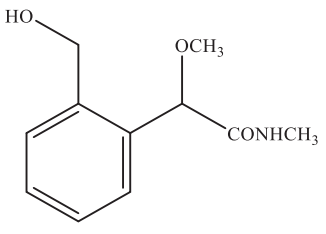
Mandestrobin is a systemic strobilurin fungicide. It acts on various fungi and also on the control of bacterial nuclear disease. Mandestrobin acts by inhibiting mitochondrial respiration. It binds at the Qo-centre on cytochrome b and blocks electron transfer between cytochrome b and cytochrome c1, disrupting the energy cycle within the fungus by halting the production of ATP.

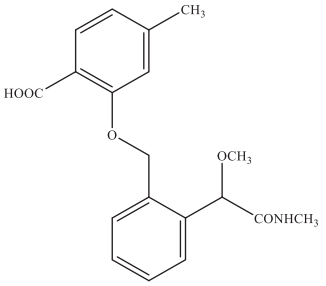
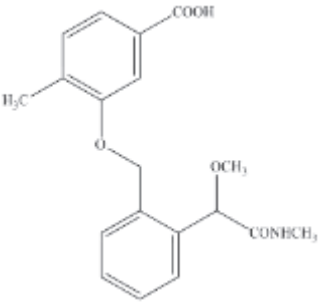
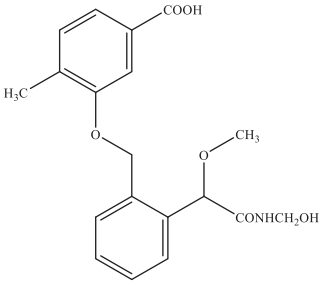
The Meeting received information from the manufacturer on identity, physical and chemical properties, metabolism in plant and livestock, confined and field rotational crop studies, soil degradation studies, field dissipation studies, residue analysis, storage stability, use patterns, supervised residue trials, fate of residues during processing and livestock feeding studies.

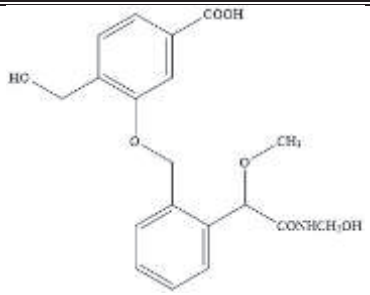
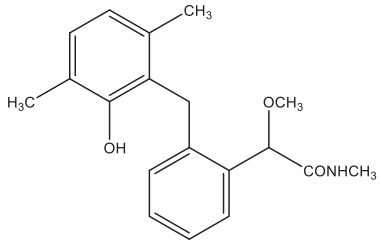
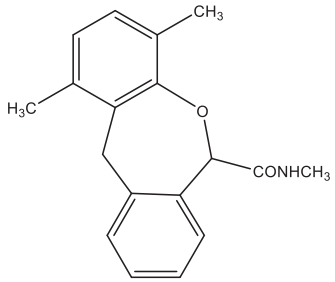
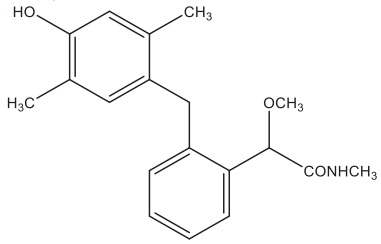
The IUPAC name of mandestrobin is (RS)-2-methoxy-N-methyl-2-[α-(2,5-xylyloxy)-o-tolyl]acetamide. The CAS name is 2-[(2,5-dimethylphenoxy)methyl]-α-methoxy-N-methylbenzeneacetamide. Mandestrobin (S-2200) is a racemic mixture of S-2167 (R-isomer) and S-2354 (S-isomer). All compounds referred to in the appraisal are listed in the table below.

Table 1 Abbreviations for the relevant compounds referred to in the appraisal

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
Mandestrobin	IUPAC: (2RS)-2-((2,5-dimethylphenoxy)methyl)phenyl)-2-methoxy-N-methylacetamide  MW = 313.39	Hydrolysis in water Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Tomato fruits; Tomato leaves Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60); Rape seed [application at bloom] Rotated wheat grain; Rotated wheat straw Rotated wheat forage; Rotated wheat hay Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
4-OH- mandestrobin	IUPAC: (2*RS*)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-*N*-methylacetamide	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat grain; Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	 MW = 329.39	Rotated carrot leaves Egg Poultry liver Poultry muscle Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney
2-CH_2OH-mandestrobin	IUPAC: (2*RS*)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay;Wheat straw Wheat grain Green rape seed fodder (BBCH > 60) Rape seed [application before or at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry liver Poultry muscle Poultry skin Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
5-CH_2OH-mandestrobin	IUPAC: (2*RS*)-2-[2-(5-hydroxymethyl-2-methylphenoxymethyl)phenyl]-2-methoxy-*N*-methylacetamide  MW = 329.39	Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Green rape seed fodder (BBCH > 60) Rape seed [application at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot leaves Milk fat Ruminant liver; Ruminant kidney
De-Xy- mandestrobin	IUPAC: (2*RS*)-2-(2-hydroxymethylphenyl)-2-methoxy-*N*-methylacetamide  MW = 210.253	Photolysis in water Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Immature lettuce; Mature lettuce Wheat forage; Wheat hay; Wheat straw Wheat grain Egg Poultry liver Poultry skin; Poultry fat Milk fat; Skimmed milk Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
2-COOH-mandestrobin	IUPAC: 2-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid  MW = 343.38	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Egg Poultry liver Poultry skin Ruminant liver; Ruminant kidney Ruminant muscle; Ruminant fat
5-COOH-mandestrobin	IUPAC: 3-({2-[(1*RS*)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid  MW = 343.38	Photolysis in water (tentative; not confirmed) Soil surface photodegradation Laboratory aerobic soil degradation Field dissipation Wheat straw Rape seed [before and at bloom] Rotated wheat forage; Rotated wheat hay Rotated wheat straw Rotated immature lettuce; Rotated mature lettuce Rotated carrot root; Rotated carrot leaves Poultry skin Ruminant liver; Ruminant kidney Ruminant fat
5-CA-mandestrobin-NHM	3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)-4-methylbenzoic acid  MW = 359.38	Egg Poultry skin; Poultry fat Skimmed milk Ruminant liver
5-CA-2-HM-mandestrobin-NHM	4-(hydroxymethyl)-3-((2-(2-((hydroxymethyl)amino)-1-methoxy-2-oxoethyl)benzyl)oxy)benzoic acid	Poultry skin Ruminant liver; Ruminant kidney

Abbreviation	Trivial and systematic chemical names Other abbreviations used in study reports Structural formula	Found as or in
	 MW = 375.38	
Mandestrobin-OR	(*RS*)-2-(2-(2-hydroxy-3,6-dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide 	Photolysis in water Soil surface photodegradation The benzyl radical recombined at the o- position of the phenoxy radical.
Mandestrobin-ORC	(*RS*)-N,1,4-trimethyl-6,11-dihydrodibenzo[b,e]oxepine-6-carboxamide 	Photolysis in water Analysed in soil surface photodegradation but not detected Formed from mandestrobin-OR by cyclisation.
Mandestrobin-PR	(*RS*)-2-(2-(4-hydroxy-2,5-dimethylbenzyl)phenyl)-2-methoxy-N-methylacetamide 	Photolysis in water Analysed in soil surface photodegradation but not detected The benzyl radical recombined at the p- position of the phenoxy radical.

