5.22 SPIROMESIFEN (294)

TOXICOLOGY

Spiromesifen is the ISO-approved common name for 3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate (IUPAC), which has the CAS number 283594-90-1. Spiromesifen is an insecticidal compound belonging to the chemical class of cyclic ketoenoles. It is an acetyl coenzyme A carboxylase inhibitor. The biological activity of cyclic ketoenoles correlates with the inhibition of lipogenesis, resulting in decreased lipid contents, especially of triglycerides and free fatty acids, in treated insects.

Spiromesifen has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request of CCPR.

All critical studies contained statements of compliance with GLP and were conducted in accordance with relevant national or international test guidelines, unless specified otherwise.

Biochemical aspects

Following the administration of a single oral dose of 2 mg/kg bw of [^{14}C]spiromesifen to rats, absorption was rapid, although incomplete. At 2 and 500 mg/kg bw, urinary excretion was 39% and 9%, respectively, whereas faecal excretion was 55–57% and 90%, respectively. Bile duct–cannulated rats treated with 2 mg/kg bw excreted approximately 7% of the administered dose with the bile. At 2 mg/kg bw, at least 48% of the dose was absorbed; at 500 mg/kg bw, absorption appeared to be much lower. Maximum concentrations in blood were reached in 2 hours in males and 1 hour in females after a dose of 2 mg/kg bw and in 6 hours (both sexes) after a dose of 500 mg/kg bw. Distribution was widespread. Highest tissue concentrations were found in liver, and concentrations in liver were higher in males than in females. The calculated half-lives for radiolabel in plasma and whole blood, assessed in three experiments, ranged from 7 to 18 hours, without a clear effect of dose or sex. Spiromesifen and its metabolites do not accumulate. The absorbed spiromesifen is extensively metabolized. No parent compound was found in urine or bile.

The first step in the biotransformation of spiromesifen is the cleavage of the alkyl ester group, resulting in spiromesifen-enol, which is subsequently excreted or further transformed via hydroxylation of the cyclopentyl ring or the methyl side-chain of the phenyl ring, carboxylation of the methyl side-chain of the phenyl ring and oxidation in the cyclopentyl ring or the methyl side-chain of the aromatic ring. A sex difference was apparent in excretion profiles. The main metabolite in the excreta of female rats of the low-dose groups was spiromesifen-enol (BSN 0546, M01), whereas the main metabolite in the excreta of the male rats of the low-dose groups was 4-hydroxymethyl-BSN 0546 (M02). The excretion profiles in males and females were not affected by the size of the dose or predosing the rats with unlabelled spiromesifen for 14 days.

Toxicological data

The acute toxicity of spiromesifen in rats is low (oral LD_{50} > 2000 mg/kg bw; dermal LD_{50} > 2000 mg/kg bw; inhalation LC_{50} > 4.9 mg/L). Spiromesifen was not irritating to the skin or the eyes of rabbits. Spiromesifen was a skin sensitizer in a Magnusson and Kligman test in guinea-pigs.

In repeated-dose oral toxicity studies with spiromesifen in mice, rats and dogs, the most sensitive effect was reduction of plasma cholesterol. This is probably secondary to the inhibition of lipogenesis by spiromesifen. Common findings were effects on body weight, liver (including liver enzyme induction), thyroid and adrenals.

In a 14-week study in mice using dietary concentrations of spiromesifen of 0, 140, 700 and 3500 ppm (equal to 0, 22, 105 and 589 mg/kg bw per day for males and 0, 35, 191 and 1010 mg/kg

bw per day for females, respectively), a NOAEL could not be identified. The LOAEL was 140 ppm (equal to 22 mg/kg bw per day), based on decreased haemoglobin levels and increased alkaline phosphatase levels in females and decreased cholesterol levels, discoloration of the adrenals and an increased incidence of cytoplasmic eosinophilia in zona fasciculata cells with reduced or absent normal fine vesiculation in adrenals in both sexes.

In a second 14-week study in mice using dietary concentrations of spiromesifen of 0, 20 and 80 ppm (equal to 0, 3.2 and 11.5 mg/kg bw per day for males and 0, 5.1 and 20.3 mg/kg bw per day for females, respectively), the NOAEL was 20 ppm (equal to 3.2 mg/kg bw per day), based on decreased cholesterol levels in both sexes and adrenal cytoplasmic eosinophilia in zona fasciculata in one female at 80 ppm (equal to 11.5 mg/kg bw per day).

In a 14-week study in rats using dietary concentrations of spiromesifen of 0, 100, 500 and 3000 ppm (equal to 0, 6.3, 32 and 204 mg/kg bw per day for males and 0, 7.7, 37 and 232 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 6.3 mg/kg bw per day), based on a slight reduction in body weight gain and water intake in males, an increased thromboplastin time, increased alkaline phosphatase activity, decreased concentrations of plasma cholesterol and triglycerides, a tendency to higher TSH values, increased relative kidney weights in males, white jejunal mucosa coverings and cytoplasmic vacuolation of the jejunal mucosa in females and increased incidences of thyroidal follicular cell hypertrophy in females and thyroidal colloidal alterations in males observed at 500 ppm (equal to 32 mg/kg bw per day).

In a 29-day dietary study in dogs using spiromesifen concentrations of 0, 25, 100, 500 and 2000 ppm (equal to 0, 0.9, 3.7, 19.3 and 72.6 mg/kg bw per day for males and 0, 0.9, 3.9, 18.1 and 76.5 mg/kg bw per day for females, respectively), the NOAEL was 2000 ppm (equal to 72.6 mg/kg bw per day), the highest dose tested. The Meeting considered the effects (increased absolute and relative liver weights, increased alkaline phosphatase activity, hepatic enzyme induction, increased T_4 elimination and hepatocellular cytoplasmic changes) observed at 500 and 2000 ppm to reflect hepatic enzyme induction.

In a 3-month dietary study in dogs using spiromesifen concentrations of 0, 20, 50, 250 and 2000 ppm (equal to 0, 0.7, 1.8, 9.2 and 71 mg/kg bw per day for males and 0, 0.8, 1.9, 9.3 and 71 mg/kg bw per day for females, respectively), the NOAEL was 2000 ppm (equal to 71 mg/kg bw per day), the highest dose tested. The Meeting considered the effects (increased plasma alkaline phosphatase activity and triglyceride levels and hepatocellular cytoplasmic changes) observed at 250 and 2000 ppm to reflect hepatic enzyme induction.

In a second 3-month dietary study in dogs using spiromesifen concentrations of 0, 3000 and 5000 ppm (equal to 0, 101 and 172 mg/kg bw per day, respectively, for both sexes), the NOAEL was 3000 ppm (equal to 101 mg/kg bw per day), based on a 9-fold increase in plasma alkaline phosphatase activity and vomiting at 5000 ppm (equal to 172 mg/kg bw per day).

In a 1-year study in dogs using dietary concentrations of spiromesifen of 0, 50, 400 and 4000 ppm (equal to 0, 1.4, 11.5 and 109 mg/kg bw per day for males and 0, 1.4, 10.8 and 117 mg/kg bw per day for females, respectively), the NOAEL was 400 ppm (equal to 10.8 mg/kg bw per day), based on decreased body weights in females, decreased T_4 (due to increased hepatic T_4 elimination), increased alkaline phosphatase activity, hepatic inclusions/vacuoles (hyaline bodies) and a small cell type in adrenocortical zona fasciculata observed in both sexes at 4000 ppm (equal to 109 mg/kg bw per day).

The overall NOAEL for the 3-month and 1-year toxicity studies in dogs was 400 ppm (equal to 10.8 mg/kg bw per day), and the overall LOAEL was 4000 ppm (equal to 109 mg/kg bw per day).

In an 18-month carcinogenicity study in mice using dietary concentrations of spiromesifen of 0, 20, 140, 1000 and 2000 ppm (equal to 0, 3.3, 22, 157 and 335 mg/kg bw per day for males and 0, 3.8, 30, 201 and 401 mg/kg bw per day for females, respectively), the NOAEL was 20 ppm (equal to 3.3 mg/kg bw per day), based on effects on the adrenal glands (i.e. macroscopic discoloration, microscopic cytoplasmic eosinophilia in the zona fasciculata and decreased incidences and/or severities of cortical ceroid deposits and normal diffuse fatty changes) observed at 140 ppm (equal to

22 mg/kg bw per day). No treatment-related increase in the incidence of tumours was observed in mice in this study.

In a 1-year toxicity study in rats using dietary concentrations of spiromesifen of 0, 50, 125, 300 and 800 ppm (equal to 0, 2.6, 6.5, 16 and 42 mg/kg bw per day for males and 0, 3.0, 7.6, 19 and 52 mg/kg bw per day for females, respectively), the NOAEL was 125 ppm (equal to 6.5 mg/kg bw per day), based on increased T_3 levels and thyroidal follicular cell hypertrophy and colloidal alteration in males and a reduction in cholesterol in females at 300 ppm (equal to 16 mg/kg bw per day).

In a 2-year carcinogenicity study in rats using dietary concentrations of spiromesifen of 0, 50, 125, 300 and 800 ppm (equal to 0, 2.5, 6.1, 15 and 40 mg/kg bw per day for males and 0, 3.3, 8.2, 20 and 54 mg/kg bw per day for females, respectively), the NOAEL was 125 ppm (equal to 6.1 mg/kg bw per day), based on increased counts of monocytes, a slight increase of posterior capsular opacities in the ocular lens for which a relationship with treatment cannot be excluded, and decreased plasma cholesterol concentration in females at 300 ppm (equal to 15 mg/kg bw per day). No treatment-related increase in the incidence of tumours was observed in rats in this study.

The Meeting concluded that spiromesifen is not carcinogenic in mice or rats.

Spiromesifen was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. There was no evidence of genotoxicity.

The Meeting concluded that spiromesifen is unlikely to be genotoxic.

In view of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that spiromesifen is unlikely to pose a carcinogenic risk to humans.

In a two-generation reproductive toxicity study in rats using spiromesifen at dietary concentrations of 0, 30, 120 and 500 ppm (equal to premating doses of 0, 2.6, 10.2 and 47 mg/kg bw per day for F_0 males, 0, 3.3, 14.7 and 56 mg/kg bw per day for F_0 females, 0, 3.1, 13.6 and 58 mg/kg bw per day for F_1 males and 0, 4.7, 21 and 86 mg/kg bw per day for F_1 females, respectively), the NOAEL for parental toxicity was 120 ppm (equal to 10.2 mg/kg bw per day), based on decreased body weights in F_1 males and in F_0 and F_1 females, decreased relative weights of liver, spleen and kidneys in F_0 males, decreased absolute spleen weight in F_0 females, decreased absolute brain weight in F_1 males, slight effects on the thyroid gland (follicular cell hypertrophy, altered follicular colloid) in males and females of both generations, decreased vacuolation of the adrenal zona glomerulosa cells and decreased hepatic periportal fat content in F_0 females observed at 500 ppm (equal to 47 mg/kg bw per day). The NOAEL for offspring toxicity was 120 ppm (equal to 14.7 mg/kg bw per day), based on decreased body weights (F_1, F_2, F_{2b}) during lactation and decreased absolute (F_1 males, F_2 males and females) and increased relative (F_1 and F_2 males and females) brain weights, decreased absolute spleen and thymus weights (F_1 and F_2 males and females, F_{2b} males) and decreased absolute thymus weight in F_{2b} females observed at 500 ppm (equal to 56 mg/kg bw per day). The NOAEL for reproductive toxicity was 500 ppm (equal to 47 mg/kg bw per day), the highest dose tested.

