

**Levels relevant to risk assessment**

Species	Study	Effect	NOAEL	LOAEL
Dog	Acute toxicity <sup>a</sup>	Diarrhoea	2 mg/kg bw	5 mg/kg bw
	Three-month studies of toxicity <sup>b</sup>	Clinical signs and reduced body-weight gain in females	—	2 mg/kg bw per day

<sup>a</sup> Gavage administration<sup>b</sup> Capsule administration.*Estimate of acute reference dose*

0.02 mg/kg bw

*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.

**5.14 FLUSILAZOLE (165)****TOXICOLOGY**

Flusilazole is the ISO approved name for 1-[[bis(4-fluorophenyl)methyl]silyl]methyl]-1*H*-1,2,4-triazole (CAS No. 85509-19-9). It is a broad-spectrum fungicide that belongs to the triazole subclass of ergosterol biosynthesis inhibitors. Flusilazole was previously evaluated by the Joint Meeting in 1989 (Annex 5, references 56, 58) and in 1995. An ADI of 0–0.001 mg/kg bw was allocated in 1989, based on a NOAEL of 0.14 mg/kg bw per day (5 ppm) for liver toxicity in a 1-year feeding study in dogs. This was confirmed in 1995. The compound was re-examined by the present Meeting as part of the Periodic Re-evaluation Programme of CCPR. Three new studies were provided, two studies of developmental toxicity in rats (one of oral and one of dermal administration) and a 28-day mechanistic study in dogs.

Owing to the age of the database, some studies predate GLP; however, all critical studies complied with GLP.

**Biochemical aspects**

In rats, orally administered [<sup>14</sup>C] labelled flusilazole was readily absorbed from the gastrointestinal tract and rapidly excreted in urine (72% of triazole label) and faeces (up to 87% of phenyl label), with little or no radioactivity recovered in the expired air. The excretion half-life was approximately 34 h and > 90% of the administered dose was eliminated within 96 h. Tissue retention of radiolabelled material was low. Total tissue residues excluding the carcass (which accounted for approximately 2% of the administered dose) was < 1%, therefore demonstrating no evidence of bioaccumulation.

[<sup>14</sup>C]Flusilazole was extensively metabolized in rats. Recovered parent compound accounted for only 2–11% of the given dose, found predominantly in the faeces (urinary concentration, < 1%). After absorption, flusilazole was cleaved at the triazole ring. With phenyl-labelled test material, the major faecal metabolites identified were [bis(4-fluorophenyl)methyl] silanol, [bis(4-fluorophenyl)methyl] methanol and its fatty acid conjugates, and disiloxane. Except for the fatty acid conjugates, the same metabolites were found in the urine. With triazole-labelled material, the main metabolite identified was 1*H*-1,2,4-triazole, which was found predominantly in the urine (63.8% of the administered dose in males, 51.6% in females); faeces contained only a small amount of the metabolite.

### *Toxicological data*

Flusilazole is moderately to slightly toxic in rats when given as a single oral dose; and minimally toxic to rats and rabbits when administered as a single dose dermally or by inhalation. The oral LD<sub>50</sub> in rats was 672–1216 mg/kg bw, the dermal LD<sub>50</sub> in rabbits was > 2000 mg/kg bw and the inhalation LC<sub>50</sub> in rats was 6.8–7.7 mg/L. Flusilazole was found to be minimally irritating to the eyes and the skin of New Zealand White rabbits. It was practically non-irritating to the skin and was not a dermal sensitizer in guinea-pigs in a Buehler test.

Short- and long-term studies of repeated oral doses of flusilazole in mice (90-day dietary study), rats (90-day studies of gavage and dietary administration) and dogs (90-day and 1-year dietary studies) resulted primarily in lesions of the liver (hepatocellular hypertrophy, fatty change, focal inflammation/necrosis (mouse only) and vacuolation) and urinary bladder (urothelial hyperplasia and vacuolation). In addition, the gastrointestinal tract was a target in dogs. Clinical chemistry was not assessed in the studies in mice, the only finding in the studies in rats was a decrease in cholesterol in both sexes in the 90-day study and increase in cholesterol in females only in the long-term studies. On the basis of the hepatic and/or urinary bladder histopathology, the NOAEL was 75 ppm (equal to 12 mg/kg bw per day) in mice, 125 ppm (equal to 9 mg/kg bw per day) in rats and 20 ppm (equal to 0.7 mg/kg bw per day) in dogs (1-year study). Lymphoid hyperplasia of the gastric mucosa was observed in all treated dogs in the 90-day study, but not in the controls. In the 1-year study, this finding was observed in all dogs, including controls, with severity increasing in a dose-related manner. Effects at the LOAEL in the 1-year study in dogs included hepatocellular hypertrophy, inflammatory infiltration and vacuolation (males only), decreased cholesterol, total protein and albumin, and increased alkaline phosphatase activity and leukocyte counts. A mechanistic study in male dogs at the doses used in the 1-year study indicated that after 28 days of exposure, the effects observed on the liver were adaptive and reversible (weight, increased aspartate aminotransferase activity and cytochrome P450). The dog appeared to be the most sensitive species in these studies, with a NOAEL of 0.7 mg/kg bw per day in the 1-year study, on the basis of histopathology changes in the liver and stomach and changes in clinical chemistry.

After repeated short-term (21-day) dermal application of flusilazole, there was no evidence of any treatment-related systemic toxicity in rabbits given doses of up to 200 mg/kg bw per day.

Flusilazole was tested for genotoxicity in an adequate range of assays *in vitro* and *in vivo*. It was not genotoxic in mammalian or microbial systems. The Meeting concluded that flusilazole was unlikely to be genotoxic.

Two 18-month dietary studies with flusilazole were conducted in mice. In the first study in which flusilazole was administered at concentrations of up to 200 ppm in the diet, the target organs identified were the liver (hepatocellular fatty changes), kidney (decreased weight), and urinary bladder (histopathological change). There was no evidence of carcinogenicity in this study. Concentrations from 100 to 2000 ppm were used in the second 18-month study. Systemic toxicity was observed at all doses. At doses of 500 and 1000 ppm in males (73.1 and 144 mg/kg bw per day, respectively) or 1000 and 2000 ppm in females (200 and 384 mg/kg bw per day, respectively), overt hepatic lesions (increased foci of hepatocellular alteration and hepatocellular hypertrophy with cytoplasmic vesiculation and/or vacuolation) and cellular hyperplasia in the urinary bladder were observed. Increased incidences of liver tumours (hepatocellular adenomas and carcinomas) were observed at concentrations of more than 1000 ppm. Liver tumours occurred at doses in excess of the maximum tolerated dose (MTD) and were preceded at lower concentrations by clear histopathological changes in the liver. The overall NOAEL for systemic toxicity was 25 ppm, equal to 3.4 mg/kg bw per day, on the basis of hepatotoxicity and urinary bladder hyperplasia at 100 ppm (14.3 mg/kg bw per day) in males and hepatocellular fatty changes at 200 ppm (27 mg/kg bw per day) in both sexes. The overall NOAEL for carcinogenicity was 200 ppm (equal to 36 mg/kg bw per day) in females and 1000 ppm (equal to 144 mg/kg bw per day) for males. The incidence of tumours at the NOAEL was within the range for historical controls.

The toxicity and carcinogenicity of flusilazole were investigated in two 2-year studies in rats. The target organs identified were the liver and bladder. The overall NOAEL for systemic toxicity was 50 ppm, equal to 2.0 mg/kg bw per day, on the basis of mild nephrotoxicity (pyelonephritis in females) and hepatotoxicity (hepatocellular hypertrophy in both sexes), acidophilic foci, and diffuse fatty change (females only). There was no treatment-related increase in the incidence of any tumour type AT up to 250 ppm (the highest dose tested in the first study). Concentrations of between 125 and 750 ppm, the latter exceeding the MTD, were used in the second study. Flusilazole was found to be tumorigenic at the highest dose of 750 ppm (30.8 mg/kg bw per day) causing bladder transitional cell neoplasia in both sexes and testicular Leydig cell tumours in males. There was no evidence of any treatment-related increase in tumour incidence at a dietary concentration of 375 ppm. The overall NOAEL for carcinogenicity was 375 ppm (14.8 mg/kg bw per day)

A special 2-week study to investigate the possible mechanism for the induction of testicular Leydig cell tumours was conducted in rats. The results demonstrated that flusilazole caused a dose-dependent lowering of estradiol concentrations at 20 mg/kg bw per day and above, and of serum and interstitial testosterone concentrations at 150 mg/kg bw per day in vivo after subcutaneous exposure ( $n=10$ ) and a dose-related decrease in testosterone and androstenedione production in testicular Leydig cell cultures by inhibition of enzymes involved in steroid biosynthesis in vitro at less than 5  $\mu\text{mol/l}$ . In the 90-day mechanistic study in rats given flusilazole at doses similar to those used in the second long-term study in rats (0, 10, 125, 375 or 750 ppm), there were no changes in serum concentrations of testosterone, estradiol or LH, which would be expected for this mode of action. However, there was appreciable inter-animal variability in the hormone measurements. Overall, the data suggested that flusilazole may induce Leydig cell tumours via an endocrine-related mechanism— inhibition of testosterone and estradiol biosynthesis could contribute to disruption of the hypothalamus–pituitary–testis axis, resulting in over stimulation of the testicular endocrine tissues. Exposure to flusilazole at doses not causing disruption of the hypothalamus–pituitary–testis axis would, therefore, be unlikely to induce an increase in Leydig cell tumours. Although this mode of action is relevant to humans, there was good evidence to suggest that humans are less sensitive to chemically-induced Leydig cell tumours than are rats, owing to differences in sensitivity to LH on the basis of number of Leydig-cell receptors and control of LH-receptor expression (e.g., by prolactin in rodents but not in humans).