Physical and chemical properties

Mandestrobin is not volatile (3.4×10^{-5} mPa at 20 °C). Solubility in water is low (15.8 mg/L) and higher in various organic solvents (up to 522 g/L in acetone). Hydrolysis is unlikely to be a significant route of degradation in the environment, but photodegradation in water to 3% TRR was shown within 30

days.

Plant metabolism

The Meeting received plant metabolism studies for mandestrobin under greenhouse conditions (topical and soil applications on tomato plants and foliar spray applications on lettuce and wheat), under outdoor conditions (foliar application on oilseed rape), and on seed treatments of maize grains and soya bean seeds.

In all plant metabolism studies the R/S ratio of mandestrobin remained approximately 1:1.

Where two values are separated by "/", they account for the phenoxy [Ph-^{14}C]- and benzyl [Bz-^{14}C] labelled mandestrobin, respectively.

Plant metabolism after greenhouse applications

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was also studied in greenhouse grown tomato plants following 3 topical applications, using syringes, to fruits or leaves at a rate approximating 0.3 kg ai/ha each, with a 10-day interval. Plants were harvested 3 days after the last treatment. Total radioactive residues in fruit and leaves were 7.4–14 mg eq/kg and 87–199 mg eq/kg, respectively. Fruit and leaves were surface rinsed with acetonitrile and extracted with acetone/water. No residues were detected in the untreated leaves and fruits indicating no translocation from the treated fruits or leaves to the other parts of the plants. The majority of the radioactivity (95–99%) of the total radioactive residue (TRR) remained on the fruit or leaf surface, suggesting mandestrobin is not systemic. The major compound was identified as parent: 99–100% TRR in fruits and 98–99% TRR in leaves.

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown tomato plants following a single soil application at a rate of 0.9 kg ai/ha at fruit developing stage. Plants and soil were collected at 24 days after treatment. Insignificant translocation from soil to plant was observed: 0.06–0.08% of the total applied radioactivity (TAR) was found in leaves and none in fruits.

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown lettuce following two foliar applications each at 0.82 kg ai/ha with a 10-day interval. Lettuce was treated at BBCH 43 and BBCH 48. Samples of immature and mature lettuce were taken 5 days after each treatment, respectively. Total radioactive residues in immature and mature lettuce were 35 and 43 mg eq/kg for the phenoxy label and 28 and 42 mg eq/kg for the benzyl label, respectively. A high proportion of the residue was removed by acetonitrile surface wash (78–88% TRR) and the total amount extracted with acetone/water, including the acetonitrile surface wash, was > 98% TRR. The principal component of the residue in the immature and mature lettuce plant was the parent compound (89–94% TRR).

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in greenhouse grown wheat following a single foliar spray application at 0.31 or 0.30 kg ai/ha, respectively. Wheat was treated at BBCH 32. Samples of wheat forage and wheat hay (each BBCH 37) were taken 7 and 14 days after treatment, respectively. Grain and straw samples were collected at maturity at 104 days after treatment. Total radioactive residues in wheat forage, hay, straw and grain were 11/10 mg eq/kg, 6.2/9.0 mg eq/kg, 1.9/2.5 mg eq/kg and 0.012/0.089 mg eq/kg, respectively.

Acetonitrile surface wash removed a total of 41/34% TRR in wheat forage, 23/19% TRR in wheat hay, and 3.7/2.8% TRR in wheat straw samples, harvested at 7, 14 and 104 days after treatment, respectively. A total of 91/92, 84/90, 45/49 and 54/53% TRR could be extracted from wheat forage, hay, straw and grain with acetone/water (including the surface wash), respectively. The remaining residues from hay and straw could be released with enzymes, acid and base until less than 2% TRR remained as solids.

In wheat grains a total of 64% TRR was identified for the benzyl label only. Parent mandestrobin was not detected and De-Xy-mandestrobin was the major component with 61% TRR

(0.054 mg eq/kg). None of the ^{14}C-phenoxy labelled material could be identified, noting the low total residues.

A total of 71/81% TRR was identified in wheat forage, 59/51% TRR in wheat hay, and 18/30% TRR in wheat straw. Parent was the major component in wheat forage (51/60% TRR or 5.7–6.2 mg eq/kg) and hay (26/23% TRR or 1.6/2.1 mg eq/kg). In wheat straw, parent mandestrobin accounted only for 1.4/2.0% TRR (0.026/0.050 mg eq/kg). The residue in wheat straw consisted of several low level components with De-Xy-mandestrobin (up to 12% TRR) being the major component. De-Xy-mandestrobin accounted for 3.2% TRR in wheat forage and 1.5% TRR in wheat hay. Free and malonylglucoside conjugated 4-OH-mandestrobin was detected in wheat straw, forage and hay with levels ranging from 1.2/1.5% TRR in straw to 13/5.5% TRR in hay. Free and malonylglucoside conjugated 2-CH_2OH-mandestrobin accounted for 11/5.5% TRR in wheat forage, 11/13% TRR in hay and 9.5/6.4% TRR in straw. Other identified and unidentified metabolites accounted for less than 10% TRR (< 0.83 mg eq/kg).

Plant metabolism after outdoor applications

The metabolism of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in field grown oilseed rape following either one application at 0.39 kg ai/ha at BBCH 55–61 or following two foliar spray applications of 0.39–0.38 kg ai/ha each, applied at BBCH 55–61 (pre-bloom) and BBCH 66–67 (full bloom), with an interval of 14 days. Rape seed samples were harvested at maturity (BBCH 89) at 54 days after the single treatment or at 40 days after the double treatment. Green rape fodder samples (BBCH > 60) were collected 14 days after the double treatment only. Total radioactive residues were 3.4–4.0 mg eq/kg in green rape fodder, 0.47–0.64 mg eq/kg in rape seed at DAT 40 (double treatment) and 0.050–0.11 mg eq/kg in rape seed at DAT 54 (single treatment).

In rape seeds (DAT 40) after two applications, hexane combined with acetone/water extracted 78/92% TRR, while 58/46% TRR was identified. Parent was the major compound with 31/25% TRR (0.14/0.16 mg eq/kg). The major metabolite was malonylglucoside conjugated 4-OH-mandestrobin, which was found at 14/11% TRR (0.068–0.071 mg eq/kg). Other identified and unidentified metabolites were below 10% TRR.

In rape seed (DAT 54) after one application, hexane combined with acetone/water extracted 72/79% TRR, while only 20/0% TRR was identified. None of the components were found at levels above 10% TRR. Parent was not detected. Malonylglucoside conjugated 4-OH-mandestrobin (8.0% TRR), malonylglucoside conjugated 2-CH_2OH-mandestrobin (3.6% TRR) and free 5-COOH-mandestrobin (8.7% TRR) were the major components identified in the phenoxy label only. None of the residues in the ^{14}C-benzyl label experiment were identified.

In green rape fodder (DAT 14) after two applications, acetone/water extracted 87/89% TRR (including 34–37% TRR that was removed with an acetonitrile surface wash). A total of 73/65% TRR was identified. The principal component of the residue was the malonylglucoside conjugated form of 4-OH-mandestrobin (36/27% TRR), followed by parent (20/22% TRR) and the free and malonylglucoside conjugated form of 2-CH_2OH-mandestrobin (12/13% TRR). Other identified and unidentified metabolites were below 10% TRR.