In a second two-generation reproductive toxicity study in rats using dietary concentrations of spiromesifen of 0, 30, 120 and 500 ppm (equal to premating doses of 0, 2.2, 8.8 and 37 mg/kg bw per day for F_0 males, 0, 3.8, 14.2 and 64 mg/kg bw per day for F_0 females, 0, 3.3, 13.2 and 76 mg/kg bw per day for F_1 males and 0, 4.6, 18.0 and 91 mg/kg bw per day for F_1 females, respectively), the NOAEL for parental toxicity was 30 ppm (equal to 3.3 mg/kg bw per day), based on decreased body weights in F_1 males and F_1 females and decreased absolute spleen weights in F_1 males observed at 120 ppm (equal to 13.2 mg/kg bw per day). The NOAEL for offspring toxicity was 30 ppm (equal to 3.8 mg/kg bw per day, maternal intake), based on decreased body weights during lactation in male and female F_1 and F_2 pups and on decreased absolute spleen and thymus weights in male F_1 pups observed at 120 ppm (equal to 14.2 mg/kg bw per day). The NOAEL for reproductive toxicity was 500 ppm (equal to 37 mg/kg bw per day), the highest dose tested.

The overall NOAEL for parental toxicity was 30 ppm (equal to 3.3 mg/kg bw per day); for reproductive toxicity, 500 ppm (equal to 47 mg/kg bw per day), the highest dose tested; and for offspring toxicity, 30 ppm (equal to 3.8 mg/kg bw per day).

In a developmental toxicity study in rats using gavage doses of spiromesifen of 0, 10, 70 and 500 mg/kg bw per day, the NOAEL for maternal toxicity was 10 mg/kg bw per day, based on reduced feed intake and body weight development at 70 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 70 mg/kg bw per day, based on a marginal decrease in fetal weight and slightly more progressed ossification of phalangeal and single skull bones of equivocal toxicological significance at 500 mg/kg bw per day. No evidence for a teratogenic potential of spiromesifen was identified.

In a developmental toxicity study in rabbits administered spiromesifen by gavage at a dose of 0, 5, 35 or 250 mg/kg bw per day, the NOAEL for maternal toxicity was 5 mg/kg bw per day, based on decreased feed intake and amount of faeces, transient body weight loss and decreased body weight gain at 35 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 250 mg/kg bw per day, the highest dose tested. There was no evidence for teratogenic potential.

The Meeting concluded that spiromesifen is not teratogenic.

In an acute neurotoxicity study in rats using gavage doses of spiromesifen of 0, 200, 700 and 2000 mg/kg bw, the NOAEL was 2000 mg/kg bw, the highest dose tested.

In a 13-week neurotoxicity study in rats using dietary concentrations of spiromesifen of 0, 100, 500 and 2000 ppm (equal to 0, 6.4, 32 and 123 mg/kg bw per day for males and 0, 7.9, 38 and 149 mg/kg bw per day for females, respectively), the NOAEL was 500 ppm (equal to 32 mg/kg bw per day), based on decreased body weight and feed consumption and behavioural findings at 2000 ppm (equal to 123 mg/kg bw per day).

The Meeting concluded that spiromesifen is not neurotoxic.

The immunotoxic properties of spiromesifen were assessed in plaque-forming cell assays in mice and rats. In a 4-week study in mice using spiromesifen at dietary concentrations of 0, 100, 500 and 3500 ppm (equal to 0, 31, 163 and 1230 mg/kg bw per day for males and 0, 48, 279 and 1510 mg/kg bw per day for females, respectively), a slight increase in plaque-forming cells in the spleen was observed at 500 and 3500 ppm (equal to 163 and 1230 mg/kg bw per day, respectively). In a 4-week study in rats using spiromesifen at dietary concentrations of 0, 100, 500 and 3000 ppm (equal to 0, 9.6, 52.83 and 292 mg/kg bw per day for males and 0, 10.7, 45.7 and 289 mg/kg bw per day for females, respectively), no effect on plaque-forming cells in the spleen was observed. It is noted that there was considerable interindividual variability in both experiments.

The Meeting concluded that spiromesifen is not immunotoxic.

Toxicological data on metabolites and/or degradates

The major residues in crops and livestock were spiromesifen, spiromesifen-enol (M01), 4-hydroxymethyl-spiromesifen-enol (M02) and its glucoside, and 4-carboxy-3-hydroxy-spiromesifen-enol (M07). No specific toxicity studies on metabolites of spiromesifen are available. However, M01, M02 and M07 occur in rats at about 10% of the absorbed dose or higher. The toxicity of the rat metabolites M01, M02 and its glucoside and M07 is therefore considered to be covered by that of spiromesifen.

Human data

There were no reports of adverse health effects from use in agriculture or from manufacturing sites.

The Meeting concluded that the existing database on spiromesifen was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw for spiromesifen on the basis of a NOAEL of 3.3 mg/kg bw per day for macroscopic and histopathological effects on the adrenal glands in an 18-month mouse study and a NOAEL for parental toxicity of 3.3 mg/kg bw per day, based on decreased body weights in F_1 males and F_1 females and decreased absolute spleen weights in F_1 males in a two-generation reproductive toxicity study in rats. This ADI is supported by a NOAEL for offspring toxicity of 3.8 mg/kg bw per day, based on decreased body weights in male and female F_1 and F_2 pups during lactation and on decreased absolute spleen and thymus weights in male F_1 pups observed in a two-generation reproductive toxicity study in rats. A safety factor of 100 was used.

The Meeting concluded that the ADI would apply to spiromesifen and the metabolites spiromesifen-enol (M01), 4-hydroxymethyl-spiromesifen-enol (M02) and its glucoside and 4-carboxy-3-hydroxy-spiromesifen-enol (M07).

The Meeting concluded that it was not necessary to establish an ARfD for spiromesifen in view of its low acute oral toxicity and the absence of any toxicological effects, including developmental toxicity, that would likely be elicited by a single dose.

Levels relevant to risk assessment of spiromesifen

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	20 ppm, equal to 3.3 mg/kg bw per day	140 ppm, equal to 22 mg/kg bw per day
		Carcinogenicity	2 000 ppm, equal to 335 mg/kg bw per day[b]	–
Rat	One-year study of toxicity[a]	Toxicity	125 ppm, equal to 6.5 mg/kg bw per day	300 ppm, equal to 16 mg/kg bw per day
	Two-year study of toxicity and carcinogenicity[a]	Toxicity	125 ppm, equal to 6.1 mg/kg bw per day	300 ppm, equal to 15 mg/kg bw per day
		Carcinogenicity	800 ppm, equal to 40 mg/kg bw per day[b]	–
	Two-generation studies of reproductive toxicity[a,c]	Reproductive toxicity	500 ppm, equal to 47 mg/kg bw per day[b]	–
		Parental toxicity	30 ppm, equal to 3.3 mg/kg bw per day	120 ppm, equal to 13.2 mg/kg bw per day
		Offspring toxicity	30 ppm, equal to 3.8 mg/kg bw per day	120 ppm, equal to 14.2 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	10 mg/kg bw per day	70 mg/kg bw per day
		Embryo and fetal toxicity	70 mg/kg bw per day	500 mg/kg bw per day
	Acute neurotoxicity study[d]	Neurotoxicity	2 000 mg/kg bw[b]	–

Species	Study	Effect	NOAEL	LOAEL
	Thirteen-week neurotoxicity study[a]	Neurotoxicity	2 000 ppm, equal to 123 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	5 mg/kg bw per day	35 mg/kg bw per day
		Embryo and fetal toxicity	250 mg/kg bw per day[b]	–
Dog	Thirteen-week and 1-year studies of toxicity[a,c]	Toxicity	400 ppm, equal to 10.8 mg/kg bw per day	4 000 ppm, equal to 109 mg/kg bw per day

[a] Dietary administration.

[b] Highest dose tested.

[c] Two or more studies combined.

[d] Gavage administration.

Acceptable daily intake (ADI; applies to spiromesifen and the metabolites spiromesifen-enol [M01], 4-hydroxymethyl-spiromesifen-enol [M02] and its glucoside and 4-carboxy-3-hydroxy-spiromesifen-enol [M07], expressed as the parent compound)

0–0.03 mg/kg bw

Acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to spiromesifen

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; moderate at low doses, low at high doses (rats and dogs)
Dermal absorption	No data
Distribution	Widespread distribution, highest concentrations found in liver
Potential for accumulation	Low potential for accumulation
Rate and extent of excretion	Rapid; 88–95% in 48 h
Metabolism in animals	Extensively metabolized; no parent compound in urine or bile; seven metabolites identified
Toxicologically significant compounds in animals and plants	Spiromesifen, spiromesifen-enol (M01), 4-hydroxymethyl-spiromesifen-enol (M02) and its glucoside, 4-carboxy-3-hydroxy-spiromesifen-enol (M07)

Acute toxicity	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 4.9 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Cholesterol reduction, adrenal
Lowest relevant oral NOAEL	3.2 mg/kg bw per day (mouse)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day, highest dose tested (rat)
Lowest relevant inhalation NOAEC	5.0 mg/m^3
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Adrenal
Lowest relevant NOAEL	3.3 mg/kg bw per day (mouse)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	No reproductive effects; decreased body weight and spleen and thymus weights in pups
Lowest relevant parental NOAEL	3.3 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	3.8 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	47 mg/kg bw per day, highest dose tested (rat)
Developmental toxicity	
Target/critical effect	Progressed ossification, marginally decreased body weight (rat)
Lowest relevant maternal NOAEL	5 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	70 mg/kg bw per day (rat)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested (rat)
Subchronic neurotoxicity NOAEL	123 mg/kg bw per day, highest dose tested (rat)
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	Not immunotoxic
Studies on toxicologically relevant metabolites	No data
Human data	
	No adverse effects reported

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

Summary

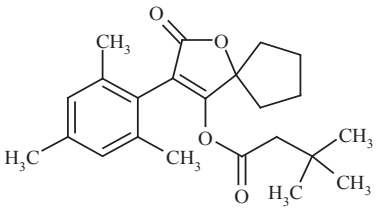
	Value	Study	Safety factor
ADI[a]	0–0.03 mg/kg bw	Two-generation reproductive toxicity study (rat); 18-month toxicity and carcinogenicity study (mouse)	100
ARfD	Unnecessary	–	–

[a] Applies to spiromesifen and the metabolites spiromesifen-enol (M01), 4-hydroxymethyl-spiromesifen-enol (M02) and its glucoside and 4-carboxy-3-hydroxy-spiromesifen-enol (M07), expressed as the parent compound.