The Meeting concluded that the weight of evidence indicated that the mode of action for bladder tumours was via cell injury and regenerative hyperplasia.

In view of the lack of genotoxicity and the finding of hepatocellular tumours in mice and testicular and bladder transitional cell tumours in rats only at doses at which marked toxicity was observed, the Meeting concluded that flusilazole is not likely to pose a carcinogenic risk to humans at dietary levels of exposure.

The effect of flusilazole on reproduction in rats was investigated in two two-generation studies. The first was a part of a 2-year feeding study. No parental toxicity was observed at doses of up to 250 ppm. The same doses were used in the second definitive two-generation study. The NOAEL for parental systemic toxicity was 50 ppm, equal to 4.04 mg/kg bw per day, on the basis of slightly lower body-weight gain in F<sub>1</sub> females. The main reproductive effects at 250 ppm included increased duration of gestation and increased maternal mortality during parturition. The NOAEL for reproductive toxicity was 50 ppm, equal to 3.46 mg/kg bw per day. Toxicity observed in offspring at 250 ppm included a reduced number of live pups per litter and decreased pup growth. The NOAEL for offspring toxicity was 50 ppm, equal to 4.04 mg/kg bw per day.

Nine studies of developmental toxicity were carried out with flusilazole administered orally, of which five were in rats (one dietary study and four with gavage administration) and four (one dietary study and three with gavage administration) in rabbits to characterize potential teratogenicity observed in some studies.

In most of the studies in rats, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and decreased food consumption. In one study, the NOAEL for maternal toxicity was 2 mg/kg bw per day on the basis of increased incidence of red vaginal discharge

during the latter part of gestation and an increase in placental weights at 10 mg/kg bw per day, which was not assessed in the other studies. At maternally toxic doses, specific malformations noted were cleft palate, nares atresia and absent renal papillae. An increased incidence of anomalies (extra cervical ribs, patent ductus arteriosus) was also observed. The incidence of rudimentary cervical ribs was slightly, but not statistically significantly increased at 2 mg/kg bw per day (3 out of 3, 4 out of 4, 9 out of 6, 27 out of 15, and 141 out of 22 fetuses per litter in the groups at 0, 0.5, 2, 10, 50 mg/kg bw per day, respectively). The overall NOAEL for embryo/fetotoxicity was 2 mg/kg on the basis of a higher incidence of skeletal variations (extra cervical ribs) at 10 mg/kg bw per day. No malformations were found at doses of less than 50 mg/kg bw per day.

In four studies of developmental toxicity in rabbits, the NOAEL for maternal and embryo/foetal toxicity was 7 mg/kg bw per day on the basis of clinical signs of toxicity, increased incidence of abortion and total resorption at 15 mg/kg bw per day. There was no evidence for any teratogenic potential in rabbits given flusilazole at doses of up to 15 mg/kg bw per day, the maximum tolerated dose in this study.

A major metabolite identified was 1*H*-1,2,4-triazole, which was found predominantly in the urine (63.8% of the administered dose in males, 51.6% in females). Studies with this metabolite are summarized in the evaluation of difenoconazole in the present report.

No neurotoxic effects were seen during conventional repeat-dose studies with flusilazole.

There were no reports of adverse health effects in manufacturing plant personnel or in operators and workers exposed to flusilazole formulations during their use. Also, there was no evidence or data to support any findings in relation to poisoning with flusilazole.

The Meeting concluded that the existing database on flusilazole was adequate to characterize the potential hazards to foetuses, infants and children.

### Toxicological evaluation

The Meeting established an ADI of 0–0.007 mg/kg bw based on the NOAEL of 0.7 mg/kg bw per day for lymphoid hyperplasia in the gastric mucosa, liver histopathology (hypertrophy, inflammatory infiltration in males and females, and vacuolation in males only), and clinical chemistry (decreased concentrations of cholesterol, total protein and albumin and increased alkaline phosphatase activity and leukocyte counts) in the 1-year dietary study in dogs and a safety factor of 100.

The Meeting established an ARfD of 0.02 mg/kg bw based on the NOAEL of 2 mg/kg bw per day for skeletal anomalies in the study of developmental toxicity in rats treated orally and a safety factor of 100.

A toxicological monograph was prepared.

#### *Levels relevant to risk assessment*

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity <sup>a</sup>	Toxicity	25 ppm, equal to 3.4 mg/kg bw per day	200 ppm, equal to 27 mg/kg bw per day
		Carcinogenicity <sup>d</sup>	200 ppm equal to 36 mg/kg bw per day (females)	1000 ppm equal to 384 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity <sup>a,c</sup>	Toxicity	50 ppm, equal to 2 mg/kg bw per day	250 ppm, equal to 10 mg/kg bw per day
		Carcinogenicity	375 ppm, equal to 14.8 mg/kg bw per day	750 ppm, equal to 30.8 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	Multigeneration reproductive toxicity <sup>a,c</sup>	Parental toxicity	50 ppm, equal to 4.04 mg/kg bw per day	250 ppm, equal to 19.6 mg/kg bw per day
		Offspring toxicity	50 ppm, equal to 4.04 mg/kg bw per day	250 ppm, equal to 19.6 mg/kg bw per day
		Reproduction	50 ppm, equal to 4.04 mg/kg bw per day	250 ppm, equal to 19.6 mg/kg bw per day
	Developmental toxicity <sup>a,b,c</sup>	Maternal toxicity	2 mg/kg bw per day	10 mg/kg bw per day
		Embryo/fetotoxicity	2 mg/kg bw per day	10 mg/kg bw per day
Rabbit	Developmental toxicity <sup>a,b,c</sup>	Maternal toxicity	7 mg/kg bw per day	15 mg/kg bw per day
		Embryo/fetotoxicity	7 mg/kg bw per day	15 mg/kg bw per day
Dog	One-year study of toxicity <sup>a</sup>	Toxicity	20 ppm, equal to 0.7 mg/kg bw per day	75 ppm, equal to 2.4 mg/kg bw per day

<sup>a</sup> Dietary administration.

<sup>c</sup> Two or more studies combined.

<sup>b</sup> Gavage administration.

<sup>d</sup> Greater than the maximum tolerated dose (MTD).

#### *Estimate of acceptable daily intake for humans*

0–0.007 mg/kg bw

#### *Estimate of acute reference dose*

0.02 mg/kg bw

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

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

#### *Critical end-points for setting guidance values for exposure to flusilazole*

##### *Absorption, distribution, excretion and metabolism in mammals*

Rate and extent of oral absorption	Rapid and extensive (up to 80%)
Dermal absorption	Data not available
Distribution	Widely
Potential for accumulation	Low
Rate and extent of excretion	Rapidly excreted
Metabolism in animals	Extensively metabolized
Toxicologically significant compounds in animals, plants and the environment	Parent compound, 1,2,4-triazole

##### *Acute toxicity*

Rat, LD <sub>50</sub> , oral	674 mg/kg bw
Rat, LD <sub>50</sub> , dermal	> 2000 mg/kg bw
Rat, LC <sub>50</sub> , inhalation	2.7–3.7 mg/L, 4 h