The Meeting noted that the two studies on rape seeds had different metabolic profiles. The single application and the first of the two applications were conducted before flowering, i.e. prior to seed formation. Since the GAP indicates that application should be conducted at flowering (BBCH 62–65), the study where the second application was conducted at BBCH 66–67 is considered more relevant for the definition of the residue.

Metabolism after seed treatment

The metabolic fate of [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was studied in maize grains and soya bean seeds following seed treatment at 9.3–12 g ai/100 kg of seeds. Seeds were planted 6 days after treatment. Harvest occurred at appropriate growth stages. The soya bean food RACs and the maize food and feed RACs did not take up mandestrobin related residues to any significant extent

(< 0.005 mg eq/kg). Significant residues were found only in maize fodder (< 0.005–0.008 mg eq/kg; benzyl label only), soya bean forage (0.038–0.061 mg eq/kg) and soya bean hay (0.030–0.050 mg eq/kg).

Extraction with acetonitrile and acetonitrile/water released a total of 71% TRR from maize fodder (benzyl label only), 87/89% TRR from soya bean forage and 48/53% TRR from soya bean hay, while only 8.2, 29/7.6, 3.7/3.4% TRR was identified, respectively. The major compound identified in soya bean forage was De-Xy-mandestrobin at 5.3–12% TRR (0.005 mg eq/kg). Identified components in maize fodder and soya bean hay were all below 10% TRR. Mandestrobin was found at 1.2–1.9% TRR (soya bean hay), 2.3–7.9% TRR (soya bean forage) and 2.8% TRR (maize fodder), but never exceeded 0.003 mg eq/kg. HPLC profiles showed two major unidentified fractions which accounted for 14–29% TRR (0.001–0.022 mg eq/kg) and 9.6–22% TRR (0.002–0.016 mg eq/kg).

Metabolism in confined rotational crops

In a confined rotational crop study, [Ph-^{14}C]-mandestrobin or [Bz-^{14}C]-mandestrobin was applied to bare soil at a single rate of 1.6 kg ai/ha. Rotational crops (lettuce, carrots and wheat) were planted at intervals (PBI) of 30, 120 and 365 days after application. Wheat forage, hay, straw and grain, lettuce, carrot roots and leaves were harvested at appropriate growth stages. Significant residues (0.019–4.5 mg eq/kg) were found in all crop commodities at all plant back intervals, except in wheat grains and carrot roots at PBI 365 where total radioactivity did not exceed 0.01 mg eq/kg. Total radioactivity of residues in rotational crops generally declined from PBI 30 to PBI 120 and PBI 365.

Acetone/water extracted 77–87% TRR from carrot roots, 59–92% TRR from lettuce, carrot leaves and wheat forage, 45–62% TRR from wheat hay, and 34–64% TRR from wheat straw. Residues from wheat grain could not be extracted to a significant extent, with only 5.2–5.8% TRR for the phenoxy label and 31–37% TRR for the benzyl label. Only 47–65% TRR (carrot roots, benzyl label, but was higher for the phenyl-label), 6.9–53% TRR (wheat forage), 16–71% TRR (lettuce), 5.4–26% TRR (carrot leaves), 9.3–45% TRR (wheat hay), and 14–33% TRR (wheat straw) was identified.

Parent was present at low concentrations in all crop commodities, with a maximum of 78% TRR (0.040 mg eq/kg) in carrot roots at PBI 30 days or 1.8% TRR (0.045 mg eq/kg) in wheat forage at PBI 30 days. Major metabolites found in free and malonylglucoside or glucoside conjugated form were 4-OH-mandestrobin (at 2.7–34% TRR in all samples, except carrot roots), 5-CH_2OH-mandestrobin (1.7–32% TRR in all samples, except wheat grain) and to a minor extent 2-CH_2OH-mandestrobin (0–16% TRR in all samples, except wheat grain). Metabolite De-Xy-mandestrobin was not observed in the confined rotational crop study. Other identified and unidentified metabolites were below 10% TRR.

Summary and conclusion of metabolism in primary and rotational crops

Mandestrobin remains at the treated surface of the plant with very little translocation, absorption and degradation during the first 5 days after treatment under greenhouse conditions as was shown for tomato and lettuce (> 80% TRR in surface wash). With increasing time, parent compound is absorbed and metabolized as was shown by the decreasing amounts of residues found in the surface washes of field grown green rape fodder (34–37% TRR at DALT 14) and greenhouse grown wheat forage (34–41% TRR at DAT 14) and wheat straw (2.8–3.7% TRR at DAT 104).

In lettuce and tomato the TRR consisted nearly completely of unchanged parent (98–100% TRR). Parent (23–60% TRR) was also the major compound in wheat forage, wheat hay, and green rape fodder after foliar treatment, but not in wheat straw and wheat grain (DAT 104). In rotational root crop commodities (carrot roots) at PBI 30 and 120 days, parent accounted for 53–78% TRR, albeit at low absolute levels (0.015–0.040 mg eq/kg). For applications with increasing PHI and/or where uptake via the soil is possible, a larger variety of metabolites occur. De-Xy-mandestrobin was the major and only identified compound in wheat grain (61% TRR) and it was also the major compound in primary wheat straw (12% TRR). Both parent and De-Xy-mandestrobin were found at low levels (< 0.005 mg eq/kg) in seed treated feed commodities (soya bean forage, maize fodder). Compounds at levels above 10% TRR were free and (malonyl)glucoside conjugated 4-OH-mandestrobin (3.4–36% TRR), 2-CH_2OH-mandestrobin (3.6–14% TRR), and 5-CH_2OH-mandestrobin (4.3–6.8% TRR). Major compounds in the

confined rotational crops were free and (malonyl)glucoside conjugated 4-OH-mandestrobin (1.7–24% TRR), 5-CH_2OH-mandestrobin (2.9–27% TRR) and 2-CH_2OH-mandestrobin (0.69–16% TRR). The identity of the residues in the wheat grain from field grown rotational crops remains unknown, with parent compound and 4-OH-mandestrobin detected at trace levels (< 0.001–0.008 mg eq/kg).

The metabolism of mandestrobin in primary and rotational crops is essentially the same, although quantitative levels of parent and metabolites differ.

The metabolic pathway of mandestrobin included monohydroxylation of the dimethylphenoxy ring to form 4-OH-mandestrobin followed by formation of the glucoside and (malonyl)glucoside conjugates. Oxidation of the methyl groups attached to the 2- and 5-positions on the dimethylphenoxy ring to form 2-CH_2OH-mandestrobin, 5-CH_2OH-mandestrobin and 5-COOH-mandestrobin was followed by formation of the corresponding (malonyl)glucoside conjugates. Minor metabolic pathways included the O-demethylation of the methoxy-group of the side chain. Another metabolic pathway includes cleavage of the ether linkage to form De-Xy-mandestrobin, which can only be detected with the benzyl label.

Since a photolysis study in water indicates that significant photo-degradation may occur, the Meeting discussed whether the metabolism studies using foliar treatments conducted in a greenhouse (lettuce, wheat) were representative for uses in the field. Comparison of the nature and magnitude of residues found in green rape fodder (outdoor, DALT 14 days) and in wheat hay (greenhouse, DALT, 14 days) shows no accelerated degradation by photolysis, since parent compound accounted for 20/22% TRR and 26/22% TRR, respectively for green rape fodder and wheat hay. Although no photolytic degradation products were used as reference compounds in any of the metabolism studies, all unknowns in the metabolism studies were below 10% TRR or < 0.01 mg eq/kg, indicating that photolytic degradation products may be present at trace levels only. From this, the Meeting concluded that photolysis is not a significant form of degradation. Therefore, the metabolism studies in the greenhouse can be used in support of outdoor uses.