RESIDUE AND ANALYTICAL ASPECTS

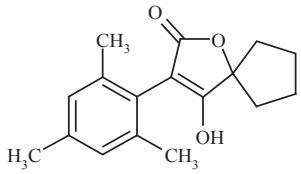
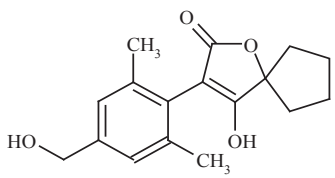
Residue and analytical aspects of spiromesifen were considered for the first time by the present Meeting. The residue evaluation was scheduled for the 2016 JMPR by the 47th Session of the CCPR.

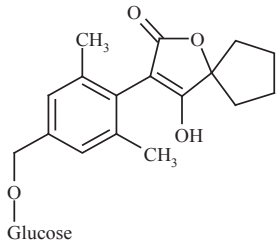
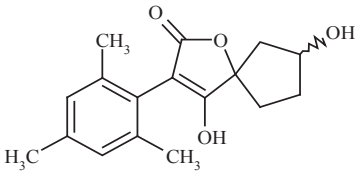
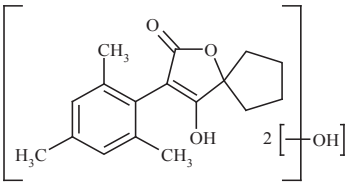
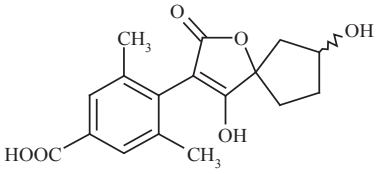
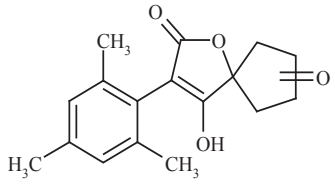
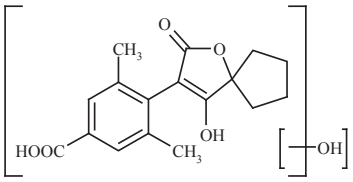
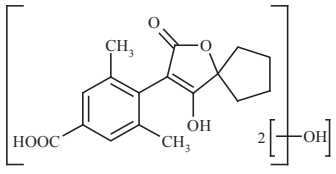
Spiromesifen is a contact insecticide-acaricide belonging to the titronic acid class of compounds. The pesticidal mode of action is inhibition of lipid biosynthesis, especially triglycerides and free fatty acids. The product is mixed with water and applied as a foliar spray using ground, aerial, or chemigation equipment. The Meeting received information on identity, animal and plant metabolism, environmental fate in soil, rotational crops, analytical methods, storage stability, use pattern, supervised trials, dairy cattle feeding studies, and fate of residues in processing.



Butanoic acid, 3,3-dimethyl-, 2 oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl ester

In this appraisal, the following abbreviated names were used for metabolites.

Identifier	Chemical Structure
Spiromesifen-enol Sp-enol	
4-Hydroxymethyl-Sp-enol	

Identifier	Chemical Structure
4-Hydroxymethyl-glucoside-Sp-enol	
3-Pentanol-Sp-enol	
Dihydroxy-Sp-enol	
4-Carboxy-3-hydroxy-Sp-enol	
Oxo-cyclopentyl-Sp-enol	
4-Carboxy-hydroxy-Sp-enol	
4-Carboxy-dihydroxy-Sp-enol	

Plant metabolism

The Meeting received studies conducted with spiromesifen radiolabelled at the 3-hydrofuranone carbon depicting metabolism of spiromesifen in tomato, lettuce, and cotton.

In the tomato metabolism study, plants growing in a plastic tunnel received two applications of spiromesifen at ca. 400 g ai/ha. The interval between applications was 24 days, and the second application was seven days prior to harvest. Additional treatments were made to plants with protected fruits and to fruits directly, to evaluate translocation. Application to the fruits was equivalent to ca. a three-fold exaggerated application rate.

The translocation portion of the study indicates that residues did not translocate following foliar application. This finding is supported by the wash data showing that ca. 80% of the total radioactive residue (TRR) was associated with the surface wash fraction of both unripe (0.36 mg eq/kg) and ripe fruits (0.67 mg eq/kg). Solvent extraction (acetonitrile followed by acetonitrile:H_2O) removed ca. 0.13 mg eq/kg from unripe and ripe fruits, accounting for 25% and 17% of the whole fruit TRR, respectively. Surface washing and extraction, combined, accounted for ca. 97% of the whole fruit TRR. Spiromesifen was the only major residue (≥ 10% TRR and ≥ 0.01 mg/kg) in both surface washes and in extracts, accounting for 86–87% of the total residue in unripe (0.43 mg/kg) and ripe fruits (0.73 mg/kg). The next most abundant residue was the glucoside conjugate of 4-hydroxymethyl-Sp-enol. This metabolite was observed in extracts only (not surface washes), and made up 5.4 to 7.0% of the TRR (0.035 to 0.046 mg/kg). Treatment of the unripe fruit extracts with ß-glucosidase resulted in a decrease in 4-hydroxymethyl-glucoside-Sp-enol and a nearly quantitative increase in the non-conjugated 4-hydroxymethyl-Sp-enol.

In the lettuce metabolism study, lettuce plants grown in a plastic tunnel were treated with radiolabelled spiromesifen, at 300 g ai/ha, 26 days after planting and 1 week prior to harvest.

Of the radioactivity in the lettuce leaves, 0.41 mg eq/kg (98%) was extracted (acetonitrile + acetonitrile:H_2O). Spiromesifen accounted for 58% of the TRR (0.24 mg/kg) in the extract. The only other major residue was 4-hydroxymethyl-glucoside-Sp-enol (12% TRR, 0.049 mg/kg).

In the cotton metabolism study, cotton plants were treated three times, on a 7-day interval during boll-set to boll-split growth stages. Cotton was harvested 21 days after the last application (DALA). Two treatment regimes were established, one at 300 g ai/ha/application and the other at 1000 g ai/ha/application. Some bolls were protected during treatment to assess translocation. Cotton was harvested and separated into gin trash and undelinted seed. The undelinted seed was processed into delinted seed and seed lint.

Total radioactive residue in treated boll components was approximately an order of magnitude greater than that in protected bolls. Solvent extraction (acetonitrile followed by acetonitrile:H_2O) removed 94 and ca. 100% of the radioactivity from undelinted seed and cotton gin trash, respectively. For cotton seed, radioactivity was associated more with seed lint (73% TRR, 0.37 mg eq/kg) than with delinted seed (21% TRR, 0.011 mg eq/kg). In seed lint and delinted seed, spiromesifen and Sp-enol, combined, accounted for ca. 100% of the TRR [seed lint: 52% TRR (0.020 mg/kg) spiromesifen + 48% TRR (0.018 mg/kg) Sp-enol; delinted seed: 82% TRR (0.009 mg/kg) spiromesifen + 18% TRR (0.002 mg/kg) Sp-enol]. As with cotton seed, the only major residues in cotton gin trash were spiromesifen (26% TRR, 1.7 mg/kg) and Sp-enol (49% TRR, 3.1 mg/kg); however, in cotton gin trash, a higher proportion of the residue was the Sp-enol metabolite. Enzymatic treatment did not result in substantial changes in the residue profile.

In the confined rotational crop study, radiolabelled spiromesifen was applied to bare soil at 800 g ai/ha and rotational crops of spring wheat, spinach, and turnip were planted into the treated soil 30, 120–187, and 365 days after application.

Total radioactive residues ranged from 0.027 mg eq/kg (wheat grain) to 1.1 mg eq/kg (wheat straw) at the 30-day plant-back interval (PBI), and decreased with increasing PBI for all matrices except wheat grain. Wheat grain showed a peak TRR (0.18 mg eq/kg) at the 187-day PBI, which

declined to 0.082 mg eq/kg at the 365-day PBI. Unlike primary crops, spiromesifen was not a major residue in any rotational crop sample from any PBI. Identified residues in wheat grain were < 0.01 mg eq/kg at all PBIs. The principal residue, especially at earlier PBIs, in all matrices was 4-hydroxymethyl-Sp-enol. With the exception of wheat grain, 4-hydroxymethyl-Sp-enol (including the glucoside conjugate) ranged from 50–65% TRR (0.042–0.61 mg/kg) at the 30-day PBI to < 9–21% TRR (< 0.004–0.067 mg/kg) at the 365-day PBI. Of that, up to 83% was made up of the glucoside conjugate. The other major residues in confined rotational crops were 3-pentanol-Sp-enol (spinach: up to 29% TRR and 0.057 mg/kg; turnip leaves: up to 18% TRR and 0.025 mg/kg; turnip roots: up to 13% TRR and 0.01 mg/kg; and wheat straw: up to 11% TRR and 0.08 mg/kg) and dihydroxy-Sp-enol (spinach: up to 19% TRR and 0.038 mg/kg; wheat forage: up to 11% TRR and 0.071 mg/kg, wheat hay: up to 11% TRR and 0.032 mg/kg; and wheat straw: up to 13% TRR and 0.094 mg/kg).

Field accumulation in rotational crops

Field rotational crop studies were conducted with bulb onion, green onion, sugar beet, barley, wheat, sugar cane, and alfalfa. In addition, greenhouse rotational crop studies were conducted with carrot, lettuce, and tomato. For all of the field studies, spiromesifen was applied to bare soil at a total rate of ca. 840 g ai/ha (as one application for sugar cane or as three applications for other crops). The maximum seasonal rate from any of the registered uses is 864 g ai/ha (as four applications, each at 216 g ai/ha). Rotational crops were planted into the treated soil ca. 30 days after the last application (14 days for sugar cane). In the greenhouse trials, tomato was treated as a primary crop, receiving four applications at 216 g ai/ha. Approximately 30 days after the last application, treated tomato plants were removed and rotational carrot, lettuce, or tomato were planted. In the case of rotational tomato, additional plantings were made at PBIs of 45 days and 127 or 140 days. With the exception of tomato, rotational crops were analysed for spiromesifen, Sp-enol, and 4-hydroxymethyl-Sp-enol; tomato samples were assayed for 4-hydroxymethyl-Sp-enol only. For all crops except sugar cane, the analytical method included a hydrolysis step; therefore, reported concentrations of 4-hydroxymethyl-Sp-enol include the residue contribution from the glucoside metabolite.