Guinea-pig, skin sensitization (test method used)	Non-sensitizing (Buehler)		
<i>Short-term studies of toxicity</i>			
Target/critical effect	Liver and urinary bladder		
Lowest relevant oral NOAEL	0.7 mg/kg bw per day (1-year study in dogs)		
Lowest relevant dermal NOAEL	5 mg/kg bw per day (21-day study in rabbits)		
Lowest relevant inhalation NOAEC	No data presented		
<i>Genotoxicity</i>			
	Not genotoxic		
<i>Long-term studies of toxicity and carcinogenicity</i>			
Target/critical effect	Liver and bladder		
Lowest relevant NOAEL	2.0 mg/kg bw per day (2-year study in rats)		
Carcinogenicity	No carcinogenic concern at levels of dietary exposure		
<i>Reproductive toxicity</i>			
Reproduction target/critical effect	Increased gestation length, reduced live born pups/litter and decreased pup growth		
Lowest relevant reproductive NOAEL	50 ppm (4.04 mg/kg bw per day)		
Developmental target/critical effect	Skeletal anomalies, malformations at higher doses		
Lowest relevant developmental NOAEL	2 mg/kg bw per day (rats)		
<i>Neurotoxicity/delayed neurotoxicity</i>			
	No indications of neurotoxicity in studies of acute toxicity or repeated doses		
<i>Other toxicological studies</i>			
Mechanistic studies	Disruption of the hypothalamus–pituitary–testis axis Necrosis and hyperplasia in the rat bladder		
<i>Medical data</i>			
	No occupational or accidental poisoning reported		
<b>Summary</b>			
	<b>Value</b>	<b>Study</b>	<b>Safety factor</b>
ADI	0–0.007 mg/kg bw	Dog, 1-year study	100
ARfD	0.02 mg/kg bw	Rat, study of developmental toxicity	100

### RESIDUE AND ANALYTICAL ASPECTS

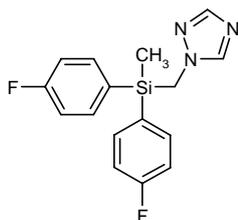
Flusilazole is a fungicide belonging to the ergosterol biosynthesis inhibitor class. It was evaluated by the JMPR for residues in 1989, 1990, 1991 and 1993. Toxicology was reviewed in 1989 and 1995, establishing an ADI of 0–0.001 mg/kg bw in 1989 (confirmed in 1995). Flusilazole was listed for the Periodic Re-Evaluation Programme at the 38<sup>th</sup> Session of the CCPR for periodic review by the 2007 JMPR for toxicology and residues.

#### **Chemical name:**

Flusilazole

Bis(4-fluorophenyl)(methyl)(1H-1,2,4-triazol-1-ylmethyl)silane (IUPAC)

1-[ [Bis(4-fluorophenyl)methyl)silyl]methyl]-1H-1,2,4-triazole (CA)



### ***Animal metabolism***

The Meeting received results of animal metabolism studies in rats, lactating goats, and laying hens.

Flusilazole is extensively metabolized in rats. Eight metabolites were identified. In addition to unchanged flusilazole, the major metabolites identified in urine and faecal samples were [bis(4-fluorophenyl)methyl]silanol (IN-F7321); [bis(4-fluorophenyl)methylsilyl]methanol (IN-H7169) and its glucuronide; 1H-1,2,4-triazole (IN-H9933); and 1,3-dimethyl-1,1,3,3-tetrakis(4-fluorophenyl)disiloxane (IN-G7072). Cleavage and rapid excretion of IN-H9933 (1H-1,2,4 triazole) was the primary step in the metabolism of flusilazole in rats. The silane molecule may then be excreted or further metabolized to fatty acid metabolites,  $\beta$ -D-gluco-pyranuronic acid conjugate and further degrade to more polar molecules.

Two lactating goats received daily doses of [ $^{14}$ C] labelled flusilazole orally by gelatine capsule at a level equivalent to 50 ppm in their diet. One lactating goat was dosed daily for 6 consecutive days (phenyl label) and one goat was dosed for 5 consecutive days (triazole label) with 50 mg of [ $^{14}$ C]-labelled flusilazole. For the goat dosed with phenyl-labelled flusilazole, muscle contained 0.05–0.07% of the total dose (0.41–0.70 mg/kg flusilazole equivalents); liver accounted for 5.3% (13.5 mg/kg); kidney had 1.2% of the dose (8.7 mg/kg); and fat contained 0.15–0.50% of the total administered dose (4.07–5.15 mg/kg). For the goat dosed with triazole-labelled flusilazole, muscle contained 0.10–0.15% of the total dose (0.52–0.53 mg/kg parent equivalents); liver accounted for 1.5% (3.5 mg/kg); kidney had 0.05% of the dose (0.75 mg/kg); and fat contained 0.01–0.07% of the total administered dose (0.15–0.94 mg/kg).

The transfer of radioactive residues to milk and tissues was low. Only 0.34 and 1.3% of the dose was present in milk for the phenyl and triazole labels, respectively. After 6 and 5 consecutive days of dosing, concentrations of total residues in milk were only 0.74 and 0.63 mg/kg (flusilazole equivalents) for the phenyl- and triazole-dosed goats, respectively. Residue levels in milk reached a plateau 2–5 days after the initial dose.

Percentages of extractable radioactivity varied from 89 to > 99% of the total radioactivity in the tissues for the goat dosed with phenyl-labelled flusilazole, and 90 to 94% for the goat dosed with triazole-labelled flusilazole. Flusilazole was extensively metabolized. Cleavage between the triazole and the silicon moieties was the predominant early metabolic transformation, followed by glucuronidation of one of the products. Except in the liver, unchanged flusilazole accounted for less than 10% of the tissue radioactivity. IN-F7321 (silanol) and IN-H9933 (1H-1,2,4-triazole) were the major metabolites found in tissues of goats dosed with phenyl- and triazole-labelled flusilazole, respectively.

In milk, unchanged flusilazole varied between 13–30% of TRR for the goat dosed with phenyl-labelled flusilazole, and < 1–13% for the goat dosed with triazole-labelled flusilazole. In the latter, metabolite IN-H9933 (1H-1,2,4-triazole) accounted for 87 to > 99% of the TRR in milk, which represented 0.16–0.30% of the administered dose. Metabolites IN-F7321 (silanol) and IN-G7072 (disiloxane) together accounted for 34 to 63% of the TRR in milk from the goat dosed with phenyl-labelled flusilazole. Polar material accounted for 7 to 28% of the radiolabel present in the milk of the goat dosed with phenyl-labelled flusilazole.

Laying hens were administered flusilazole ( $[^{14}\text{C}]$ -labelled at either the phenyl group or at the triazole group) at 0.36 or 18 mg/day, equivalent to 3 and 150 ppm in the diet. Hens from the low dose group were dosed for 14 days while those from the exaggerated dose group were dosed for 5 days (the higher dose served for metabolite isolation and identification only). Eggs and excreta were collected over the experimental period; edible tissues and blood were taken for analysis at sacrifice (approximately 6 h after the last dose).

Approximately 80% of the total radioactivity (both labels) was eliminated in the excreta. Elimination of radioactivity in the excreta became steady after 48 h. Residues in edible tissues were low, less than 1% of administered dose; thus bioaccumulation potential for flusilazole residues is low.

In hens receiving phenyl-labelled flusilazole at 0.36 mg/day dose level, highest residues were found in the liver (0.60 mg/kg flusilazole equivalents), followed by fat (0.52 mg/kg) and kidney (0.32 mg/kg). Residue levels in the muscle were the lowest. Residue levels in the hens dosed with triazole-labelled flusilazole were highest and essentially equal in whole blood (0.39 mg/kg), liver (0.38 mg/kg), kidney (0.38 mg/kg), and breast muscle (0.35 mg/kg) and much lower in fat (0.07 mg/kg). In eggs from hens dosed at 3 ppm for 14 days, radioactivity reached a steady state after about 8 days with a plateau residue level of approximately 0.2 mg flusilazole equivalents/kg (both labels).

[(4-Fluorophenyl)methyl]silanediol (IN-V5771) was the main metabolite in liver, kidney and muscle of hens dosed with phenyl-labelled flusilazole (33, 29 and 73–88% of the TRR, respectively). IN-F7321 (silanol) was the main residue in the fat (82% of the TRR) and a major one in the liver (17% of the TRR). Residues identified in the hens dosed with triazole-labelled flusilazole were IN-H9933 (1H-1,2,4-triazole), thymine and flusilazole, with IN-H9933 being the major metabolite in all tissues (76, 79 and 75–83% of the TRR in liver, kidney and muscle, respectively). 1H-1,2,4-triazole residues ranged from 0.057 mg triazole/kg in liver to non-detectable levels in fat. Flusilazole levels ranged from 0.018 mg/kg in kidney to 0.049 mg/kg in fat. No flusilazole was detected in muscle.

In eggs, the two major metabolites from the phenyl label dosed hens were IN-F7321 (silanol) and IN-V5771 (silanediol), at 32 and 38% of the TRR, respectively at 12 days. The major metabolite in eggs from the triazole label dosed hens was 1H-1,2,4-triazole (IN-H9933), with much smaller amounts of thymine and unchanged flusilazole. At 12 days, triazole, thymine and flusilazole residues were 0.197, 0.023 and 0.006 mg flusilazole equivalents/kg, respectively (77, 9 and 2% of the TRR, respectively). When calculated on a molar equivalent basis, the triazole and thymine residues were 0.043 and 0.009 mg/kg in the 12 day egg samples.