All plant metabolites identified in the primary and rotational crop studies were found in the rat metabolism study.

Animal metabolism

The Meeting received information on the fate of orally-dosed mandestrobin in rat, lactating goats and laying hens. Where two values are given divided by "/", they account for the [Ph-^{14}C]- and [Bz-^{14}C]-mandestrobin labels, respectively.

Metabolism in laboratory animals was summarized and evaluated by the WHO panel of the JMPR in 2018.

Lactating goats were orally dosed with [Phenoxy-^{14}C]-mandestrobin or [Benzyl-^{14}C]-mandestrobin, equivalent to 13–14 ppm in the feed for 7 consecutive days. Goats were sacrificed 6 hrs after the last dose. The majority of the cumulative administered dose was recovered in urine and faeces at 35–40 and 38–42% TAR, respectively. Radioactivity recovered in tissues (liver, kidney, muscle and fat) accounted for a total of 0.27–0.33% TAR. Radioactivity accounted for 0.002–0.005% TAR in milk fat and 0.024–0.073% TAR in skimmed milk. The highest recovery found in edible tissues was in liver (0.22–0.29% TAR).

Steady state conditions in milk were achieved within 1 day of the first dose administration, indicating that mandestrobin is rapidly eliminated. TRR levels in milk were 0.006 and 0.035 mg eq/kg in the aqueous and fat fraction of milk, respectively. Highest TRR levels were found in liver (0.32/0.61 mg eq/kg), followed by kidney (0.17/0.41 mg eq/kg), fats (0.012–0.031 mg eq/kg) and loin and flank muscle (0.010–0.015 mg eq/kg).

Extractability of radioactivity from milk, kidney, muscle and fat with solvents (hexane, acetonitrile and ethyl acetate) ranged from 72–100% TRR, except liver (60–70% TRR). Only liver post-extracted solids were treated with enzymes, acid and base until less than 6% TRR remained as solids.

Only 53/47 (milk fat), -/32 (skimmed milk); 31/36 (muscle), 65/37 (fat), 48/41 (liver) and 59/53 (kidney) as % TRR was identified.

Parent was the major compound identified in milk fat (33/32% TRR), goat muscle (23/18% TRR) and fat (50/23% TRR). In liver, kidney and skimmed milk lower levels of parent were found (1.6 to 7.7% TRR). A major metabolite identified in goat liver (20/11% TRR) and kidney (25/20% TRR) was free 5-COOH-mandestrobin. A second major metabolite identified in kidney was free and glucuronide conjugated 4-OH-mandestrobin (17/13% TRR). Free 2-CH_2OH-mandestrobin was found in muscle up to 10% TRR (0.0015 mg eq/kg). The major compound identified in skimmed milk was 5-CA-mandestrobin-NHM at 15% TRR (0.003 mg eq/kg). Several other metabolites were identified in various goat matrices but only at levels < 10% TRR and below 0.01 mg eq/kg, apart from 2-CH_2OH-mandestrobin (up to 0.038 mg eq/kg in liver) and 2-COOH-mandestrobin (up to 0.014 mg eq/kg in liver). The fat extract from the benzyl label contained one region of 38% TRR (0.011 mg eq/kg) that could not be identified, despite exhaustive efforts.

Two groups of laying hens were orally dosed once daily for 14 consecutive days via capsules equivalent to 13 ppm in the feed. Hens were sacrificed 6–7 hours after dosing. The majority of the administered dose was recovered in excreta (83/98% TAR). A minor part of the total radioactivity was recovered in eggs (0.21/0.18% TAR) and tissues (0.070/0.090% TAR). The highest residue concentration in tissues was found in the liver (0.29/0.30 mg eq/kg) followed by skin (0.048/0.054 mg eq/kg), fat (0.032/0.032 mg eq/kg) and muscle (0.014/0.024 mg eq/kg). Total radioactive residues in whole eggs achieved a plateau concentration of 0.11 mg eq/kg [Ph-^{14}C]-label after 11 days of dosing. Total radioactive residues in egg whites and egg yolk were not determined separately.

The residue after solvent extraction (hexane, ethyl acetate and acetonitrile) accounted for 86–96% TRR in fat, eggs and skin. In liver and muscle the extracted residue accounted for 63–66% TRR and 52–59% TRR, respectively. An additional 28–32% TRR was released from liver by sequential extraction with water, 1 M HCl, 1 M NH_3 and protease digestion. Only 19–23% (liver), 5.0–5.9% TRR (muscle), 42–59% TRR (eggs, fat) and 10–33% TRR (skin) was identified.

In eggs and hen fat, the main component of the radioactive residue was free parent, accounting for 51/33% TRR (0.058/0.025 mg eq/kg) and 50/34% TRR (0.016/0.011 mg eq/kg), respectively. All metabolites identified in egg and fat were present at levels < 0.01 mg eq/kg. In muscle and skin, parent mandestrobin and identified metabolites were found at trace levels (< 0.001 mg eq/kg).

In hen liver, parent mandestrobin accounted for 3.0/2.1% TRR (equivalent to 0.009/0.006 mg eq/kg). The main components in liver were free (8.6% TRR) and conjugated (3.6% TRR) De-Xy-mandestrobin (total of 12% TRR, equivalent to 0.036 mg eq/kg) with the [^{14}C-Bz]-label only and free (13% TRR/-) and conjugated (2.0/2.7% TRR) 4-OH-mandestrobin (total 15/2.7% TRR, equivalent to 0.045/< 0.001 mg eq/kg). Other metabolites were present at trace levels (< 0.01 mg eq/kg).

Summary and conclusion of metabolism in livestock

Parent was the major compound identified in milk fat, goat muscle and goat and poultry fat and eggs (18–51% TRR). In poultry muscle and skin, goat and poultry liver, goat kidney and skimmed milk lower levels of parent were found (1.3–7.7% TRR). Free 5-COOH-mandestrobin was identified as major metabolite in goat liver and kidney (11–25% TRR). A second major metabolite identified in kidney was free and glucuronide conjugated 4-OH-mandestrobin (13–17% TRR). Free 2-CH_2OH-mandestrobin was found in muscle up to 10% TRR. The major compound identified in skimmed milk was 5-CA-mandestrobin-NHM (15% TRR). Several other metabolites were identified in various goat matrices at levels < 10% TRR and mostly below 0.01 mg eq/kg, apart from 2-CH_2OH-mandestrobin (up to 0.038 mg eq/kg in liver) and 2-COOH-mandestrobin (up to 0.014 mg eq/kg in liver).

In summary, the primary metabolic process observed in lactating goats and laying hens included a series of hydroxylations and oxidations, N-demethylation, O-demethylation, ether hydrolysis and (glucuronide) conjugation. Hydroxylation of the phenoxy ring gives 4-OH-mandestrobin, and

hydroxylation of the methyl groups on the phenoxy ring gives 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. Further oxidation of the hydroxymethyl groups to the carboxylic acid gives 2-COOH-mandestrobin, 5-COOH- mandestrobin and 5-CA-2-HM-mandestrobin. Hydroxylation also occurs on the N-methyl group, to give 5-CA-mandestrobin-NHM and 5-CA-2-HM-mandestrobin-NHM. Mandestrobin is also subject to hydrolysis of the phenoxy ether link, yielding De-Xy-mandestrobin, N-demethylation of 5-COOH-mandestrobin, and O-demethylation. The primary metabolites are further metabolized by conjugation.