Residues of spiromesifen, *per se*, were non-quantifiable in all field rotational crop matrices. Residues of Sp-enol were as follows < 0.01 mg/kg in all matrices except for one sample of bulb onion (0.033 mg/kg) and one sample of green onion (0.039 mg/kg). Residues of 4-hydroxymethyl-Sp-enol in rotational crops were as follows: bulb onion (n = 5), < 0.01 (5) mg/kg; green onion (n = 3), < 0.01 (2), and 0.032 mg/kg, sugar beet roots (n = 11), < 0.01 (11) mg/kg; sugar beet tops (n = 11), < 0.01 (10), and 0.16 mg/kg; barley hay (n = 12), 0.02 (2), 0.04 (3), 0.06 (3), 0.11, 0.12, 0.15, and 0.18 mg/kg; barley straw (n = 12), < 0.01, 0.01, 0.02 (2), 0.03 (3), 0.04, 0.06 (2), 0.09, and 0.11 mg/kg; wheat forage (n = 20), < 0.01 (6), 0.01 (4), 0.02, 0.03 (4), 0.04, 0.08 (2), 0.09, and 0.14 mg/kg; wheat hay (n = 20), < 0.01 (3), 0.01, 0.02 (5), 0.03 (2), 0.04, 0.05, 0.06, 0.07 (2), 0.09, and 0.10 (3) mg/kg; wheat straw (n = 20), 0.01 (6), 0.02 (3), 0.03 (2), 0.04 (3), 0.05, 0.06, 0.07, 0.09, 0.11, and 0.21 mg/kg; sugar cane stalks (n = 6), < 0.01 (6) mg/kg; alfalfa forage (n = 12), < 0.01 (3), 0.02, 0.05, 0.09, 0.14, 0.22, 0.23, 0.33, 0.36, and 0.85 mg/kg; alfalfa hay (n = 12); 0.01 (3), 0.03, 0.12, 0.22, 0.39, 0.64, 0.75, 0.80, 1.0, and 2.2 mg/kg; carrot (greenhouse; n = 4), < 0.02 (4) mg/kg; lettuce (greenhouse; n = 4), < 0.02 (2), 0.03 (2) mg/kg; and tomato (greenhouse; n = 4): < 0.01 (4) mg/kg.

Overall, the residue profile in primary crops and rotational crops differ, with spiromesifen and Sp-enol being the principal residues in primary crops and 4-hydroxy-Sp-enol generally being the principal residue in rotational crops. Both primary and rotational crop residues include the glucose-conjugated 4-hydroxymethyl-Sp-enol metabolite.

Animal metabolism

The Meeting received animal metabolism studies with spiromesifen in rats, lactating goat and laying hens. The metabolism and distribution of spiromesifen in animals were investigated using test material radiolabelled at the 3-dihydrofuranone moiety.

The metabolism of spiromesifen in rats was evaluated by the WHO Core Assessment Group of the 2016 JMPR.

A single lactating goat was dosed with [^{14}C]spiromesifen at a dose equivalent to approximately 344 ppm in the diet daily for three consecutive days. Approximately 50% of the applied dose (AD) was recovered: 33% in urine, 15% in faeces, 1% in cage wash, and < 1% in milk and tissues.

Total radioactive residues (TRR) in the milk accounted for 0.02% AD. Residues in milk may have plateaued; however, the duration of dosing was not sufficient to make a definitive conclusion. In tissues residue levels were highest in kidney (8.4 mg eq/kg), followed by liver (3.8 mg eq/kg), fat (ca. 0.5 mg eq/kg) and muscle ca. 0.23 mg eq/kg.

The principal residue in all matrices was Sp-enol, ranging from 29% TRR (1.0 mg/kg) in liver to 77% TRR (6.9 mg/kg) in kidney. Spiromesifen was not a major residue in tissues. In milk spiromesifen and Sp-enol were reported as combined residues and represented 33% TRR (0.03 mg/kg). Analysis of milk and tissue samples at ca. 1–2 months after sacrifice and ca. 11 months after sacrifice indicated some degradation of spiromesifen and a concomitant increase in Sp-enol in fat and milk; spiromesifen was generally stable in other matrices. 4-hydroxymethyl-Sp-enol was also a significant residue in milk (24% TRR, 0.02 mg/kg). The only other major residue that was reported was a glucuronide conjugate of Sp-enol at 21% TRR (0.77 mg/kg) in liver.

Laying hens were orally dosed with [^{14}C]spiromesifen at a dose equivalent to 190 ppm in the diet daily for three consecutive days. Sixty-three percent of the AD was recovered. The majority of the dose was eliminated in the excreta (58%) and cage wash (4%). Retained residues were highest in liver (1.7 mg eq/kg), lower in skin (0.32 mg eq/kg), fat (0.09 mg eq/kg), muscle (0.067 mg eq/kg), and eggs (0.026 mg eq/kg). Total ^{14}C residues in eggs did not plateau during the dosing period and were 0.032 mg eq/kg on the third day of dosing.

Spiromesifen was the only major residue in fat (51% TRR, 0.046 mg/kg) and a large percentage of the TRR in egg (28% TRR, 0.007 mg/kg). In liver, the only major residue was Sp-enol (38% TRR, 0.026 mg/kg), which was also a major residue in skin (18% TRR, 0.06 mg/kg), liver (18% TRR, 0.30 mg/kg), and egg (44% TRR, 0.011 mg/kg). In skin and liver, 4-carboxy-hydroxy-Sp-enol was a major residue, occurring at 12% TRR (0.04 mg/kg) and 16% TRR (0.26 mg/kg, respectively. The 4-carboxy-3-hydroxy-Sp-enol metabolite was a major residue in liver (20% TRR, 0.34 mg/kg). In other matrices, the 4-carboxy-3-hydroxy-Sp-enol metabolite could not be analytically resolved from oxo-cyclopentyl-Sp-enol. The unresolved residues were major only in skin (42% TRR, 0.13 mg/kg).

Metabolism of spiromesifen in animals is generally similar, with desterification leading to spiromesifen-enol, followed by multiple oxidations to the alkane portions of the molecule. Some differences, however, are noted. While both goats and hens form glucuronide conjugates of metabolised spiromesifen, in the hen, conjugation was to spiromesifen-enol whereas in goats it was to the more oxidized 4-hyroxymethyl-Sp-enol; glucuronide conjugates were not identified in the rat.

Spiromesifen plus spiromesifen-enol (free and glucuronide conjugate) made up the majority of residues in all matrices except poultry skin and liver. In those matrices, the principal residues were comprised of carboxylated and hydroxylated forms of spiromesifen-enol.

Environmental fate in soil and water

The Meeting received information on the environmental fate of spiromesifen in laboratory and field soil systems and on aqueous hydrolysis and photolysis dissipation pathways.

In an aqueous hydrolysis study, spiromesifen was quantitatively hydrolysed to Sp-enol, with hydrolysis occurring more rapidly under higher pH conditions. Estimated hydrolysis half-lives at 20 °C were 4.8 days (pH 9), 45 days (pH 7), and 107 days (pH 4). Spiromesifen is labile to photolysis. In two studies, spiromesifen had a photolysis half-life of 2 to 11 days. A photolysis study

with Sp-enol indicated that it is relatively stable to photolysis (93% of the test substance remained after photo radiation equivalent to 31 days at 33° latitude).

In aerobic laboratory soil dissipation studies using four soils, major residues were spiromesifen (81–88% TRR at Day 0; < 5% TRR by Day 30 or 120), Sp-enol (30–60% TRR, Day 7 or Day 14), 4-carboxy-Sp-enol (3–11% TRR, Day 14 or Day 30), and CO_2 (58–70% TRR, Day 120). Other residues, categorized as unknown, were ≤ 2% TRR throughout the study period. At the 120-day time point, unextracted residues accounted for 16 to 21% of the radioactivity in the system. Field dissipation studies resulted in half-life estimates of 2–6 days for spiromesifen, 6–21 days for Sp-enol, and 7–15 days for total residues (spiromesifen, Sp-enol, 4-carboxy-Sp-enol, and photo isomers of spiromesifen and Sp-enol).

The results of the environmental fate studies, including rotational crop studies, indicate that spiromesifen and its major metabolites (Sp-enol and its carboxylated and/or hydroxylated degradation products) are not likely to be persistent in the environment, and mineralisation to CO_2 is likely to be significant.

Methods of analysis

The Meeting received description and validation data for analytical methods for residues of spiromesifen, Sp-enol, 4-hydroxymethyl-Sp-enol, and 4-hydroxymethyl-Sp-enol glucoside in plant and livestock matrices.

For most data gathering methods, residues are extracted with acetonitrile:H_2O (4:1, v/v). Methods designed to include analysis of 4-hydroxymethyl-Sp-enol generally included a hydrolysis step to cleave the glucose conjugate from the molecule. Extracted residues undergo clean-up by solid-phase extraction (SPE). Residue separation and analysis is by HPLC-MS/MS using deuterated internal standards or matrix-matched standards. For most matrices, the limits of quantification (LOQs) for each analyte, defined as the lowest limit of method validation, is 0.01 mg/kg. Papaya and tea have LOQs of 0.02 mg/kg for each analyte.

The method for livestock matrices specifies extraction with acetonitrile:H_2O (4:1, v/v) at elevated temperature and pressure (70 °C, 10300 KPa) and includes both acid and alkaline hydrolysis steps in order to assay free and conjugated 4-hydroxymethyl-Sp-enol. The reported LOQs for each analyte in livestock matrices are 0.005 mg/kg for milk, 0.01 mg/kg for fat and muscle, and 0.05 mg/kg for kidney and liver.

All of the submitted methods are adequate for the direct analysis of residues of spiromesifen and Sp-enol. 4-hydroxymethyl-Sp-enol and its conjugates are also supported with adequate methods, provided that a hydrolysis step is included in order to assay both free and conjugated forms of the hydroxymethyl metabolite.

Stability of pesticide residues in stored analytical samples

The Meeting received data on the stability of residues of spiromesifen and Sp-enol in multiple crop commodities, and the stability of 4-hydroxymethyl-Sp-enol in turnip root and wheat commodities. In the case of spiromesifen and Sp-enol, both compounds were added to homogenized test matrix. Samples were placed into frozen storage and analysed by the method(s) used in the supervised residue trial. For 4-hydroxymethyl-Sp-enol, separate samples were prepared and fortified with the test substance. During the storage periods, the percent remaining of spiromesifen decreased in most commodities, with corresponding increases in the percent remaining of Sp-enol, indicating that the parent compound was degrading in storage and forming the enol metabolite, which remained stable.

Spiromesifen was stable for less than or up to ca. 160 days in mustard greens, maize stover, potato tuber, undelinted cotton seed, and cotton gin trash; for up to or at least 316 to 376 days in cucumber, tomato fruit and processed commodities, maize grain and forage, and potato processed

commodities; and at least 679 to 727 days in melon peel, wheat grain, forage, and hay, and turnip root.

The sum of spiromesifen and Sp-enol amounted to ca. 100% of the applied material remaining throughout the storage periods for the various storage stability samples, indicating that in total, those residues are stable for at least ca. 365 days in tomato (fruit and processed commodities), mustard greens, maize (grain and forage), potato (tuber and processed commodities), and cotton (seed and gin trash); and at least ca. 700 days in cucumber, melon peel, French beans, wheat grain, and turnip root.

Residues of 4-hydroxymethyl-Sp-enol were stable during frozen storage for at least ca 450 days in wheat and sugar beet raw commodities.

Analyses from livestock feeding studies were completed within 35 days of sample collection; therefore, supporting storage stability data for residues of spiromesifen in livestock commodities are not required. The Meeting noted, however, that analyses of livestock matrices in the goat metabolism study at 1–2 months after slaughter and again ca. 11 months after slaughter indicated that spiromesifen likely degraded to Sp-enol in fat and milk.