The residues found in goats and hens indicated a similar metabolic pathway to the rat. Generally, unchanged flusilazole was present at levels lower than the metabolites. In goat liver and chicken fat of animals dosed with triazole-labelled flusilazole, flusilazole levels were higher than levels of the metabolite 1,2,4-triazole, (IN-H9933) perhaps due to the polar nature of the triazole. Except in goat liver and chicken fat, 1,2,4-triazole was the major metabolite arising from triazole-labelled flusilazole. The silanol metabolite (IN-F7321) was also common to both goats and hens. The main difference between the goat study and the hen studies was the occurrence of the silanediol (IN-V5771) as a major metabolite in hens. Other phenyl-labelled metabolites, resulting from hydroxylation and conjugation reactions, were present at relatively low levels in chicken tissues and eggs.

Based on the results of the submitted studies, the Meeting concluded that, in rats, goats, and hens, flusilazole was rapidly and extensively converted to polar metabolites.

### ***Plant metabolism***

The Meeting received plant metabolism studies for flusilazole in wheat, sugar beet, apples, grapes, bananas and peanuts. The wheat, bananas and sugar beet were greenhouse grown. The grapes, apples and peanuts were grown in the field.

Wheat was treated with [phenyl(U)- $^{14}\text{C}$ ]flusilazole and [triazole-3- $^{14}\text{C}$ ]flusilazole at 200 g ai/ha. In forage, labelled residues (expressed as flusilazole) fell from initial values of 32 and

8.6 mg/kg for phenyl and triazole labels, respectively, to approximately 6 mg/kg by days 5 to 12. Flusilazole accounted for 56–59% of the residue in forage for days 5–12. Residues in straw were 8.6 and 7.9 mg/kg for phenyl and triazole labels, respectively. Unchanged flusilazole accounted for only about 14% of the residue in mature straw, and there was extensive metabolism to at least seven phenyl-labelled and six triazole-labelled metabolites. No single straw or forage metabolite accounted for more than 13.5% of the total radioactivity present. Unidentified minor metabolites were present in triazole and phenyl [ $^{14}\text{C}$ ]flusilazole treated wheat straw; however, no unidentified metabolites exceeded 4% of the total radioactive residue.

There were negligible radioactive residues (0.01 mg/kg) in the grain from phenyl-labelled wheat. In the triazole-labelled wheat, grain residues of 4.4 mg/kg flusilazole equivalents (at 52 days after the treatment) were comprised of triazolyl alanine (IN-V9462) and triazole acetic acid (IN-D8722). No flusilazole was found in triazole-labelled grain samples harvested 69 days after the treatment. This data indicates that although metabolites containing the triazole ring can be translocated, intact flusilazole is not translocated to grain.

The metabolic pathway of flusilazole in wheat included hydroxylations, conjugations, and cleavage of the silicon-methylene bond. The major phenyl-labelled metabolites in straw and forage were glucose-6-phosphate of IN-37722 (2-fluoro-5-[(4-fluorophenyl) (methyl) (1H-1,2,4-triazol-1-ylmethyl)silyl]phenol); mono[6-deoxy-2-O-[2-fluoro-5-[(4-fluorophenyl) (methyl) (1H-1,2,4-triazol-1-ylmethyl) silyl] phenyl]- $\beta$ -D-glucopyranos-6-yl] propanedioate (IN-37735); a conjugate of IN-37738 (2-fluoro-5-[(4-fluorophenyl) (hydroxy) (methyl) silyl] phenol) ; and [bis(4-fluorophenyl)methyl] silanol (IN-F7321). The major triazole-labelled metabolites were triazolyl alanine (IN-V9462); triazole acetic acid (IN-D8722); the glucose-6-phosphate of IN-37722; IN-37735; and IN-37722. Triazolyl alanine and triazole acetic acid accounted for 69 and 24% of the radioactivity in the grain, respectively.

The leaves and detached unpeeled green fruits of immature banana plants growing under greenhouse conditions were treated directly with phenyl- or triazole- labelled flusilazole, each formulated as an emulsifiable concentrate and diluted to a final concentration six times the label rate. The bananas were analysed at intervals of 0, 2, 4, 7, and 11 days and the leaves were analysed at intervals of 0, 7, 14, and 18 days. Autoradiographs showed that flusilazole applied to banana leaves did not translocate from the treated areas. In the case of banana fruit, flusilazole distribution from the peel to the pulp was negligible since 98–99% of the radioactivity applied to the peel remained in the washings and peel. Intact flusilazole accounted for more than 87% of the radioactivity in the peel rinses and peels.

Sugar beets were treated post-emergence with either phenyl- or triazole-labelled flusilazole as an over the top spray at application rates of 124–131 g ai/ha (three times at 14-day intervals). The sugar beets were harvested at 0, 14, 28, and 59 or 77 days (maturity). At each sampling interval, radioactive residues were consistently higher in the foliage than in the roots. Immediately after the third treatment, total radioactive residues in the foliage ranged between 1.5 and 7.2 mg/kg for triazole- and phenyl-labelled flusilazole, respectively. At each sampling interval, total radioactive residues in the roots were lower for the phenyl-treated plants (0.008 mg/kg maximum) than for the triazole-treated plants (0.15 mg/kg maximum). With time, the total radioactive residues in both the foliage and roots decreased.

Flusilazole was the major residue in the foliage, accounting for a maximum of 89% of the total radioactivity present in the foliage. Minor metabolites found included 1,3-dimethyl-1,1,3,3-tetrakis(4-fluorophenyl) disiloxane (IN-G7072) and 2-fluoro-5-[(4-fluorophenyl) (methyl) (1H-1,2,4-triazol-1-ylmethyl)silyl]phenol (IN-37722). No flusilazole was detected in root extracts. Other residues in the foliage and roots consisted of polar materials that were not resolved by HPLC.

Grape vines (separate branches of foliage and grapes) were treated with phenyl- or triazole-labelled flusilazole under field conditions. The berries were harvested 41 days after the application. Flusilazole was the predominant residue, extracted from grape berries, treated with either the phenyl-labelled or triazole-labelled compounds, comprising between 57 and 31% of the recovered radioactivity, respectively. The principal degradation product from phenyl-labelled flusilazole was

[bis(4-fluorophenyl)methylsilyl] methanol (IN-H7169), accounting for 11% of the residue. Four identified minor metabolites containing the phenyl label together accounted for < 10% of the recovered radioactivity. Those four minor metabolites included [bis(4-fluorophenyl)methyl] silanol (IN-F7321); [(4-fluorophenyl)methyl]silanediol (IN-V5571); bis(4-fluorophenyl) (1H-1,2,4-triazol-1-yl)silanol (IN-A7634); and bis(4-fluorophenyl)silanediol (IN-T7866). In addition to flusilazole, triazolyl alanine (IN-V9462) was a major degradation product in triazole-labelled grape berries, accounting 30% of the total radioactivity. Unextractable residues from fruit accounted for between 5 and 14% of the recovered radioactivity.

Apple trees were treated four times at 14-day interval with either phenyl- or triazole-labelled flusilazole at rates of approximately 8 mg/100 mL. Mature fruit were harvested 14 days after the final application. Flusilazole was the predominant residue extracted from apple fruit treated with either phenyl-labelled or triazole-labelled compounds, comprising between 71 and 48% of the recovered radioactivity, respectively. Three identified minor metabolites containing the phenyl label (IN-F7321, IN-V5571, and IN-H7169) together accounted for approximately 11% of the recovered radioactivity. Triazolyl alanine (IN-V9462) was a significant triazole-containing metabolite, accounting for 22% of TRR. Unextractable residues from the apple fruit accounted for between 8 and 14% of the recovered radioactivity.

Peanuts were treated with [phenyl (U)-<sup>14</sup>C]flusilazole applied to the foliage at 140 g ai/ha, 52 days prior to harvest. Peanuts (nut and shells) were harvested at 52 days (maturity). Total radioactive residues in the foliage of peanut plants declined from 3.4 mg/kg at day 0 to 0.38 mg/kg at day 52. There was no significant translocation of phenyl-labelled metabolites to the peanut seed (total residue in the seed was 0.018 mg/kg) or peanut shell (0.03 mg/kg). Flusilazole was the major residue in the foliage at all sampling intervals, declining from 3.2 mg/kg at day 0 to 0.19 mg/kg at day 52. Flusilazole at 0.006 mg/kg and “water soluble metabolites,” also at 0.006 mg/kg, were present in the seed with the remaining residue unextractable.