The metabolic profile of mandestrobin in ruminants and poultry is very similar to that of rats.

Environmental fate

The Meeting received information on hydrolysis and aqueous photolysis, aerobic degradation in soil under laboratory conditions, soil field dissipation and on field rotational crops.

Mandestrobin was shown to be stable to hydrolysis at pH 4–9. No change in ratio between the R- and S-isomers was observed. Hydrolysis is not a significant route of degradation.

Mandestrobin was extensively degraded under simulated sunlight in pH 7 water. The DT_{50} values ranged from 3 to 5 days. Major degradation products identified were mandestrobin-OR at 22–24% TAR, mandestrobin-ORC at 16–18% TAR and mandestrobin-PR at 9.3–9.6% TAR. Mandestrobin-OR and mandestrobin-PR degrade quickly with DT_{50} values of 5.1 and 2.5 days, respectively. Photolysis forms a significant route of degradation in water.

With DT_{50} values ranging from 49–64 days photolysis is not considered to be a significant route of degradation on the soil surface.

DT_{50} values for mandestrobin in aerobic soil (n = 10) under laboratory conditions ranged from 49–378 days, with a geomean DT_{50} of 151 days, indicating possible accumulation in soil. No isomerisation between the R- and S-isomer of mandestrobin under aerobic soil conditions was observed. Major degradation products identified were 5-COOH mandestrobin at 3.5–20% TAR, 2-COOH-mandestrobin at 1.6–8.6% TAR, 5-$CONH_2$-mandestrobin at 1.0–14% TAR and 2-$CONH_2$-mandestrobin at 0.4–14% TAR. Degradation products 5-COOH-mandestrobin and 2-COOH-mandestrobin degraded rapidly with DT_{50} values of 22–41 days and 18–26 days.

DT_{50} values for mandestrobin in soil (n = 9) from field dissipation studies ranged from 2.3–165 days, with the high value indicating possible accumulation of mandestrobin in soil and the need to consider a plateau value for evaluating residues in rotational crops.

The maximum seasonal rate of 0.42 kg ai/ha is based on a cGAP for rape seed for application at flowering (BBCH 62–65). Although the crop may intercept the full application at this stage, rape fodder is not fed to livestock at BBCH > 60 and will be ploughed into the soil after harvest of the seeds. Therefore the full application may end up in the soil and the crop interception factor is 1.

Based on the highest DT_{50} of 165 days in the field dissipation studies and using the formulas presented in OECD guidance (2018)[11], the soil accumulation factor is 0.275, leading to a plateau value of 0.12 kg ai/ha (0.275 x 0.42 kg ai/ha). The subsequent corrected maximum seasonal rate of 0.54 kg ai/ha (0.42 kg ai/ha + 0.12 kg ai/ha) can be used for assessing potential residues in rotational crops.

Field rotational crop studies

The Meeting received two field rotational crop studies. One study was conducted in France and Spain where mandestrobin was applied at a single rate of 0.21 kg ai/ha to the preceding crop oilseed rape at BBCH 65 (full bloom). Oilseed rape was crushed and incorporated into the soil 14 days after application. Follow-up crops lettuce, carrot, broccoli, and barley were planted at PBIs of 14, 120 and 365 days.

[11] GUIDANCE DOCUMENT ON RESIDUES IN ROTATIONAL CROPS, OECD Environment, Health and Safety Publications, Series on Pesticides No. 97, Series on Testing & Assessment No. 279, ENV/JM/MONO(2018)9

The second study was conducted in 2011–2012 in Fresno, CA, USA. Plots with primary crop leaf lettuce were treated with four foliar applications at BBCH 15, 17, 19 (i.e. 5, 7, 9 or more true leaves unfolded) and 49 (typical size, form and firmness) of mandestrobin at a rate of 0.42 kg ai/ha (each, with an interval of 7 days) and a total seasonal rate of 1.7 kg ai/ha. The leaf lettuce was removed one day after the last application. Follow-up crops spinach, red beets, wheat or sorghum were planted at PBIs of 101, 253 and 356 days.

Succeeding crops were harvested at their appropriate growth stages. No residues (< 0.01 mg/kg in EU trials; < 0.02 mg/kg in USA trials) of parent or metabolites De-Xy-mandestrobin and free and (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, and 5-CH_2OH-mandestrobin were found in any succeeding crop samples at any plant back interval.

The Meeting noted that in the first field rotational crop study the application rate (0.21 kg ai/ha) was below the corrected maximum seasonal rate of 0.54 kg ai/ha and that application was on primary crop instead of bare soil. In the second field rotational crop study the application rate (4 × 0.42 kg ai/ha = 1.7 kg ai/ha) is higher than the corrected maximum seasonal rate of 0.54 kg ai/ha. However, the product was not applied to bare soil and the treated lettuce was removed from the plot 1 day after the last application. Though mandestrobin was not applied to bare soil, the Meeting concluded that the crop coverage at the first three applications would together have allowed for a total dose rate approximating 0.54 kg ai/ha of reaching the bare soil and concluded that residues in rotational crops are unlikely.

Methods of Analysis

The Meeting received description and validation data for analytical methods of plant and animal commodities for the determination of mandestrobin, De-Xy-mandestrobin and free and (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites.

Multi-residue enforcement methods were available for plant commodities. Multi-residue method QuEChERS with HPLC-MS/MS detection was valid for the determination of mandestrobin in crops with high acid content (oranges, grapes), high water content (peaches), high oil content (soya bean seeds) and high starch content (wheat grains). Multi-residue method DFG-S19 with GC-MS detection was valid for the determination of mandestrobin in crops with high oil content (rape seeds). The limit of quantification (LOQ) was 0.01 mg/kg for each method. When the DFG-S19 method was combined with chiral HPLC-MS/MS detection, the R- and S-isomers of mandestrobin could be determined individually with an LOQ of 0.005 mg/kg for each isomer (lettuce, carrot roots and leaves, broccoli, green rape seed fodder, rape seed, barley grain and straw).

The analytical method for enforcement of animal commodities used acetone/water (7:3, v/v) for extraction. After clean-up, mandestrobin and De-Xy-mandestrobin were determined by HPLC-MS/MS with an LOQ of 0.01 mg/kg for each analyte. The method was successfully validated for free forms of mandestrobin and De-Xy-mandestrobin in liver, eggs and cream. Validation for muscle, fat and whole milk is desirable. The Meeting noted that for quantitative extraction of the residues from liver samples further treatment with 1 M HCl and protease is needed to liberate parent and De-Xy-mandestrobin.

Analytical methods used in the study reports used acetone/water (4:1, or 7:3, v/v) for extraction of residues from various plant commodities. The extracts were treated sequentially with 0.06–0.1 M NaOH (1–2 hours at room temperature) and beta-glucosidase (3 hours at 37 °C) to release the aglycons of (malonyl)glucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites. After clean-up residues were determined by HPLC-MS/MS. For all methods, final quantification is achieved using HPLC-MS/MS, with an LOQ of 0.01–0.02 mg/kg for each analyte.

Radiovalidation with ^{14}C labelled green rape seed fodder and rape seed from a metabolism study indicated that the extraction efficiency for acetone/water 4:1 and 7:3 is sufficient. Efficient hydrolysis of the (malonyl)glucoside conjugated hydroxylated mandestrobin metabolites could be demonstrated in green rape seed fodder but not in rape seed.