Definition of the residue

Plants

In metabolism studies with tomato, lettuce, and cotton as primary crops, spiromesifen, Sp-enol, and 4-hydroxymethyl-Sp-enol (free and conjugated) consistently accounted for 75 to 100 percent of the total radioactive residues in the harvested commodities at maturity. Spiromesifen was the predominant residue in tomato fruit (86% TRR), lettuce leaves (58% TRR), and cotton seed (82% TRR in delinted seed). Sp-enol was the predominant residue in cotton gin trash (49% TRR), followed by spiromesifen (26% TRR). 4-Hydroxymethyl-Sp-enol was > 10% TRR only in lettuce leaves. Spiromesifen concentrations were at quantifiable levels (0.24–1.7 mg/kg) in all matrices except cotton seed, where the TRR was low (ca. 0.05 mg eq/kg). In crop field trials, spiromesifen was also consistently observed to occur at levels greater than Sp-enol; although in multiple cases, the proportion of Sp-enol increased relative to spiromesifen at longer time periods between final application and harvest. Residues of 4-hydroxymethyl-Sp-enol were not measured in crop field trials.

In confined and field rotational crop studies, spiromesifen and Sp-enol were < LOQ in all crop samples, and 4-hydroxymethyl-Sp-enol (free and conjugated) was the predominant residue (ca. 50% TRR; up to 0.16 mg/kg in food crops and 2.2 mg/kg in feed crops at a 30-day PBI). Residues of 4-hydroxymethyl-Sp-enol in wheat grain were still the predominant residues, but were < 0.005 mg/kg at all PBIs. Two additional metabolites occurred as major residues in most commodities in the confined rotational crop study: 3-pentanol-Sp-enol and dihydroxy-Sp-enol. Maximum residues for both compounds were observed at the 30-day PBI, and ranged across all matrices from 0.004 to 0.09 mg/kg each. Levels were generally lower at longer PBIs and were observed at 10 to 40% of the levels of free and conjugated 4-hydroymethyl-Sp-enol. These compounds were not assayed in the field rotation studies.

In the high-temperature hydrolysis study, spiromesifen was converted to Sp-enol. The conversion was ca 25% under pasteurisation conditions, more extensive (ca. 85%) under baking, boiling, and brewing conditions, and essentially complete under conditions mimicking sterilisation. No other residues were identified in the study. In many crop matrices, spiromesifen was not demonstrated to be stable during storage. Data indicate that the parent compound degrades to the Sp-enol metabolite, which is stable during storage. As a result, when considered together, residues of spiromesifen and Sp-enol are stable during frozen storage.

The analytical method for plant matrices is able to determine residues of spiromesifen, Sp-enol, and free 4-hydroxymethyl-Sp-enol, and with the addition of a hydrolysis step, glucose-conjugated 4-hydroxymethyl-Sp-enol.

Although spiromesifen was the most predominant residue in primary crops and would be a suitable marker for compliance purposes, its breakdown during storage to Sp-enol necessitates that residues of the Sp-enol metabolite be taken into account in stored analytical samples. The Meeting agreed that combined residues of spiromesifen and spiromesifen-enol {4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one}, expressed as parent spiromesifen, are suitable for enforcement purposes in plant commodities.

In considering residues for dietary risk assessment, crop field trials reported residues of spiromesifen and Sp-enol. However, the trials did not include analysis of free and conjugated 4-hydroxymethyl-Sp-enol. Based on the data from the lettuce metabolism study, residues of 4-hydroxymethyl-Sp-enol (free + conjugated) are expected to be at one fourth the concentration of spiromesifen and Sp-enol (combined) in leafy crops (significant residues of 4-hydroxymethyl-Sp-enol are not expected in other crops). A comparison of dietary exposure estimates with and without residues of 4-hydroxymethyl-Sp-enol in leafy crops indicates that exposure to that compound is not negligible. The Meeting determined that Sp-enol and 4-hydroxymethyl-Sp-enol are no more toxic than spiromesifen, and that for risk assessment, dietary exposure is adequately covered by the ADI for spiromesifen. Therefore, the Meeting determined that combined residues of spiromesifen, Sp-enol, and free and conjugated 4-hydroxymethyl-Sp-enol {4-hydroxy-3-[4-(hydroxymethyl)-2,6-dimethylphenyl]-1-oxaspiro[4.4]non-3-en-2-one} expressed as spiromesifen, are appropriate for assessing dietary risk from residues in plant commodities.

Animals

In the lactating goat metabolism study (dose = 344 ppm in the feed), TRR were higher in kidney (8.9 mg eq/kg) and liver (3.6 mg eq/kg) than in fat (0.47 mg eq/kg), muscle (0.26 mg eq/kg), and milk (0.11 mg eq/kg). The principal residue in all goat matrices was Sp-enol, making up 29 to 77% of the total residue. The glucose conjugate of Sp-enol was also a major residue in liver (21% TRR), and 4-hydroxymethyl-Sp-enol was a major residue in milk (24% TRR). All other residues were < 10% TRR. In the feeding study with lactating cattle (dosing up to 50 ppm in the feed), residues of spiromesifen + Sp-enol were found at quantifiable levels in samples of milk, fat, kidney, and liver from the highest dose group, and in fat and kidney samples from the middle dose group; residues of spiromesifen + Sp-enol were < LOQ in samples of muscle from all dose groups and in all other matrices at the lowest dosing level. Residues of 4-hydroxymethyl-Sp-enol (free and conjugated) were < LOQ in all samples from the feeding study.

In the laying hen metabolism study, TRR were highest in liver (1.7 mg eq/kg) and skin (0.32 mg eq/kg), with lower residues in muscle (0.067 mg eq/kg), fat (0.09 mg eq/kg), and egg (0.026 mg eq/kg). Spiromesifen was the only major residue in fat (51% TRR) and a major residue in egg (28% TRR). The Sp-enol metabolite was a major residue in all matrices except fat, ranging from 18% TRR in skin to 44% TRR in egg. The only other major residues were 4-carboxy-3-hydroxy-Sp-enol in liver (20% TRR), unresolved 4-carboxy-3-hydroxy-Sp-enol + oxo-cyclopentyl-Sp-enol (42% TRR) in skin, and 4-carboxy-hydroxy-Sp-enol in liver (16% TRR) and skin (12% TRR).

Analytical methods for animal matrices are available for the analysis of spiromesifen, Sp-enol, and free and glucuronide-conjugated 4-hydroxymethyl-Sp-enol. Some degradation of spiromesifen to Sp-enol was noted in samples from the lactating goat metabolism study.

The Meeting agreed that combined residues of spiromesifen and Sp-enol are suitable markers for compliance with MRLs in livestock commodities.

In the feeding study, total residues of spiromesifen and spiromesifen-enol were ca. 11 fold greater in fat than in muscle and ca. 22 fold greater in cream than in skim milk. On that basis, the Meeting concluded that the residue is fat soluble.

For assessing dietary risk, the Meeting noted that residues of 4-hydroxymethyl-Sp-enol were not detected in any sample from the cattle feeding study. Therefore, it was excluded from the residue definition for dietary risk assessment. 4-carboxy-3-hydroxy-Sp-enol was as significant residue only in liver and skin (both of which are modelled as edible offal for dietary risk assessment). The Meeting determined that 4-carboxy-3-hydroxy-Sp-enol is not more toxic than spiromesifen and that dietary risk assessment to that metabolite is adequately covered by the ADI for spiromesifen. A similar conclusion could not be made for the related, non-specific hydroxyl analogues (4-carboxy-hydroxy-Sp-enol). For the specific 4-carboxy-3-hydroxy-Sp-enol metabolite, a comparison of long-term dietary exposure estimates for spiromesifen with and without inclusion of 4-carboxy-3-hydroxy-Sp-enol residues are indistinguishable, leading to the conclusion that relative exposure to the metabolite is negligible and that it can be excluded from the residue definition for dietary risk assessment. The Meeting determined that the residue definition for assessing dietary risk from livestock commodities is the sum of residues of spiromesifen and Sp-enol, expressed as spiromesifen.

The non-specific hydroxyl analogues observed in the poultry metabolism study were 4-carboxy-hydroxy-Sp-enol and 4-carboxy-dihydroxy-Sp-enol. The Meeting decided to use the TTC approach to evaluate dietary risk from exposure to these compounds (as combined residues). The ratio of the hydroxy analogues in liver (0.33 mg/kg) to spiromesifen + Sp-enol (0.31 mg/kg) is 1.1. The estimated residue for evaluating both one-day and long-term dietary exposure is 0.055 mg/kg. The IESTI resulted in one-day exposure estimates of ≤ 0.36 µg/kg bw/day. The long-term dietary exposure estimates were ≤ 0.005 µg/kg bw/day. As the Cramer Class III TTC thresholds are 5 µg/kg bw/day for one-day intake and 1.5 µg/kg bw/day for long-term dietary exposure, public-health concerns were considered unlikely for 4-carboxy-hydroxy-Sp-enol and 4-carboxy-dihydroxy-Sp-enol based on the uses evaluated by this Meeting.

Definition of the residue for plant and animal commodities (for compliance with the MRL): *sum of spiromesifen and 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (spiromesifen-enol), expressed as spiromesifen.*

Definition of the residue for plant commodities (for dietary risk assessment): *sum of spiromesifen, 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (spiromesifen-enol), and 4-hydroxy-3-[4-(hydroxymethyl)-2,6-dimethylphenyl]-1-oxaspiro[4.4]non-3-en-2-one (4-hydroxymethyl-spiromesifen-enol) (free and conjugated), all expressed as spiromesifen.*

Definition of the residue for animal commodities (for dietary risk assessment): *sum of spiromesifen and 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one (spiromesifen-enol), expressed as spiromesifen.*

The residue is fat soluble.

Results of supervised residue trials on crops

The Meeting received supervised trial data for the foliar application of spiromesifen on strawberry, papaya, broccoli, cabbage, cucumber, melon, summer squash, peppers, tomato, sweet corn, head and leaf lettuce, spinach, mustard greens, common bean (pods and/or immature seeds), dry bean, cassava, potato, maize, popcorn, cotton, coffee, and tea.

Labels for end-use products containing spiromesifen were available from Belgium, Brazil, Canada, Central America, Colombia, Ecuador, France, Greece, India, Italy, Japan, Kenya, Mexico, the Netherlands, New Zealand, Peru, Spain, and the United States describing the registered uses of spiromesifen.

For all trials, residues were determined by a method involving extraction with acetonitrile:H_2O and analysis by HPLC-MS/MS with either deuterated internal standards or matrix-matched standards. For most crops, the extracts underwent clean-up by solid-phase extraction.

For all trials, combined residues of spiromesifen and Sp-enol (i.e., total spiromesifen) are supported by adequate storage stability data.

In determining combined residues of spiromesifen and Sp-enol, the following convention was used: residue > LOQ = finite residue, residue between LOQ and LOD = LOQ (if both compounds are in this category, then combined is < 2 × LOQ), and residue < LOD = 0 contribution (if both compounds < LOD, then combined is < LOQ).

Based on the ratio of free and conjugated 4-hydroxymethyl-Sp-enol to spiromesifen + Sp-enol from the lettuce metabolism study (0.25), residue estimates of spiromesifen + Sp-enol have been multiplied by 1.25 to derive residue estimates for assessing dietary intake from leafy vegetables and Brassica leafy vegetables. An adjustment is not necessary for other crops.