Based on the results of the submitted studies on wheat, apples, grapes and sugar beets, the Meeting concluded that qualitatively similar metabolism occurred among these crops. The metabolic pathway of flusilazole in plants involves hydroxylations, conjugations, and cleavage between the silicon and the triazole ring. As the interval between treatment and sampling increases, the residues of unchanged flusilazole decreased and the metabolism and conjugation increased.

Due to the extensive degradation of flusilazole by multiple mechanisms to many minor metabolites, there are no major flusilazole metabolites in plants, other than triazolyl alanine. With the exception of triazolyl alanine and triazole acetic acid, individual metabolites generally account for less than 14% of the total radioactivity in the plants.

### ***Environmental fate***

#### *Soil*

The Meeting received information on aerobic and anaerobic degradation of flusilazole in soil; photolysis on soil surface; mobility in soil; field dissipation studies; and flusilazole residues in rotational crops.

The aerobic degradation of [phenyl(U)-<sup>14</sup>C] and [triazole-3-<sup>14</sup>C] flusilazole was studied in two soils (sandy and silt loam soils) incubated in the dark at 25 °C for 1 year.

The primary route of degradation in non-sterile soils was cleavage of the methylene-silicon bond to form IN-F7321 (silanol) which was found < 5% of applied radioactivity after one year and IN-H9933 (triazole) which was not detected.

The anaerobic degradation of [phenyl(U)-<sup>14</sup>C] and [triazole-3-<sup>14</sup>C]-flusilazole was studied in two pond water/sediment systems (silt loam and a sand) under anaerobic conditions at 25 °C at a nominal concentration of 1.0 mg/kg sediment.

The major radiolabelled metabolite (found at 2% of the applied radioactivity) was identified as bis(4 fluoro-phenyl)methyl silanol (IN-F7321).

The photodegradation of flusilazole was studied using silt loam soils under artificial and natural sunlight. No significant degradation was observed in the studies. Under the artificial sunlight conditions, the observed half-life was greater than 30 days. Under the natural sunlight conditions, flusilazole degraded slowly with a  $DT_{50}$  of about 97 days. Based on these results, the Meeting concluded that photolysis on soil is not an important mode of degradation for flusilazole.

Field dissipation studies on bare soil and cropped soils were performed in the United States, Canada and Europe.

The studies showed substantial metabolism of flusilazole with the majority of the applied radioactivity found near the top of the soil (5–15 cm). The major metabolite was the silanol (IN-F7321) which was present at no more than 14% of the applied radioactivity while the triazole metabolites reached a maximum of < 3%. In all studies, very limited mobility was observed. The  $DT_{50}$  values ranged from 71–755 days. However, the residue in soil remained low after multiple applications, and the soil residues continued to decline after application of flusilazole was discontinued.

A field study designed to measure the potential for off-target movement of flusilazole into water-bodies adjacent to orchards showed low to undetectable levels of flusilazole detected in water and sediments adjacent to orchards. The study concluded that environmental exposure to non-target areas would be extremely low under normal use conditions.

A similar pattern was seen in the presence of a wide range of crops (e.g., cereals, oilseed rape and sugar beets). Soil samples of flusilazole remained low (< 0.09 mg/kg) even after a six year accumulation study (up to 3 kg flusilazole applied) and continued to decline after discontinuation of application. No accumulation was seen in soil or crops when used according to recommended use rates. Based on these results, the Meeting concluded that there is a little potential for flusilazole accumulation in soil or crops after multiple years of continuous use.

### ***Residues in rotational crops***

The Meeting received results of two confined [ $^{14}C$ ]flusilazole rotational crop studies. The first study examined the potential for uptake of phenyl-containing residues into four crops (barley, beets, cabbage, and soya beans) from soil (sandy loam) treated with phenyl-labelled flusilazole at rates of 289 or 543 g ai/ha and aged for 30 or 120 days under greenhouse conditions. The second study examined the potential for uptake of phenyl- or triazole-containing residues into three crops (cabbage, wheat and beets) from soils (silt loam) treated with phenyl- or triazole-labelled flusilazole at 1129 g ai/ha and then aged for 120 or 360 days in the field.

During both confined rotational crop studies, radioactive residue levels in the soil remained relatively constant during the aging and plant growth periods. Soil residues ranged from 0.04 to 0.12 mg/kg (289 g ai/ha application rate), 0.12 to 0.20 mg/kg (543 g ai/ha application rate) and 0.21 to 0.44 mg/kg (1129 g ai/ha). Flusilazole levels and the percentage of extractable radioactivity decreased with time. Major soil residues included flusilazole and the silanol (IN-F7321).

There was no significant accumulation of residues from either label in cabbage, soya beans or beets in the confined rotation studies. Accumulation did occur in mature small grain fractions of wheat grown in soil treated with [triazole-3- $^{14}C$ ]flusilazole. Parts of matured wheat grown in 360-day aged soil contained phenyl and triazole labelled residues, respectively: chaff 0.60–9.5 mg/kg, straw 1.4–7.9 mg/kg and grain 0.081–17.5 mg/kg. The extent of accumulation was similar in comparable samples from all aging periods. A major wheat metabolite was triazolyl alanine with flusilazole comprising < 20% of the radioactivity in the wheat grain or straw. This suggests that a triazole-containing fragment, rather than intact flusilazole, translocates from soil into wheat.

The Meeting concluded that there is no significant uptake of flusilazole into rotational (succeeding) crops, except cereal grains.

### ***Methods of Analysis***

The Meeting received description and validation data for analytical methods for flusilazole and its important metabolites, mainly [bis(4-fluorophenyl)methyl] silanol (IN-F7321), in samples of plant and animal origin.

The described methods are mostly based on extraction with an organic solvent (usually ethyl acetate or acetone); followed by a partition step, gel permeation chromatography (GPC) clean-up, and often also a silica solid-phase extraction (SPE) clean-up. The determination step employs mainly capillary GC with nitrogen-phosphorus detection (NPD), followed by a mass spectrometric (MS) confirmation, or a single-step GC-MS determination.

The typical LOQ is 0.01 mg/kg for most plant and animal matrices, with mean recoveries typically ranging between 70–120%.

Multiresidue methods, such as the DFG S19, are available for flusilazole.

The Meeting concluded that adequate multi- and single-residue methods exist for both gathering data in supervised trials and other studies and for monitoring and enforcing flusilazole MRLs in samples of plant and animal origin.

### ***Stability of pesticide residues in stored analytical samples***

The Meeting received information on the stability of flusilazole and its silanol IN-F7321 metabolite in freezer-stored samples (at approximately -20 °C) of plant and animal origin, including apples, grapes, wheat grain, wheat straw, oilseed rape (seed and shoots) and bovine matrices (milk, muscle, kidney, liver and fat). Fortified samples were stored up to the following intervals: wheat grain: 40 months; wheat straw: 40 months; apples: 48 months (flusilazole) and 26 months (IN-F7321); grapes: 17 months (flusilazole) and 25 months (IN-F7321); oilseed rape: 14 months (flusilazole only); whole milk: 6 months (flusilazole) and 11 months (IN-F7321); bovine muscle: 6 months (flusilazole) and 15 months (IN-F7321); bovine kidney: 3.5 months (IN-F7321); bovine liver: 6 months (flusilazole) and 14.25 months (IN-F7321); and bovine fat: 6 months (flusilazole) and 16 months (IN-F7321).

No significant degradation of flusilazole and its silanol metabolite IN-F7321 was observed in the tested plant and bovine matrices and storage intervals, with the exception of IN-F7321 in liver (residues remained and corrected for recoveries were 35, 84, and 38% for 1, 3, and 14.25 months of storage, respectively). In the case of 3 month-storage of IN-F7321 in liver, samples were only partially thawed and rapidly refrozen after fortification, whereas the other samples (1 and 14.25 months of storage) were completely thawed and remained in contact with the fortification solution at ambient temperature for at least 30 minutes. The partially thawed and rapidly refrozen samples showed limited degradation, probably due to a much lower rate of enzyme activity at lower temperatures. While this does not directly reflect the stability of incurred residues of IN-F7321 in liver, it emphasises the need, when analysing residues in liver, to ensure that samples are processed expeditiously and are not allowed to remain at elevated temperatures prior to extraction and analysis.

### ***Residue definition***

Flusilazole is extensively metabolized in animals and plants. The major metabolic reaction is cleavage of the Si-CH<sub>2</sub> bond to form silanol and triazole related metabolites.

In plants, there are no predominant metabolites with the exception of triazole alanine and triazole acetic acid. These plant metabolites are produced by all fungicides in the triazole class and are therefore excluded from the definition of the residue for flusilazole.