All methods for plant commodities were successfully validated for mandestrobin, De-Xy-mandestrobin and free 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin metabolites, demonstrating good reproducibility in the concentration range up to 0.2 mg/kg per analyte. Considering the single validation results in the different high acid matrices as a whole ($n > 5$), would lead to acceptable validation of the levels expected in the residue trials (up to 3.6 mg/kg in grapes).

Analytical methods used in the study reports on animal commodities used acetonitrile and hexane for extraction of mandestrobin. After clean-up, mandestrobin was determined by HPLC-MS/MS with an LOQ of 0.02 mg/kg. The method was successfully validated for the free forms of mandestrobin in liver, kidney, muscle, fat and milk. The Meeting noted that for quantitative extraction of residues from liver samples further treatment with 1 M HCl and protease is needed to liberate parent.

Stability of pesticide residues in stored analytical samples

The Meeting received storage stability studies in plant and animal commodities. No change in the ratio between R- and S-isomers was detected during storage.

The R- and S-isomers of mandestrobin and metabolite De-Xy-mandestrobin are stable for at least 12 months in crop commodities representative of high water (lettuce), high acid (orange, strawberry, grape, grape juice), high starch (barley grain), high protein (dry bean seed) commodity groups as well as in barley straw and at least 39 months in commodities with high oil content (rape seed) when stored at or below -18 °C. Storage stability studies with incurred residues suggest that storage stability for mandestrobin and De-Xy-mandestrobin can be extended to 538 days for grapes and grape juice, 385 days for grape raisins and 571 days for strawberries.

Metabolites 4-OH-mandestrobin and 2-CH_2OH-mandestrobin are stable for at least 26 months in crop commodities representative of high acid content (orange) and at least 12 months in crop commodities representative of high water (lettuce), high starch (barley grain), high protein (dry bean seed), high oil (rape seed) commodity groups as well as in barley straw when stored frozen at or below -18 °C.

Metabolite 5-CH_2OH-mandestrobin is stable for at least 12 months in crop commodities representative of the high water (lettuce) and high protein (dry bean seed) commodity groups as well as in barley straw when stored at or below -18 °C.

Mandestrobin (racemic mixture) is stable for at least 62 days in milk, 78 days in fat and 93 days in liver, kidney and muscle.

Definition of the residue

Residue definitions for plant commodities

Parent was the major compound (20–100% TRR) in the majority of crop commodities (tomatoes, lettuce, rape seed, green rape seed fodder, wheat forage, wheat hay). Parent was not detected or only at very low concentrations in wheat grain and wheat straw (DAT 104). De-Xy-mandestrobin was the major compound (12–61% TRR) in wheat grain and wheat straw. Both parent and De-Xy-mandestrobin were found at low levels in feed commodities grown from treated seed (soya bean forage, maize fodder).

No residues are expected in rotational crops and mandestrobin is stable under the processing conditions representing pasteurisation, cooking and sterilisation.

Supervised field trials on fruits (grapes, strawberries), oilseeds (rape seeds) and feed (green rape seed fodder, soya bean forage and fodder) show that parent compound is the major compound found. Residues at or below the LOQ were found in pulses (dry soya bean seeds). GAPs and trials on cereals were not submitted.

Suitable analytical methods for enforcement are available for mandestrobin.

The Meeting concluded that mandestrobin can be considered a suitable marker compound for enforcement purposes and decided to define the residue for enforcement/monitoring as mandestrobin.

If uses are extended to include uses on cereals the residue definition for plant commodities may need to be revisited.

Besides parent, several compounds observed in the metabolism studies were considered for dietary risk assessment. These compounds include De-Xy-mandestrobin and free and malonylglucoside conjugated 4-OH-mandestrobin, 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin. These metabolites were not observed in supervised field trials on dry soya bean seeds and rape seeds and they were incidentally found at low levels up to 0.02–0.05 mg/kg in supervised field trials on grapes and strawberries at cGAP rates, but below 4% of parent. In the metabolism study on lettuce these metabolites were found at absolute levels between 0.10–1.2 mg eq/kg, but the sum of metabolites was below 6.3% of parent. The metabolites were only found at significant levels (> 10% parent) in metabolism studies and supervised trials on feed items (such as forage/fodder crops of pulses, oilseeds and cereals).

Both the free and conjugated forms of metabolite 4-OH-mandestrobin are found in rat (free form < 1% of the applied dose; conjugated form > 30% of the applied dose in bile). Limited toxicity studies were available. Based on these studies and the high levels in bile, the (acute and long term) toxicity of the free and conjugated 4-OH-mandestrobin metabolite is considered to be covered by the parent compound. Furthermore, field residue trial data show that exposure from plant commodities is very low relative to parent compound, either <LOQ or approximating 1% of parent mandestrobin, with the exception of 1 trial.

Metabolite De-Xy-mandestrobin and the free form of metabolites 2-CH_2OH-mandestrobin and 5-CH_2OH-mandestrobin were found in rat (< 1% of the applied dose). Limited toxicity studies were available leading to the conclusion that the acute toxicity of De-Xy-mandestrobin, free and conjugated 2-CH_2OH-mandestrobin is considered to be similar to that of the parent compound. No experimental data were available for 5-CH_2OH-mandestrobin. Quantitative structure-activity relationship (QSAR) analysis indicates that the metabolite is of similar toxicity to parent. Furthermore, the similarity with the chemical structure of 2-CH_2OH-mandestrobin allows for a read-across approach, also concluding its similarity with parent mandestrobin. Residue trial data show that exposure to these metabolites through the current uses is low relative to parent compound (< 5%).

For long-term toxicity, the TTC approach could be applied for those three metabolites using Cramer Class III.

The estimated long-term exposure based on uses on fruits, fruiting vegetables, pulses and oilseeds and using maximum values found in the supervised residue trials resulted in the following maximum long-term dietary exposures:

De-Xy-mandestrobin	0.58 µg/kg bw per day;
2-CH_2OH-mandestrobin (including conjugates)	0.29 µg/kg bw per day;
5-CH_2OH-mandestrobin (including conjugates)	0.43 µg/kg bw per day.

The Meeting noted that all estimated exposures are below the threshold of toxicological concern for Cramer Class III compounds (1.5 µg/kg bw per day for long-term risk assessment).

The Meeting concluded that De-Xy-mandestrobin and the hydroxy-mandestrobin compounds and their conjugates would not contribute significantly to the dietary exposure of mandestrobin and decided to define the residue for dietary risk assessment for plant commodities as mandestrobin.

The Meeting noted that the current uses do not lead to livestock exposure. For future uses, De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin (including conjugates) and 5-CH_2OH-mandestrobin (including conjugates) should be reconsidered, as these compounds may contribute significantly to the dietary burden and may contribute to residues of concern in animal commodities.

Residue definitions for commodities of animal origin

In animal metabolism studies parent was identified in all animal commodities, albeit at low levels in goat liver, kidney of goat and hen, hen muscle and skin (1.3–7.7% TRR). The Meeting concluded that

mandestrobin parent (free) is therefore a suitable marker compound for enforcement. A suitable analytical method for determining free forms of mandestrobin is available.

The Meeting decided to define the residue for enforcement/monitoring in animal commodities as mandestrobin.

The log K_{ow} for mandestrobin is 3.4–3.5. The goat and hen metabolism studies showed a clear tendency of the parent compound to partition into the fat tissues, with a ratio of approximately 40:1 observed in hen fat and muscle and a ratio between fat and aqueous fraction in milk of 15:1. The effect was less pronounced in goat fat and muscle, but also showed a tendency to concentrate in the fat fraction. The Meeting concluded that the residue is fat-soluble.