Berries and other small fruits

Spiromesifen is registered in the US for outdoor use on low-growing berry, which includes bearberry, bilberry, blueberry (lowbush), cloudberry, cranberry, lingonberry, muntries, partridgeberry, and strawberry. The US GAP is for up to three applications, on a 7-day interval, each at 0.28 kg ai/ha. The PHI is 3 days. Spiromesifen is also registered for greenhouse use on strawberry in the Netherlands, with an unspecified number of applications at 0.12 kg ai/ha and a 1-day PHI.

Strawberry

Eight supervised trials were conducted in the US according to the US GAP. The trials resulted in the following independent residue values (n = 8): 0.26, 0.27, 0.47 (2), 0.57, 0.70, 1.5, and 1.6 mg/kg.

Four trials were conducted in Europe according to the Netherlands GAP. The trials resulted in the following independent residue values (n = 4): 0.11, 0.23, 0.26, and 0.62 mg/kg.

The residue data show the US GAP to be the critical GAP. The Meeting estimated a maximum residue level and STMR for spiromesifen residues in strawberries of 3 mg/kg and 0.52 mg/kg, respectively.

As the berries covered by the registered use correspond to the Codex subgroups for low-growing berries (FB 2009), the Meeting recommends extrapolating the estimates from strawberry to subgroup FB 2009.

Tropical and sub-tropical fruit – inedible peel

Papaya

The GAP for papaya is from registrations in Ecuador, with two applications at 0.12 kg ai/ha and a 7-day PHI. An alternative GAP exists from registrations in Colombia and Mexico, consisting of a single application at 0.12 kg ai/ha and a 7-day PHI. Six residue trials were conducted in Ghana wherein 1 to 3 applications were made at 0.12 kg ai/ha. Fruits were harvested 0 to 56 DALA, with harvest 7 DALA for only two trials. Residues were reported for spiromesifen only.

Two trials were conducted matching the Ecuador GAP and two trials were conducted matching the Columbia and Mexico GAP. The number of trials reflecting either GAP is insufficient. Furthermore, the residues that were reported do not address the residue definition for either enforcement or dietary intake. For these reasons, the Meeting is not making a recommendation for residues of spiromesifen in papaya.

Brassica (cole or cabbage) vegetables, Head cabbage, Flowerhead brassica

Spiromesifen is registered in Canada and the US for use on Brassica leafy vegetables–broccoli and Chinese (gai lon) broccoli, Brussels sprouts, cabbage, Chinese mustard (gai choy) cabbage,

cauliflower, cavalo broccolo, kohlrabi, mustard spinach, and rape greens. The GAP is three applications, each at 0.144 kg ai/ha and a 7-day PHI.

Broccoli

Seven supervised trials were conducted in the US at GAP. The two trials at Hillsboro, Oregon are not considered to be independent. The trials resulted in the following independent residue values (n = 6): 0.02, 0.056, 0.091, 0.15, 0.21, and 0.58 mg/kg.

Cabbage

Six supervised trials were conducted in the US, five of which were at GAP. The trials resulted in the following independent residue values (n = 5): < 0.02, 0.18 0.44, 1.6, and 1.8 mg/kg.

As the registered use and available data correspond to the Codex group for Brassica (cole or cabbage) vegetables, head cabbages, flowerhead Brassicas (VB 0040); the median residues from the broccoli and cabbage trials are within 5-fold of each other; and analysis by the Kruskal-Wallis test showed no evidence of a difference between the residue populations, the Meeting decided to make a recommendation for the group based on the following combined broccoli and cabbage data (n = 11): < 0.02, 0.02, 0.056, 0.091, 0.15, 0.18, 0.21, 0.44, 0.58, 1.6, and 1.8 mg/kg.

The Meeting estimated a maximum residue level STMR, and highest residue for spiromesifen residues in Brassica (cole or cabbage) vegetables, head cabbages, flowerhead Brassicas of 3 mg/kg, 0.18 mg/kg, and 1.8 mg/kg, respectively.

Fruiting vegetables, Cucurbits

Spiromesifen has registration for use on field-grown cucurbit vegetables as a group, as well as greenhouse-grown cucumber and melon as individual crops. The field and greenhouse uses are considered to be different GAPs. As such the Meeting has evaluated the greenhouse data against the individual-crop greenhouse GAPs and considered the field data against the crop group field GAP.

Cucumber

Spiromesifen is registered in Greece for use on greenhouse-grown cucumbers, with four applications, each at 0.216 kg ai/ha and a 3-day PHI. In addition, spiromesifen is registered for use on field-grown cucurbit vegetables in Canada and the US, with three applications at 0.144 kg ai/ha and a 7-day PHI.

Seven supervised greenhouse trials were conducted in Europe according to the Grecian GAP. The trials resulted in the following independent residue values (n = 7): 0.03 (2), 0.04, 0.05, 0.06, 0.07, and 0.08 mg/kg.

Six supervised field trials were conducted according to the Canadian/US GAP. The trials resulted in the following independent residue values (n = 6): < 0.02 (4), 0.026, and 0.034 mg/kg.

The residue data show the greenhouse use to be the critical GAP. The Meeting estimated a maximum residue level and STMR for spiromesifen residues in cucumber of 0.15 mg/kg and 0.05 mg/kg, respectively.

Melon

Spiromesifen is registered in Greece for use on greenhouse-grown melons, with four applications, each at 0.216 kg ai/ha and a 3-day PHI. In addition, spiromesifen is registered for use on field-grown cucurbit vegetables in Canada and the US, consisting of three applications at 0.144 kg ai/ha and a 7-day PHI.

Eight supervised greenhouse trials were conducted in Europe with application rates of ca. 0.6 × the greenhouse GAP. The trials resulted in the following independent residue values (n = 8): 0.03 (2), 0.04, 0.05 (2), 0.06, and 0.07 (2) mg/kg.

The Meeting noted that the supervised greenhouse trials available did not correspond to the submitted GAP and decided to apply the proportionality approach. After scaling residues to an application rate of 0.216 kg ai/ha by using a proportionality factor of approximately 1.5 (0.216 kg ai/ha ÷ 0.144 kg ai/ha; individual trial results were scaled based on actual application rates used in the field trials), the following data set resulted (n = 8): 0.045 (2), 0.06, 0.075 (2), 0.088, and 0.11 (2) mg/kg.

In five trials also analysing the pulp, no residues of total spiromesifen above the LOQ of 0.01 mg/kg were found. However, since the proportionality approach was applied by upscaling the results, the LOQ information cannot be used for the refined estimation of the dietary intake. Therefore, the Meeting based its estimation of STMR and HR values on whole melons instead.

Six supervised field trials were conducted according to the Canadian/US GAP. The trials resulted in the following independent residue values (n = 6): < 0.02, 0.025, 0.027, 0.035, and 0.045 mg/kg.

The residue data show the greenhouse use to be the critical GAP. The Meeting estimated a maximum residue level and STMR for spiromesifen residues in melon, except watermelon of 0.3 mg/kg and 0.075 mg/kg, respectively.

Summer squash

The GAP for summer squash is from registrations in Canada and the US for cucurbit vegetables, with three applications, each at 0.144 kg ai/ha and a 7-day PHI.

Six supervised trials were conducted in the US at GAP. The two trials conducted in Vero Beach, Florida are not considered to be independent. The trials resulted in the following independent residue values (n = 5): < 0.02 (2), 0.021, 0.022, and 0.05 mg/kg.

As the registrations in Canada and the US correspond to the "fruiting vegetables, cucurbit" Codex group; the median residue values from the trials conducted according to the Canadian/US GAP with cucumber, melon, and summer squash do not differ by more than 5-fold; and analysis by the Kruskal-Wallis test showed no evidence of a difference between the residue populations, the available data support a group recommendation. The combined field residues from cucumber, melon, and summer squash are (n = 16): < 0.02 (7), 0.021, 0.022, 0.025, 0.026, 0.027, 0.034, 0.035, 0.045, and 0.05 mg/kg.

The Meeting noted the individual recommendations for cucumber and melon from the greenhouse GAPS, and estimated for fruiting vegetables, cucurbit except cucumber and melon, maximum residue level and STMR for spiromesifen residues of 0.09 mg/kg and 0.021 mg/kg, respectively.

Fruiting vegetables, other than cucurbits

Spiromesifen has registrations for use on greenhouse-grown cucumber and melon as individual crops, as well as field-grown fruiting vegetables, other than cucurbits, as a group. The field and greenhouse uses are considered to be different GAPs. As such the Meeting has evaluated the greenhouse data against the individual-crop greenhouse GAPs and considered the field data against the crop group GAP.

Tomato

Spiromesifen is registered in France and Italy for use on greenhouse-grown tomato, with four applications, each at 0.216 kg ai/ha and a 3-day PHI. In addition, there are registrations in Canada, Mexico, and the US for use on field-grown crops in the NAFTA crop group fruiting vegetables, which corresponds to the Codex group fruiting vegetables other than cucurbits—except sweet corn and mushrooms. Under the field GAP, three applications are allowed, each at 0.144 kg ai/ha, with a 1-day PHI.

Sixteen supervised greenhouse trials were conducted in Europe according to the greenhouse GAP. The trials resulted in the following independent residue values (n = 16): 0.07, 0.09 (2), 0.10, 0.11, 0.12, 0.15, 0.16, 0.17, 0.19 (2), 0.21, 0.24, 0.29, 0.42, and 0.50 mg/kg.

Twelve supervised field trials were conducted in the US according to the field GAP. The trials resulted in the following independent residue values (n = 12): 0.047, 0.056, 0.063, 0.065 (2), 0.094, 0.099, 0.11, 0.14, 0.17, 0.18, and 0.34 mg/kg.

The residue data show the greenhouse use to be the critical GAP for tomato. The Meeting estimated a maximum residue level and STMR for spiromesifen residues in tomato of 0.7 mg/kg and 0.165 mg/kg, respectively. Noting the registration for use of spiromesifen in Greece on greenhouse-grown eggplant, the Meeting decided to extrapolate these estimates to greenhouse-grown eggplant.

Peppers

Spiromesifen is registered in Greece and Italy for use on greenhouse-grown sweet peppers, with four applications, each at 0.216 kg ai/ha, and a 3-day PHI. In addition, there are registrations in Canada, Mexico, and the US for use on the NAFTA crop group fruiting vegetables, which corresponds to the Codex group fruiting vegetables other than cucurbits-except sweet corn and mushrooms. Under the Canada, Mexico, and US GAPs, three applications are allowed, each at 0.144 kg ai/ha, with a 1-day PHI.

Nine supervised greenhouse trials were conducted on sweet peppers in Europe according to the greenhouse GAP. The trials resulted in the following independent residue values (n = 9): 0.07, 0.09, 0.11, 0.12, 0.13 (2), 0.19, and 0.22 (2) mg/kg.

Twenty supervised field trials were conducted on peppers (including chilli peppers) in the US; however only ten matched the GAP with respect to the PHI. The trials resulted in the following independent residue values (n = 10): < 0.02, 0.030, 0.035, 0.040, 0.050, 0.060 (2), 0.085, 0.17, and 0.32 mg/kg.