In ruminants (goats), the most abundant metabolites in tissues and milk were flusilazole, [bis(4-fluorophenyl)methyl] silanol(IN-F7321), and 1H-1,2,4-triazole(IN-H9933). In poultry, metabolites in tissues and eggs were flusilazole, [bis(4-fluorophenyl)methyl]silanol, 1H-1,2,4-triazole, and [(4-fluorophenyl)methyl]silanediol(IN-V771). As 1H-1,2,4-triazole is a common metabolite to all triazole fungicides, it is not deemed suitable as an indicator of flusilazole exposure to ruminants or

hens. The silanediol metabolite is only found in poultry tissues, and is not expected to be detectable at anticipated dietary exposure levels to laying hens.

Based on the above, the Meeting agreed in the following residue definitions:

*Definition of the residue in plant commodities for estimation of dietary intake and for compliance with MRLs: flusilazole*

*Definition of the residue in animal commodities for estimation of dietary intake and for compliance with MRLs: flusilazole plus [bis(4-fluorophenyl)methyl]silanol (IN-F7321)*

The log  $K_{ow}$  is 3.87 (at 20 °C, pH 7), suggesting that flusilazole is fat-soluble. Both in the goat and hen metabolism studies the residues of flusilazole and its silanol, IN-F7321, in muscle was generally less than one-tenth that in the various fat depots. The Meeting concluded that the flusilazole residue is fat soluble.

### **Results of supervised trials on crops**

The Meeting received supervised trials data for flusilazole on apple, pear, apricot, nectarine, peach, grapes, banana, cucumber, sweet corn, soya bean, sugar beet (root and leaves), barley (grain, forage, and straw), rye (grain, forage, and straw), wheat (grain, forage, and straw), maize, rice, rape seed, sunflower seed and oat (forage and fodder).

#### *Pome fruit*

##### *Apple*

The Meeting received results from supervised trials with flusilazole used on apples in southern Europe (Italy, Spain and southern France), Argentina, Canada, India, New Zealand and South Africa.

None of the trials in Argentina, India, or New Zealand were conducted according to the respective GAPs of Argentina (4 applications at 4 g ai/hL with a PHI of 21 days), India (4 g ai/hL with a PHI of 10 days), and New Zealand (3 g ai/hL, up to 6 applications, with a PHI of 35 days).

The critical GAP for the southern European trials conducted in Spain, Italy and southern France is the GAP of Spain that specifies a spray concentration of 4.8 g ai/hL in high-volume applications (more than 1500 L water/ha, i.e., a maximum of  $\geq 72$  g ai/ha), maximum of 4 applications per year, and a PHI of 14 days. Flusilazole residues from ten trials according to the GAP of Spain, in ranked order, were: 0.01, 0.01, 0.02, 0.04(2), 0.05(2), 0.06, 0.12 and 0.13 mg/kg.

Two trials in Canada were conducted according to the GAP of Canada: 40 g ai/ha, maximum of 4 applications, and a PHI of 77 days. Flusilazole residues (at 88 and 130% GAP) were  $< 0.01$  mg/kg (below LOQ of the analytical method used).

The GAP of South Africa specifies a spray concentration of 2.4 g ai/hL in high-volume applications (1500–3500 L water/ha, i.e., 36–84 g ai/ha), 5 applications, and a PHI of 14 days. One trial was conducted according to this GAP. The residue of flusilazole from this trial was 0.06 mg/kg.

##### *Pear*

The Meeting received results from supervised trials with flusilazole used on pears in Italy, South Africa and China.

Trials in Italy were not conducted according to the critical GAP of the southern European region, i.e., that of Spain (the same treatment regime as for apples).

The GAP of South Africa specifies a spray concentration of 1.6 g ai/hL in high-volume applications (1500–3500 L water/ha, i.e., 24–56 g ai/ha), 5 applications, and a PHI of 14 days. Two trials were conducted at a higher application rate of 2 g ai/hL (125% GAP), 6 applications and 2-day longer PHI of 16 days. Flusilazole residues from these trials were 0.02 and 0.03 mg/kg.

Four trials in China were conducted according to the GAP of China (5 g ai/hL, 3 applications, with a PHI of 21 days), with the exception four applications were made instead of three. Flusilazole residues from these trials were: 0.01, 0.02, 0.03, and 0.13 mg/kg.

The Meeting agreed that the data on apples from southern Europe and South Africa and on pears from China appear to be from similar populations and could be used to support a “pome fruit” commodity group maximum residue level. Pome fruit is registered for use in New Zealand. Flusilazole residues in pome fruit, in ranked order, were: 0.01(3), 0.02(2), 0.03, 0.03, 0.04(2), 0.05(2), 0.06(2), 0.12 and 0.13(2) mg/kg. The Meeting estimated a maximum residue level for pome fruit of 0.3 mg/kg to replace the previous recommendation of 0.2 mg/kg, an STMR value of 0.04 mg/kg, and an HR value of 0.13 mg/kg.

#### *Apricot, nectarine and peach*

The Meeting received results from supervised trials with flusilazole used on apricots in France, on peaches in southern Europe (Greece, Italy, Spain, and southern France) and on peaches and nectarines in New Zealand.

The GAP of New Zealand for stone fruit (4 g ai/hL) does not specify a PHI. The label states that the product should not be applied after the start of shuck fall, which should be 86–113 days before harvest for most peach and nectarine cultivars. Nine trials were reported (three on peach and six on nectarine). The spray concentrations in these trials were 5, 10 and 20 g ai/hL, with very long PHIs of 91–113 days. All flusilazole residues from these trials were < 0.01 mg/kg (below LOQ of the analytical method used).

The critical GAP for the southern European trials conducted on peach in Spain, Greece, Italy and southern France is the GAP of Spain that specifies a spray concentration of 5 g ai/hL, maximum of 3 applications per year, and a PHI of 7 days. Flusilazole residues from twelve trials according to the GAP of Spain, in ranked order, were: 0.03, 0.04, 0.05(4), 0.06, 0.07, 0.08, 0.09 and 0.10 mg/kg.

The GAP of France for apricot (4 g ai/hL) does not specify a PHI. The critical GAP in the region is the GAP of Spain (5 g ai/hL, maximum of 2 applications per year, and a PHI of 7 days). Three apricot trials were conducted with a PHI of 7, one with 4 g ai/hL (8 applications) and two with 14 g ai/hL (4 and 6 applications). Flusilazole residues from these trials were 0.08, 0.05 and 0.06, respectively.

The critical GAP for the southern European trials (the GAP of Spain) is the same for peach and nectarine. The critical GAP for apricot (the GAP of Spain) specifies the same spray concentration and PHI as for peach and nectarine, with maximum of two applications instead of three. Flusilazole residues for apricot (a smaller fruit than peach) fell within the range of residues obtained for peach, even though exaggerated spray concentration (280% GAP) and/or significantly higher number of applications were used.

The Meeting decided to use the residue data from the eleven trials on peach in southern Europe to estimate a maximum residue level of 0.2 mg/kg for apricot, nectarine and peach to replace the previous recommendation of 0.5 mg/kg. The Meeting also estimated an STMR value of 0.05 mg/kg, and an HR value of 0.10 mg/kg for apricot, nectarine and peach.

#### *Grapes*

The Meeting received results from supervised trials with flusilazole on grapes in southern Europe (Greece, Italy, Portugal, Spain and southern France), Germany, Australia, China, India and South Africa.

None of the trials in India and South Africa were conducted according to the respective GAPs of India (4 g ai/hL with a PHI of 15 days) or South Africa (5 g ai/hL with a PHI of 21 days).

Flusilazole is not registered for use on grapes in Germany but it is registered in France, Switzerland and the Czech Republic. The GAPs of France and Switzerland do not specify a PHI. The

GAP of the Czech Republic for grapes specifies 30 g ai/ha (spray volume 1000 L water/ha, i.e., 3 g ai/hL), spraying interval 7–14 days (number of applications not specified), and a PHI of 42 days.

Five trials in Germany were conducted with a 42-day PHI and 32–36 g ai/ha (106–120% of GAP). Flusilazole residues in these trials were: 0.02, 0.03, 0.04, 0.10 and 0.11 mg/kg.

The GAP of Australia specifies maximum of 3 applications at 2 g ai/hL or 20 g ai/ha with a PHI of 14 days. In one trial in Australia, flusilazole was applied as a single application with 2 g ai/hL and a PHI of 14 days. Flusilazole residue from that trial was 0.11 mg/kg.

The critical GAP for the southern European trials conducted on grapes in Spain, Portugal, Greece, Italy and southern France is the GAP of Spain, specifying a spray concentration of 5 g ai/hL, a maximum of 5 applications and a PHI of 14 days. Flusilazole residues from eight trials according to the GAP of Spain (with 5–6 applications), in ranked order (median underlined), were: 0.01(2), 0.02(2), 0.03, 0.04, 0.10, and 0.11 mg/kg. One trial with a PHI of 15 days was also included as a higher residue of 0.11 mg/kg was recorded than from trials with a 14-day PHI.