Besides parent, several compounds observed in the metabolism studies were considered for dietary risk assessment. These compounds include De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, 5-CA-2-HM-mandestrobin-NHM and 5-CA-mandestrobin-NHM.

Metabolite 5-CA-mandestrobin-NHM was found to be a major metabolite (15% TRR) in skimmed milk only, but found in absolute levels below 0.01 mg eq/kg. Metabolite 5-CA-2-HM-mandestrobin-NHM was found at low levels in liver and kidney (0.88–5.2% TRR), generally < 0.01 mg eq/kg, but up to 0.021 mg eq/kg in one kidney sample (one label). The mean contribution of this compound to the toxicologically significant residue is less than 10%. Both metabolites were therefore excluded from the residue definition for dietary risk assessment.

Other metabolites, De-Xy-mandestrobin, 4-OH-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, 5-COOH-mandestrobin, and their conjugates, were found at levels above 0.01 mg eq/kg in liver and/or kidney and they were found at levels that were higher than the parent compound. Their contribution relative to parent compound amounted to 105–571% for De-Xy-mandestrobin, 11–812% for 4-OH-mandestrobin, 32–252% for 2-CH_2OH-mandestrobin, 80–221% for 2-COOH-mandestrobin and 143–1250% for 5-COOH-mandestrobin.

The acute and long-term toxicity of 4-OH-mandestrobin and its conjugates is covered by the parent. The metabolite should be included in the definition for dietary risk assessment.

Metabolites De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin are found in rat (≤ 1.3% of the applied dose). Limited toxicity studies were available leading to the conclusion that the acute toxicity of metabolites De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin and 5-COOH-mandestrobin is considered to be similar to that of the parent compound and should be included in the residue definition for acute dietary risk assessment.

The Meeting decided to define the residue for **acute** dietary risk assessment in animal commodities as: the sum of parent and 4-OH-mandestrobin, De-Xy-mandestrobin, 2-CH_2OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin and their conjugates, expressed as parent compound.

The toxicological data were insufficient to conclude on the long-term toxicity for these compounds. For long-term dietary risk assessment, the TTC approach could be applied using Cramer Class III. Since there is no exposure to livestock based on the current uses, long-term exposure is 0 µg/kg bw per day, and thus below the threshold of toxicological concern for Cramer Class III compounds (1.5 µg/kg bw per day for long-term risk assessment).

The Meeting decided to define the residue for **long-term** dietary risk assessment in animal commodities as: the sum of parent and 4-OH-mandestrobin and its conjugates, expressed as parent compound.

The Meeting calculated conversion factors based on the results of the animal metabolism studies to be used to convert parent to the residue definitions for dietary risk assessment.

Table 2 Conversion factors for dietary risk assessment for animal commodities

	Acute dietary risk assessment	Long term dietary risk assessment
Milk fat	1.3	1.1
Skimmed milk	2.8	1.5
Mammalian muscle	1.7	-
Mammalian fat	1.3	-
Mammalian liver	8.1	1.2
Mammalian kidney	26	9.1
Poultry liver	7.5	4.1
Poultry muscle	3.3	2.9
Poultry fat	1.3	1.1
Poultry skin	6.5	3.6
Eggs	1.1	1.1

In summary

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

The Meeting may revisit the residue definition with uses on cereals.

Definition of the residue for acute dietary risk assessment in animal commodities: *sum of parent, De-XY-mandestrobin, 4-OH-mandestrobin, 2-CH_2-OH-mandestrobin, 2-COOH-mandestrobin, and 5-COOH-mandestrobin and their conjugates, expressed as parent compound*.

Definition of the residue for long-term dietary risk assessment in animal commodities: *sum of parent and 4-OH-mandestrobin and its conjugates, expressed as parent compound*.

The Meeting considers the residue *fat-soluble*.

Results of supervised residue trials on crops

The Meeting received supervised trials data for the foliar application of mandestrobin on grapes, strawberry and rape seed. Residue trial data were made available from Canada, Europe and the USA. Labels were available from Canada and the USA describing the registered uses of mandestrobin.

Grapes

The critical GAP for mandestrobin on grapes in Canada and the USA is a foliar application at a rate of 3 × 0.42 kg ai/ha with an interval of 10 days and a PHI of 10 days.

In the trials on grapes in Canada and the USA matching the US GAP an adjuvant was used. Residue levels in grapes (parent) in ranked order were (n = 11): 0.74, 0.79, 1.0, 1.0, 1.3, 1.4, 1.4, 1.9, 2.0, 2.4 and 3.5 mg/kg (highest individual value: 3.7 mg/kg).

The Meeting estimated a maximum residue level 5 mg/kg, an STMR of 1.4 mg/kg and an HR of 3.7 mg/kg for mandestrobin on grapes.

Strawberry

The critical GAP for mandestrobin on strawberries in Canada and the USA is a foliar application at a rate of 4 × 0.42 kg ai/ha with an interval of 7 days and a PHI of 0 days.

In the trials from Canada and the USA matching the US GAP, an adjuvant was used. Residue levels in strawberries (parent) in ranked order were (n = 8): 0.45, 0.48, 0.70, 0.82, 0.92, 1.2, 1.2 and 2.0 mg/kg (highest individual value: 2.2 mg/kg).

Based on the Canadian and US trials for strawberries, the Meeting estimated a maximum residue level of 3 mg/kg, an STMR of 0.87 mg/kg and an HR of 2.2 mg/kg for mandestrobin on strawberries.

Rape seed

The critical GAP for mandestrobin on rape seed in Canada and the USA is a single foliar application at a rate of 0.42 kg ai/ha with an instruction for growth stage (20–50% bloom, BBCH 62–65) and PHI (35 days). The Meeting decided that trial data reflecting application at growth stage up to BBCH 69 with a PHI of 35 days are suitable for maximum residue level and STMR estimation.

Trials performed on rape seed from Canada and the USA matching the US GAP were selected based on BBCH 61–69 and a PHI of 35 days (-25%). Residue levels in rape seed (parent) in ranked order were (n = 9): < 0.01, < 0.02 (5), 0.046, 0,11, and 0.13 mg/kg.

The Meeting estimated a maximum residue level of 0.2 mg/kg and an STMR of 0.02 mg/kg.

Soya bean (dry)

Supervised trials on dry soya bean were submitted, but no GAP was provided or intended.

Soya bean forage and fodder

Supervised trials on soya bean forage and fodder were submitted, but no GAP was provided or intended.

Rape seed fodder

Supervised trials on rape seed fodder were submitted. As the GAP requires application beyond BBCH 60 and rape seed fodder beyond BBCH 60 is not suitable as livestock feed, the trials were not used to derive median or highest residues.

Fate of residues during processing

High temperature hydrolysis

The degradation of (Ph-^{14}C)-mandestrobin was studied under hydrolytic conditions at high temperatures (90–120 °C) in sterile aqueous buffers at pH 4, 5 and 6 to simulate common processing practice (pasteurisation, baking/brewing/boiling and sterilisation). No degradation was observed at any of the investigated pH and temperature ranges.

The Meeting concluded that mandestrobin is stable under hydrolytic conditions typically occurring during processing.