The residue data show the field use to be the critical GAP for peppers. Given that, and noting the GAPs for use of fruiting vegetables other than cucurbits in Canada, Mexico, and the US, the Meeting decided to explore using combined field data to evaluate residues in fruiting vegetables, other than cucurbits-except sweet corn and mushroom (excluding tomato and eggplant, as they are addressed by the greenhouse use discussed above). The residue data from field-grown peppers and tomato have median values that do not differ by more than five-fold; however, the Kruskal-Wallis test indicates that the residues are from separate populations.

As the residues in field-grown tomato are, overall, greater than those in field-grown pepper, the Meeting used the data from field-grown tomato and estimated maximum residue levels and STMRs for spiromesifen residues in peppers, okra, and pepino of 0.5 mg/kg and 0.097 mg/kg, respectively.

Of the available data from peppers, only three trials were conducted with chilli pepper varieties, which is insufficient for making a recommendation for dried chilli pepper based on chilli-pepper-specific data. Therefore, the Meeting used the generic pepper data and a default processing factor of 10 to estimate a maximum residue level and STMR for spiromesifen residues in chilli pepper (dried) of 5 mg/kg and 0.55 mg/kg, respectively.

Sweet corn

The GAP for sweet corn is from a registration in the US, with two applications, each at up to 0.3 kg ai/ha (not to exceed 0.3 kg ai/ha/season) and a 5-day PHI for sweet corn for fresh consumption.

Twelve supervised trials were conducted in the US at GAP. The trials resulted in the following independent residue values (n = 12): < 0.02 (12) mg/kg.

Noting that residues were below the limit of detection (0.0016 mg/kg) in all samples, the Meeting estimated a maximum residue level, STMR, and highest residue for spiromesifen residues in sweet corn (corn-on-the-cob) of 0.02* mg/kg, 0 mg/kg, and 0 mg/kg, respectively.

Leafy vegetables, including Brassica leafy vegetables

The GAP is from registrations in Canada and the US for leafy vegetables and Brassica leafy vegetables, with three applications, each at 0.144 kg ai/ha and a 7-day PHI.

Six supervised trials were conducted on head lettuce in the US at GAP. The trials resulted in the following independent residue values (n = 6): 0.16, 0.74, 0.97, 1.4, 2.4, and 4.5 mg/kg.

Seven supervised trials were conducted on leaf lettuce in the US at GAP. The two trials in Fresno, CA are not considered to be independent. The trials resulted in the following independent residue values (n = 6): 0.52, 0.90, 1.0, 1.7, 2.5, and 9.3 mg/kg.

Seven supervised trials were conducted on spinach in the US at GAP. The two trials in Suffolk, VA are not considered to be independent. The trials resulted in the following independent residue values (n = 6): 0.27, 1.8, 2.2, 5.0, 6.4, and 7.3 mg/kg.

Eight supervised trials were conducted on mustard greens in the US at GAP. The trials resulted in the following independent residue values (n = 8): 0.66, 1.1, 1.3, 1.5, 1.6, 2.1, 9.9, and 10 mg/kg.

The Meeting noted that the GAP in the US covers the Codex group of leafy vegetables including Brassica leafy vegetables and decided to explore the possibility of estimating a group maximum residue level for spiromesifen. As median residues in head lettuce, leaf lettuce, spinach, and mustard greens differed by less than 5-fold and the residue populations were not significantly different by the Kruskal-Wallis test, the Meeting decided to make a recommendation for leafy vegetables, including Brassica leafy vegetables based on the following combined data set (n = 26): 0.16, 0.27, 0.52, 0.66, 0.74, 0.90, 0.97, 1.0, 1.1, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 2.1, 2.2, 2.4, 2.5, 4.5, 5.0, 6.4, 7.3, 9.3, 9.9, and 10 mg/kg.

From those combined data, the Meeting estimated a maximum residue level for spiromesifen residues in leafy vegetables and in Brassica leafy vegetables of 15 mg/kg. The median and highest residues are 1.65 mg/kg and 10 mg/kg, respectively. Correcting these values to account for residues of 4-hydroxymethyl-Sp-enol (factor of 1.25) results in STMR and highest residue estimates of 2.06 mg/kg and 12.5 mg/kg, respectively.

Legume vegetables

Common beans (pods and/or immature seeds)

The GAP in the Netherlands allows for an unspecified number of applications to greenhouse-grown beans, each at up to 0.12 kg ai/ha, with a 1-day PHI.

Four supervised greenhouse trials were conducted in southern Europe approximating the Netherlands GAP were (n = 4): 0.11, 0.12, 0.27, and 0.64 mg/kg.

As four trials are not adequate for making robust residue estimates for beans, and no other trials matching the Netherlands GAP were available, the Meeting considered an alternate greenhouse GAP from Greece (up to four applications, each up to 0.144 kg ai/ha, and a 3-day PHI).

Eight supervised greenhouse trials were conducted in southern Europe matching the Greece GAP. The trials resulted in the following independent residue values (n = 8): 0.04, 0.06, 0.07, 0.08, 0.09, 0.14, 0.26, and 0.64 mg/kg.

The Meeting estimated a maximum residue level and STMR for spiromesifen residues in beans (*Phaseolus)* (green pods and/or immature seeds) of 1 mg/kg and 0.085 mg/kg, respectively.

Pulses

Dry beans

The GAP for bean (dry seed) is from a registration in Brazil, with three applications, each at 0.144 kg ai/ha and a 21-day PHI.

Three supervised trials were conducted in Brazil at GAP and at an application rate of 2 × GAP. The trials reported residues of parent spiromesifen only.

Three trials are insufficient for making robust estimates of expected residues. Furthermore, the residues that were reported do not address the residue definition for either enforcement or dietary intake. For these reasons, the Meeting is not making a recommendation for residues of spiromesifen in bean (dry seed).

Root and tuber vegetables

The GAP for is from a registration in the US for tuberous and corm vegetables, with two applications, each at 0.280 kg ai/ha and a 7-day PHI. An alternate GAP exists from registration in Canada for use on tuberous and corm vegetables, with two applications, each at 0.144 kg ai/ha and a 7-day PHI.

Cassava (manioc)

Five supervised trials on cassava (manioc) were conducted in Brazil using three applications, each at ca. 0.14 kg ai/ha and included harvest 7 DALA. The trials resulted in the following independent residue values (n = 5): < 0.02 (5) mg/kg.

Potato

Sixteen supervised trials on potatoes were conducted in the US at GAP. The trials resulted in the following independent residue values (n = 16): < 0.02 (16) mg/kg.

Based on the registrations on tuberous and corm vegetables, and the lack of quantifiable residues in cassava and the lack of detected residues in potato, the Meeting extrapolated the results to include sweet potato, and estimated a maximum residue level, STMR, and highest residue for spiromesifen residues in cassava, potato, and sweet potato of 0.02* mg/kg, 0.01 mg/kg, and 0.01 mg/kg, respectively.

Cereal grains

Maize

The GAP for maize is from a registration in the US, with two applications, each at up to 0.3 kg ai/ha (not to exceed 0.3 kg ai/ha/season) and a 30-day PHI.

Forty supervised trials were conducted in the US at GAP. The trials resulted in the following independent residue values (n = 40): < 0.02 (40) mg/kg. In addition, spiromesifen + Sp-enol residues in corn grain were < 0.02 mg/kg from three trials conducted at a 5-fold exaggerated rate.

The Meeting estimated a maximum residue level and STMR for spiromesifen residues in maize of 0.02* mg/kg and 0 mg/kg, respectively.

Popcorn

The GAP for popcorn is from a registration in the US, with two applications, each at up to 0.3 kg ai/ha (not to exceed 0.3 kg ai/ha/season) and a 30-day PHI.

Three supervised trials were conducted in the US at GAP. The trials resulted in the following independent residue values (n = 3): < 0.02 (3) mg/kg.

Three trials are insufficient for making robust estimates of expected residues; however, based on the data from maize showing residues below the LOD in all trials, including exaggerated rate trials, the Meeting estimated a maximum residue level and STMR for spiromesifen residues in popcorn of 0.02* mg/kg and 0 mg/kg, respectively.

Oilseeds

Cotton

The GAP for cotton is from a registration in the US, with applications up to 0.28 kg ai/ha each (not to exceed three sprays or 0.56 kg ai/ha per season) and a 30-day PHI.

Twelve supervised trials were conducted in the US compliant with the US GAP. The trials resulted in the following independent residue values (n = 12): < 0.02 (4), 0.034, 0.11 (2), 0.18, 0.28, 0.32, 0.34, and 0.39 mg/kg.

The Meeting determined that the trials are suitable and estimated a maximum residue level and STMR for spiromesifen residues in cotton seed of 0.7 mg/kg and 0.11 mg/kg, respectively.

Seed for beverages and sweets

Coffee

The GAP for coffee is from a registration in Brazil, with two applications, each at 0.144 kg ai/ha and a 21-day PHI.

Five supervised trials were conducted in Brazil matching the Brazil GAP. The trials resulted in the following independent residue values (n = 5): ≤ 0.02 (3), and 0.035 mg/kg and 0.11 mg/kg.

The Meeting estimated a maximum residue level and STMR for spiromesifen residues in coffee beans of 0.2 mg/kg and 0.02 mg/kg, respectively.

Derived products of plant origin

Tea

The GAP for tea is from a registration in Japan, with one application of a spray concentration of 0.015 kg ai/hL and a 7-day PHI. The label recommends spray rates of 2000 to 4000 L/ha.

Two trials were conducted in Japan according to the Japanese GAP; however, only summary reports were provided and they could not be adequately assessed. No other trials were available matching the Japanese GAP.

Six supervised trials were conducted in India with an application rate of 0.63 kg ai/ha and a 7-day PHI. The Meeting noted that the spray concentration used in the trials was exaggerated approximately 10-fold relative to the Japanese GAP. The Meeting used the spray concentration and maximum spray rate from the Japanese label to derive an application rate on a kg a.i./ha basis. As the application rate (0.63 kg ai/ha) from the India trials corresponds to the estimated maximum per-hectare rate from the Japanese label, the Meeting considered the residues resulting from the India trials approximating the Japanese GAP.

In trials conducted in India approximating the Japanese GAP, residues in fresh tea leaves were (n = 6): 0.66, 1.4, 4.4, 7.1, 7.7, and 10 mg/kg.

Sample of fresh tea (spiromesifen + Sp-enol = 7.1 mg/kg) from one trial in India were dried to form green tea (16 mg/kg) or fermented to form black tea (23 mg/kg). Applying the ratios of the residues for green tea (2.3) and black tea (3.2) results in residues as follows: green tea (n = 6), 1.5, 3.2, 10, 16, 18, and 23 mg/kg; and black tea (n = 6), 2.1, 4.5, 14, 23, 25, and 32 mg/kg.

Using the anticipated residues in black tea, the Meeting estimated a maximum residue level and STMR for tea green and black (black fermented and dried) of 70 mg/kg and 18.5 mg/kg, respectively.