The GAP of China specifies a spray concentration of 5 g ai/hL and 3 applications but does not specify a PHI or growth stage and could not be evaluated.

The Meeting decided to use the residue data from southern Europe and Germany to estimate a maximum residue level for grapes. Residues from these trials in ranked order were: 0.01(2), 0.02(3), 0.03(2), 0.04(2), 0.10(2) and 0.11(2) mg/kg. The Meeting estimated a maximum residue level of 0.2 mg/kg to replace the previous recommendation of 0.5 mg/kg, an STMR value of 0.03 mg/kg, and an HR value of 0.11 mg/kg.

### *Banana*

The Meeting received results from supervised trials with flusilazole used on bananas in the Caribbean Basin (Belize, Costa Rica, Guatemala, Honduras, Jamaica and West Indies, including Guadeloupe, Martinique and St. Lucia). The Meeting considered the GAP of Columbia (100 g ai/ha, 4–6 applications, and a PHI of 1 day) as the critical GAP for the evaluation of the submitted trials.

In the eleven submitted trials, bananas were treated with flusilazole at 100 g ai/ha (4–7 applications) using aerial application to bagged bunches with washing (normal practice) or without washing at the harvest. With a PHI of 1 day, flusilazole residues in the pulp were < 0.01 mg/kg for all the trials. With the same PHI, flusilazole residues in the peel of washed bananas (three trials) were: < 0.01 (2) and 0.01 mg/kg. Residues in the peel of unwashed bananas (eight trials) were: < 0.01 (3), 0.011, 0.012, 0.013, 0.017 and 0.02 mg/kg. Flusilazole was not analysed in the whole fruit.

Based on data in the published literature<sup>36</sup> an average pulp to peel ratio for bananas at harvest is 1.82. Assuming this ratio and combining the results from washed and unwashed bananas, flusilazole residues in whole fruit, in ranked order, were: < 0.01 (5) and 0.01 (6) mg/kg.

The Meeting estimated a maximum residue level for flusilazole in banana (whole fruit) of 0.03 mg/kg to replace the previous recommendation of 0.1 mg/kg. Based on the pulp data, the Meeting estimated an STMR value of 0.01 mg/kg and an HR value (for pulp) of 0.01 mg/kg for banana pulp.

### *Cucumber*

Flusilazole is registered for foliar application on cucumber in China (5 g ai/hL, 3 applications, a PHI of 7 days) and Korea (2.5 g ai/hL, 3 applications, and a PHI of 3 days). The Meeting received results from supervised trials with flusilazole on cucumber in China. None of the trials were conducted according to the GAPs of China or Korea. Therefore, the Meeting could not estimate a maximum residue level for flusilazole in cucumber.

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<sup>36</sup> Stover, R.H. and Simmonds, N.W., 1987, Bananas. Tropical Agriculture Series, Longman Scientific & Technical, 468 pp

*Sweet corn*

The Meeting received results from supervised trials with flusilazole on sweet corn in France and South Africa.

The GAP of France (200 g ai/ha, 2 applications) does not specify a PHI. The six reported trials on sweet corn in France were conducted as a single application at 200–420 g ai/ha (100–210% GAP) with PHIs of 10–31 days. Flusilazole residues in sweet corn kernels were < 0.01 mg/kg in all these trials.

The GAP of South Africa specifies a maximum of 2 applications at 125 g ai/ha with a PHI of 14 days. One trial conducted in South Africa at 250 g ai/ha (200% GAP) with 2 applications, resulted in flusilazole residues in cobs < 0.01 mg/kg for both tested PHIs of 0 and 14 days.

The Meeting estimated a maximum residue level for flusilazole in sweet corn (corn-on-the-cob) of 0.01\* mg/kg an STMR value of 0.01 mg/kg and an HR value of 0.01 mg/kg.

*Soya beans (dry)*

The Meeting received results from supervised trials with flusilazole used on soya beans in Argentina, Canada, France, South Africa and the United States. The trials in France could not be evaluated because there is no GAP for soya beans in Europe.

None of the six trials reported from Argentina were conducted according to the GAP of Argentina (100 g ai/ha, 2 applications, and a PHI of 35 days). In three of these trials, a single application rate of 200 g ai/ha (200% GAP) resulted in flusilazole residues < 0.005 mg/kg (below LOQ of the used analytical method) 38–60 days after the application.

The critical GAP of South Africa specifies 125 g ai/ha for aerial application or 100 g ai/ha for ground application, maximum of 2 applications, and a PHI of 30 days. Four trials in South Africa were conducted with a PHI of 34 days and using either 75 or 150 g ai/ha in two applications (75 or 150% GAP assuming ground application). Flusilazole residues were < 0.005 mg/kg (below LOD of the used analytical method) in all these trials at 34 days.

The GAP of the United States specifies 116 g ai/ha, 2 applications, and a PHI of 30 days. Twenty-one trials in the United States and two trial in Canada were conducted at 103–109% of the GAP rate, resulting in flusilazole residues of < 0.01(3), 0.01(8), 0.02(9) and 0.03(3) mg/kg.

Based on the residues obtained in the trials in the United States and Canada, the Meeting estimated a maximum residue level for flusilazole in soya beans (dry) of 0.05 mg/kg, an STMR value of 0.02 mg/kg and an HR value of 0.03 mg/kg.

*Sugar beet (root)*

The Meeting received results from supervised trials with flusilazole used on sugar beet in southern Europe (Greece, Italy, and Spain) and in northern Europe (Belgium, Denmark, northern France, Germany, the Netherlands and the United Kingdom).

Five trials in southern Europe (two in Greece, two in Italy, and one in Spain) were conducted according to the GAP of Greece (80 g ai/ha, 3 applications, and a PHI of 15 days). The Meeting noted that there were four other trials conducted in southern Europe with a shorter PHI of 14 days (3 or 6 applications) or a higher application rate (132.5% GAP) that resulted in flusilazole residues of < 0.01 mg/kg. Thus, results of these trials were also included. Flusilazole residues in sugar beet root, in ranked order, were: < 0.01 (6), and 0.01 (3) mg/kg.

Sixteen trials in northern Europe (ten in Germany, two in the UK, and one in Belgium, Denmark, the Netherlands and northern France) were conducted according to the GAP of Germany (150 g ai/ha, 2 applications, a PHI of 42 days). Among these trials, one trial in northern France had only a 35-day PHI but the flusilazole residue was < 0.01 mg/kg. Flusilazole residues in sugar beet root, in ranked order, were: < 0.01 (11), 0.01(2), 0.02, < 0.03, and 0.03 mg/kg.

The Meeting noted that the residues obtained in southern and northern Europe were from similar populations and agreed to combine the results. Flusilazole residues in sugar beet root, in ranked order, were:  $< 0.01$  (17),  $0.01$  (5), 0.02,  $< 0.03$ , and 0.03 mg/kg.

The Meeting estimated a maximum residue level for flusilazole in sugar beet root of 0.05 mg/kg to replace the previous recommendation of  $0.01^*$  mg/kg, an STMR value of 0.01 mg/kg, and a highest residue value of 0.03 mg/kg.

### *Cereal grains*

#### *Barley*

The Meeting received information on flusilazole residues in barley grains from supervised trials in Germany, the United Kingdom and South Africa.

The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 51 and a PHI of 42 days. Twelve trials in Germany on winter barley were conducted at 160–221 g ai/ha (80–111% GAP), 2 applications, with the growth stage at the last application of BBCH 51 (PHI of 57–86 days). Flusilazole residues, in ranked order, were:  $< 0.01$  (3), 0.02, 0.03, 0.04 (2), 0.05, 0.06, 0.07 (2), and 0.08 mg/kg.

The critical GAPs of the United Kingdom specify 156–160 g ai/ha, 1 application, and the BBCH 71 or 73 (watery ripe stage or early milk stage, respectively) growth stage at the last application. Four trials in the United Kingdom on spring barley were conducted at 160 g ai/ha, 2 applications, and the BBCH 71 growth stage at the last application. Flusilazole residues, in ranked order, were:  $< 0.01$  (2), 0.06, and 0.07 mg/kg.

The critical GAP of South Africa specifies 112.5 g ai/ha (aerial application) or 100 g ai/ha (ground application), 1–2 applications and a PHI of 56 days. One trial in South Africa was conducted as a single application at 125 g ai/ha with a PHI of 56 days (application method and spray volume were not specified). Flusilazole residue from this trial was  $< 0.02$  mg/kg.