Residues in processed commodities

The Meeting received information on the fate of mandestrobin during processing in grapes (juice and raisins) and rape seed (refined oil and extracted meal). Considering the $LogK_{ow}$ of 3.4–3.5 a processing factor of 1.4 was not considered representative for juice. Noting that only one trial was available, and the Meeting decided not to calculate an STMR-P for grape juice.

Table 3. Estimated processing factors for the commodities considered at this Meeting

Raw commodity [STMR/HR]	Processed commodity	Individual processing factors	Mean or best estimate processing factor	STMR-P = $STMR_{RAC}$ × PF (mg/kg)	HR-P = HR_{RAC} × PF (mg/kg)
STMR grapes (parent): 1.4 mg/kg, HR: 3.7 mg/kg					
Grapes	Raisins	2.0	2.0	2.8	7.4
STMR rape seed (parent): 0.02 mg/kg					
Rape seed	Refined oil [a]	0.06	0.06	0.0012	-
	Extracted meal	0.20 [b]	0.20 [b]	0.004 [b]	-

[a] Refined, bleached, deodorized

[b] Based on parent. Though, according to metabolism studies metabolites could be expected in rape, no metabolites were

observed in the field residue trials even when high levels of parent were found.

The Meeting estimated a maximum residue level of 10 mg/kg (MRL_{grapes} of 5 mg/kg × 2.0) for Grapes, dried.

Residues in animal commodities

Farm animal dietary burden

Grape pomace and rape seed meal are the only feed items relevant to the uses considered by the current Meeting. Based on the 2018 OECD Feed diets listed in Appendix XIV Electronic attachments to the 2016 edition of the FAO manual[12], grape pomace (dry) is only a significant feed item in Australia. As there is no registration for use of mandestrobin on grapes in Australia, and import of grape pomace would not occur, the Meeting did not include grape pomace in the dietary burden for mandestrobin. Using the median value of 0.004 mg/kg for rape seed meal in the dietary burden calculator, results in very low dietary burdens for livestock.

Table 4 Estimated maximum and mean dietary burdens of farm animals

	Animal dietary burden: mandestrobin, ppm of dry matter diet							
	US-Canada		EU		Australia		Japan	
	max	mean	max	mean	max	mean	max	mean
Beef cattle	0.0002	0.0002	-	-	0.0009	0.0009	-	-
Dairy cattle	0.0005	0.0005	0.0005	0.0005	0.0007	0.0007	-	-
Poultry – broiler	0.0007	0.0007	0.0008	0.0008	0.0002	0.0002	-	-
Poultry – layer	0.0007	0.0007	0.0005	0.0005	0.0002	0.0002	-	-

Farm animal feeding studies

The Meeting received a bovine feeding study (beef and dairy cattle), which provided information on likely residues resulting in animal tissues and milk from mandestrobin residues in animal diets.

Mandestrobin was fed via the diet to three to six dairy or beef cattle animals per dose group for 27 consecutive days. Animals were administered mandestrobin via bail feed (dairy cattle) or oral drench (beef cattle). The animals received 0 (1 animal), 25, 75, or 150 ppm in dry feed, corresponding to a calculated mean dose of 0, 0.97, 2.9 and 5.8 mg/kg bw per day in both dairy cattle (milk) and beef cattle (tissues). Animals were sacrificed 24 hours after the last dose, and tissues were analysed for residues of mandestrobin; metabolite residues were not analysed.

Mandestrobin residues were <LOQ (0.02 mg/kg) in whole milk, muscle or kidney at all dose levels, <LOQ in cream at the 25 and 75 ppm dose levels, and <LOQ in fat or liver at the 25 ppm dose level. At the 75 ppm dose level, mean and highest residues of 0.048 and 0.057 mg/kg were observed in liver and < 0.02 and 0.023 mg/kg in fat. At the 150 ppm dose level, mean and highest residues of 0.16 and 0.28 mg/kg were observed in liver, 0.033 and 0.040 mg/kg in fat and 0.021 and 0.034 mg/kg in milk cream.

The Meeting did not receive residue data from poultry feeding studies.

Animal commodity maximum residue levels

Since livestock is not significantly exposed ($< 1.0 \times 10^{-3}$ ppm, based on rape seed meal) and mandestrobin was not observed in the dietary feeding study in lactating cows dosed at 25 ppm feed, residues of mandestrobin, including all toxicologically relevant metabolites, are not expected in milk,

[12] http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/jmpr-docs/en/

eggs, and livestock tissues. As a valid analytical method for determination of parent compound in animal commodities is available, the Meeting decided to estimate maximum residue levels for animal commodities.

The Meeting recommended a maximum residue level of 0.01(*) mg/kg and an STMR and HR of 0 mg/kg in mammalian meat (muscle, fat), mammalian fat, mammalian edible offal, poultry meat (muscle, fat), poultry fat, poultry edible offal, and eggs and a maximum residue level of 0.01(*) mg/kg and an STMR of 0 mg/kg in milk.

FURTHER WORK OR INFORMATION

Desirable:

- Validated analytical methods for all relevant metabolites and their conjugates in animal commodities.
- Feeding study, where all relevant metabolites are analysed if livestock dietary burdens become significant.

RECOMMENDATIONS

On the basis of the data obtained from supervised trials the Meeting concluded that the residue levels listed in Annex 1 are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

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

The Meeting may revisit the residue definition with uses on cereals.

Definition of the residue for acute dietary risk assessment in animal commodities: the sum of parent, (2RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide (4-OH-mandestrobin) + (2RS)-2-(2-hydroxymethylphenyl)-2-methoxy-N-methylacetamide (De-XY-mandestrobin) + 2RS)-2-[2-(2-hydroxymethyl-5- methylphenoxymethyl)phenyl]-2-methoxy-N-methylacetamide (2-CH_2-OH-mandestrobin) + 2-({2-[(1RS)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid (2-COOH-mandestrobin), + 3-({2-[(1RS)-1-methoxy-2-(methylamino)-2-oxoethyl]benzyl}oxy)-4-methylbenzoic acid (5-COOH-mandestrobin) and their conjugates, expressed as parent compound.

Definition of the residue for long-term dietary risk assessment in animal commodities: the sum of parent, (2RS)-2-[2-(4-hydroxy-2,5-dimethylphenoxymethyl)phenyl)-2-methoxy-N-methylacetamide (4-OH-mandestrobin), and its conjugates, expressed as parent compound.

The residue is fat-soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The ADI for mandestrobin is 0–0.2 mg/kg bw. The International Estimated Daily Intakes (IEDIs) for mandestrobin were estimated for the 17 GEMS/Food Consumption Cluster Diets using the STMR or STMR-P values estimated by the JMPR. The results are shown in Annex 3 of the 2019 JMPR Report.

The IEDIs ranged from 0–2% of the maximum ADI. The Meeting concluded that long-term dietary exposure to residues of mandestrobin from uses considered by the JMPR is unlikely to present a public health concern.

Acute dietary exposure

The 2018 JMPR established an ARfD for mandestrobin of 3.0 mg/kg bw for women of childbearing age. The 2018 Meeting concluded that it was not necessary to establish an ARfD for mandestrobin for

the remainder of the population. The International Estimate of Short Term Intakes (IESTIs) for mandestrobin were calculated for the food commodities and their processed commodities for which HRs/HR-Ps or STMRs/STMR-Ps were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2019 JMPR Report.

The IESTIs varied from 0–4% of the ARfD for women of childbearing age. The Meeting concluded that acute dietary exposure to residues of mandestrobin from uses considered by the present Meeting is unlikely to present a public health concern.