Straw, fodder, and forage of cereal grains

Maize (including popcorn and sweet corn)

The GAP for maize is from a registration in the US, with two applications, each at up to 0.3 kg ai/ha (not to exceed 0.3 kg ai/ha/season) and PHIs of 5 days for forage and 30 days for fodder.

Maize forage

Fifty-six supervised trials were conducted with maize, popcorn, and sweet corn in the US at GAP. The trials resulted in the following independent residue values for forage (n = 27): 0.27, 0.42, 0.78, 1.0 (2), 1.3, 1.4, 1.5, 1.6, 1.7, 1.8 (3), 2.0, 2.1, 2.2 (2), 2.5 (3), 2.9, 3.0, 3.1, 3.2, 3.5, 3.6, and 4.4 mg/kg.

The Meeting estimated a median and highest residue for spiromesifen residues in maize forage (fresh) of 2 mg/kg and 4.4 mg/kg, respectively.

Maize fodder

The trials resulted in the following independent residue values for fodder (n = 34): 0.02, 0.08, 0.19 (2), 0.29, 0.31, 0.33, 0.34, 0.42, 0.49, 0.66, 0.73, 0.77, 0.84, 0.87, 0.88, 0.95, 0.97, 0.98, 1.0, 1.2, 1.3 (2), 1.4, 1.5, 1.6, 1.8, 1.9, 2.0, 2.2, 2.3, 2.6, and 4.1 mg/kg.

Based on a dry matter content of 83%, the Meeting estimated a maximum residue level for spiromesifen residues in maize fodder of 6 mg/kg.

The Meeting estimated median, and highest residues for spiromesifen residues in maize fodder (as received) of 0.96 mg/kg, and 4.1 mg/kg, respectively.

Miscellaneous fodder and forage crops

Cotton gin trash

The GAP for cotton is from a registration in the US, with three applications, each at maximum of 0.28 kg ai/ha (not to exceed 0.56 kg ai/ha/season) and a 30-day PHI.

Six supervised trials were conducted in the US at GAP. The trials resulted in the following independent residue values (n = 6): 0.41, 1.6, 2.7, 4.6, 6.0, and 11 mg/kg.

The Meeting estimated a median for spiromesifen residues in cotton gin trash of 3.65 mg/kg and a highest residue (from a single sample) of 12 mg/kg.

Fate of residues during processing

Under high-temperature hydrolysis conditions meant to mimic pasteurisation (90 °C, pH 4, 20 min.); baking, brewing, boiling (100 °C, pH 5, 60 min); and sterilisation (120 °C, pH 6, 20 min.), spiromesifen was converted to its Sp-enol metabolite. No other residues were identified. Conversion was less under the pH-4 conditions (ca. 20% formation of Sp-enol) and essentially quantitative under the pH-6 conditions.

The Meeting received data depicting the effects of processing and preparation on residue levels in strawberry, cucumber, summer squash, peppers, tomato, lettuce, spinach, beans, potato, sugar beet, maize, sorghum, wheat, cotton seed, and tea. Residue information, processing factors, and recommendations of STMR-P, HR-P, and MRLs relevant to the current evaluation are shown in the table, below.

Summary of spiromesifen residues (spiromesifen + Sp-enol) in processed commodities

	RAC residues, mg/kg				Processed residues, mg/kg	
RAC	MRL	STMR	Processed commodity	Processing factors [Median/Best estimate]	MRL	STMR-P
Strawberry	3	0.52	Jam	0.44, 0.46, 0.47, 0.58 [0.46]	–	0.24
			Preserve	0.28, 0.28, 0.32, 0.27 [0.28]	–	0.15
Broccoli	3	0.15	Cooked	1.9 [1.9]	–	0.28
Summer squash	0.09	0.02	Cooked	0.70 [0.7]	–	0.014
Tomato	0.7	0.165	Canned	0.21, < 0.06, < 0.07, 0.17, 0.35 [0.21]	–	0.035
			Juice (canned)	0.72, < 0.06, 0.13, 0.35, 0.3 [0.35]	–	0.058
			Puree	0.78, 0.72, 1.2, 2.3, 2 [1.2]	–	0.20
			Paste	2.6 [2.6]	2	0.43
			Wet pomace	4.1, 8.3, 7.4, 7.6 [7.5]	–	1.2
			Dried	5 [5]	4	0.82
Mustard greens	20	1.55	Cooked	0.14 [0.14]	–	0.22
Spinach	15	2.06	Cooked	2.1 [2.1]	–	4.3
Cotton seed	0.7	0.11	Refined oil	0.043, 0.026 [0.034]	–	0.0037
			Meal	0.08, 0.21 [0.14]	–	0.015
			Hulls	0.34 (0.34)		0.04
Tea, green, black (black, fermented and dried)	70	18.5	Black tea infusion	0.034 [0.034]	–	0.63

Residues in animal commodities

Farm animal dietary burden

The Meeting estimated the dietary burden of spiromesifen in farm animals on the basis of the diets listed in Appendix IX of the FAO Manual 2016. Calculation from highest residue, STMR (some bulk commodities) and STMR-P values provides levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle, broilers and laying poultry are provided in Appendix IX of the FAO manual. The calculations were made according to the animal diets from US-Canada, EU, Australia and Japan in the Table (Appendix IX of the FAO manual). The diets are based on residues in kale, and cotton, corn, and potato livestock feed commodities.

Livestock dietary burden for spiromesifen, ppm of dry matter diet								
	US-Canada		EU		Australia		Japan	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Beef cattle	2.3	0.97	25	6.7	9.4	4.6	–	0.015
Dairy cattle	5.0	2.3	23	5.8	40	8.6	5.5	2.5
Poultry—broiler	–	–	0.01	0.01	–	–	–	–
Poultry—layer	–	–	5.3	1.2	–	–	–	–

The bold values, above, reflect the highest burdens for both MRL estimation (maximum diet) and STMR estimation (mean diet). Burdens from dairy cattle and layer hens are being used for beef cattle and broiler poultry, respectively.

Farm animal feeding studies

The Meeting received lactating dairy cow feeding studies, which provided information on likely residues resulting in animal commodities and milk from spiromesifen residues in the animal diet.

Lactating dairy cows

Lactating dairy cows were dosed with spiromesifen for 29 days at the equivalent of 5, 15 or 50 ppm in the diet. Analysis was for residues of spiromesifen, Sp-enol, and 4-hydroxymethyl-Sp-enol. Residues for all analyses and tissues were < 0.01 for control animals.

Residues of spiromesifen in milk reached a plateau beginning on Day 4. From Day 4 through Day 28 median and maximum residues were both 0.012 mg/kg at the 50-ppm dose level (other levels not analysed). In tissues, mean (and maximum) residues at the 5, 15, and 50-ppm feeding levels, respectively were: fat < 0.01 (< 0.01), 0.03 (0.045), and 0.093 (0.12) mg/kg; kidney < 0.05 (< 0.05), 0.065 (0.096), and 0.15 (0.26) mg/kg; liver < 0.05 (< 0.05), < 0.05 (< 0.05), and 0.053 (0.059) mg/kg; and muscle < 0.01 (< 0.010), < 0.01 (0.01), and < 0.01 (< 0.01) mg/kg.

Laying hens

The Meeting did not receive a feeding study for poultry. In the metabolism study conducted with laying hens, daily dosing at a rate of ca. 190 ppm resulted in combined residues of spiromesifen and Sp-enol of 0.018 mg/kg in egg (may be less than plateau level), 0.049 mg/kg in fat, 0.3 mg/kg in liver, 0.028 mg/kg in muscle, and 0.07 mg/kg in skin.

Animal commodities maximum residue levels

For MRL estimation in animal commodities, the residue definition is the combined residues of spiromesifen and spiromesifen-enol, expressed as spiromesifen

Estimated residues in tissues and milk at the dietary burden summarized above are shown in the table below.

Spiromesifen feeding study	Feed level (ppm) for milk residues	Residues (mg/kg) in milk	Feed level (ppm) for tissue residues	Residues (mg/kg)			
				Muscle	Liver	Kidney	Fat
MRL beef or dairy cattle							
Feeding study [a]	50	0.012	15	< 0.01	< 0.05	0.096	0.045
			50	< 0.01	0.059	0.26	0.12
Dietary burden and high residue	40	0.0096	40	< 0.01	0.056	0.21	0.099
STMR beef or dairy cattle							
Feeding study [b]	50	0.012	5	< 0.01	< 0.05	< 0.05	< 0.01
			15	< 0.01	< 0.05	0.065	0.03
Dietary burden and residue estimate	8.6	0.0021	8.6	< 0.01	< 0.05	0.055	0.017

[a] Highest residues for tissues and mean residues for milk

[b] Mean residues for tissues and mean residues for milk

The Meeting estimated the following maximum residue levels: Milks = 0.015 mg/kg; mammalian fats except milk fats = 0.15 mg/kg; meat (from mammals other than marine mammals) = 0.15 (F) mg/kg; and edible offal (mammalian) 0.3 mg/kg.

The Meeting estimated the following STMR levels: Milks = 0.0021 mg/kg; mammalian fats except milk fats = 0.017 mg/kg; meat (from mammals other than marine mammals) = 0.017 (F) mg/kg, 0.01 mg/kg (meat); and edible offal (mammalian) 0.055 mg/kg.

For poultry, a comparison of the feeding level in the metabolism study (190 ppm) with the maximum dietary burden (5.3 ppm) indicates that residues of spiromesifen plus Sp-enol would not be expected to exceed 0.0073 mg/kg in any poultry commodity. For poultry meat, egg, and fat, the Meeting estimated maximum residue levels of 0.02 mg/kg and STMRs of 0.01 mg/kg. For poultry edible offal, the Meeting estimated a maximum residue level of 0.05* mg/kg, and STMR and highest residue of 0.05 mg/kg, each.

RECOMMENDATIONS

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

Definition of the residue for plant and animal commodities (for compliance with the MRL): *sum of spiromesifen and 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one, expressed as spiromesifen.*

Definition of the residue for plant commodities (for dietary risk assessment): *sum of spiromesifen, 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one, and 4-hydroxy-3-[4-(hydroxymethyl)-2,6-dimethylphenyl]-1-oxaspiro[4.4]non-3-en-2-one (free and conjugated), all expressed as spiromesifen.*

Definition of the residue for livestock commodities (for dietary risk assessment): *sum of spiromesifen and 4-hydroxy-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-2-one, expressed as spiromesifen.*

The residue is fat soluble.

DIETARY RISK ASSESSMENT

Long-term dietary exposure

The International Estimated Daily Intakes (IEDIs) of spiromesifen were calculated for the 17 GEMS/Food cluster diets using STMRs/STMR-Ps estimated by the current Meeting. The ADI is 0–0.03 mg/kg bw and the calculated IEDIs were 2–20% of the maximum ADI (0.03 mg/kg bw). The Meeting concluded that the long-term dietary exposure to residues of spiromesifen, resulting from the uses considered by the current JMPR, are unlikely to present a public health concern.

Short-term dietary exposure

The Meeting determined that an ARfD is not necessary for spiromesifen. The Meeting therefore concluded that the short-term dietary exposure to residues of spiromesifen resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.