The Meeting noted that the residues obtained in Germany, the United Kingdom and South Africa were from similar populations and agreed to combine the results. Flusilazole residues in barley grain, in ranked order, were:  $< 0.01$  (5),  $< 0.02$ , 0.02, 0.03,  $0.04$  (2), 0.05, 0.06 (2), 0.07 (3), and 0.08 mg/kg.

#### *Rye*

The Meeting received information on flusilazole residues in rye grains from supervised trials in winter rye Germany. The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 55. The Meeting noted that the growth stages (BBCH of 49, 65, 69, or 72) at the last application in the trials did not match the GAP specification. Two trials were conducted at 100–130% of the GAP rate, with 3 applications and a PHI of 42 days (BBCH 65). One additional trial resulted in a higher flusilazole residue at a PHI of 48 days vs. 35 days (BBCH 69). Flusilazole residues obtained in these trials were: 0.04 (2), and 0.05 mg/kg.

#### *Wheat*

The Meeting received information on flusilazole residues in wheat grains from supervised trials in Germany, Spain, the United Kingdom and South Africa.

None of the trials in South Africa were conducted according to the critical GAP of South Africa: 112.5 g ai/ha (aerial application) or 100 g ai/ha (ground application), 1–2 applications and a PHI of 56 days.

The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 55, and a PHI of 42 days. The Meeting agreed that the growth stage at the last application is a better indication of the GAP than the PHI. Three trials in Germany were conducted at 160–250 g ai/ha (80–125% GAP), with 2–3 applications, and the last application at the growth stage of BBCH 55 (PHI of 58–63 days). Flusilazole residues were  $< 0.01$  mg/kg. The Meeting noted that

several other trials at approx. the GAP rate (2–3 applications) but with later growth stages at the last application also resulted in flusilazole residues < 0.01 mg/kg.

The GAP of Spain specifies 200 g ai/ha, 1 application, and the BBCH 61 (beginning of flowering) growth stage at the last application. The trials in Spain were conducted at approximately 200 g ai/ha, with 2 applications, but the growth stage at the last application was in the range of 73–85 (a PHI of 28 days). Flusilazole residues from two of these trials (at BBCH 75 and 83) were < 0.01 mg/kg.

The critical GAPs of the United Kingdom specify 156–160 g ai/ha, 1–2 applications, and the BBCH 71 or 73 (watery ripe stage or early milk stage, respectively) growth stage at the last application. Four trials in the United Kingdom on winter wheat were conducted at 160 g ai/ha, 3 applications, and the BBCH 71 growth stage at the last application. Flusilazole residues were: < 0.01(4) mg/kg. Three other trials that were conducted as a single at 200 or 400 g ai/ha and later growth stages at the last application (75, 84, or 90) resulted in flusilazole residues < 0.01 (3) mg/kg.

The Meeting noted that flusilazole residues in wheat grain obtained in the sixteen trials in Germany, Spain and the United Kingdom were all < 0.01(16) mg/kg.

#### *Maize*

The Meeting received information on flusilazole residues in maize grains from supervised trials in France.

The GAP of France (200 g ai/ha, 2 applications) does not specify a PHI (the other available GAP in Europe, the GAP of Romania for cereal grains, specifies 100 g ai/ha and a PHI of 42 days). Five trials on maize were conducted at approx. 200 g ai/ha (2 applications) with a PHI of 28 days. Flusilazole residues in maize grain were < 0.01 mg/kg in all these trials.

The Meeting agreed that the data on barley, rye, wheat and maize could be used to support a “cereal grains” commodity group maximum residue level. The Meeting decided to recommend a maximum residue level of 0.2 mg/kg for cereals except rice, an STMR value of 0.04 mg/kg based on the barley data and a highest residue of 0.08 mg/kg based on the barley data.

The Meeting also agreed to withdraw its previous recommendations of maximum residue levels of 0.1 mg/kg for barley, rye and wheat grains.

#### *Rice*

The Meeting received information on flusilazole residues in rice grains from supervised trials in Spain. The GAP of Spain specifies 125 g ai/ha, 2 applications and a PHI of 30 days. Four trials were conducted at 129 g ai/ha (2 applications) with PHIs of 30 or 33 days. Flusilazole residues in rice grain, in ranked order, were: 0.06, 0.09, 0.11, and 0.18 mg/kg.

The meeting considered four trials insufficient to estimate a maximum residue level for flusilazole in rice.

#### *Rape seed*

The Meeting received results from supervised trials with flusilazole used on oilseed rape in Belgium, Denmark, France, Germany, the Netherlands and the United Kingdom. The critical GAPs in France, Germany, and the United Kingdom specify 200 g ai/ha, 1–2 applications and a PHI of 56 days (Germany) or a PHI that is not specified. The submitted trials were conducted at about the GAP rate with a PHI longer than 56 days. Flusilazole residues in these trials were generally below the LOQ of the used analytical methods: < 0.01 (9) or < 0.02 (5) mg/kg (PHIs in the range of 58–92 days). Results above LOQ: 0.01, 0.01, 0.03, and 0.04 mg/kg; were obtained with a PHI of 72, 109, 61, and 77, respectively.

Flusilazole residues in ranked order were: < 0.01(9), 0.01(2), < 0.02(5), 0.03 and 0.04 mg/kg.

The Meeting estimated a maximum residue level of 0.1 mg/kg for flusilazole in rape seed, an STMR value of 0.01 mg/kg and a highest residue of 0.04 mg/kg.

The meeting recommended withdrawal of the previous recommendation for rape seed of 0.05 mg/kg.

#### *Sunflower seed*

Flusilazole is registered for foliar application on sunflower in Czech Republic, Bulgaria, France, Hungary, Romania and Slovakia. The GAPs for sunflower in these countries specify 75–200 g ai/ha, 1–2 applications, and a PHI of 56 or 60 days (or a PHI is not specified, which is the case of the highest rate of 200 g ai/ha).

The Meeting received results from supervised trials with flusilazole used on sunflower in France. The critical GAP of France specifies 200 g ai/ha and 2 applications (1 application for late infections) but does not specify a PHI. Eight trials in France were conducted at approx. 200 g ai/ha, one application, and a PHI of 50 days (BBCH 63–71). Flusilazole residues, in ranked order, were: < 0.01 (4), 0.01, 0.03, and 0.04 (2) mg/kg.

The Meeting estimated a maximum residue level of 0.1 mg/kg for flusilazole in sunflower seed, an STMR value of 0.01 mg/kg and a highest residue value of 0.04 mg/kg.

#### *Barley, rye and wheat forage*

The Meeting received information on flusilazole residues in barley, rye and wheat forage from supervised trials in Germany. The GAP of Germany for barley, rye and wheat specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 51 (barley) or BBCH 55 (rye and wheat). In the case of livestock grazing, it is assumed that animals are unlikely to be foraging within 7 days of the application of the fungicide. Data was available for residues in forage at 0, 21, 34 and 42 days according to the above gap. For the purposes of animal exposure through grazing, a value at 7 days, interpolated from the 0 and 21 day values is a satisfactory measure of the average residue that livestock would be exposed to for a 14 day period.

The results were considered from all trials conducted at the GAP rate ( $\pm$  30%) with 2 applications (independent of the growth stage at the last application). Five trials on barley matching the criteria resulted in flusilazole residues of 0.9 (2), 1.35, 2.2, and 3.0 mg/kg. One trial on rye matched the criteria with flusilazole residues being 2.0 mg/kg. Five trials on wheat matching the criteria resulted in flusilazole residues of 0.9, 1.2, 3.3, 4.2 and 4.5 mg/kg. Combined flusilazole residues, in ranked order, were: 0.9 (3), 1.2, 1.35, 2.0, 2.2, 3.0, 3.3, 4.2 and 4.5 mg/kg; resulting in an STMR value of 2.0 mg/kg and a highest residue value of 4.5 mg/kg for flusilazole in barley, rye and wheat forage.

#### *Barley, rye, and wheat straw and fodder, dry*

The Meeting received information on flusilazole residues in barley straw from supervised trials in Germany. The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 51 and a PHI of 42 days. Thirteen trials in Germany on winter barley were conducted at 160–221 g ai/ha (80–111% GAP), 2 applications, with the growth stage at the last application of BBCH 51 (PHI of 57–86 days). Flusilazole residues, in ranked order, were: 0.11, 0.48, 0.62, 1.2, 1.4, 1.5, 2.0 (2), 2.1 (2), 2.2, 2.3, and 2.5 mg/kg.

The Meeting received information on flusilazole residues in rye straw from supervised trials on winter rye in Germany. The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 55 and a PHI of 42 days. The Meeting noted that the growth stages (BBCH of 49, 65, 69, or 72) at the last application in the trials did not match the GAP specification.

The Meeting received information on flusilazole residues in wheat straw from supervised trials in Germany, Spain and the United Kingdom.

The GAP of Germany specifies 200 g ai/ha, 2 applications, the second application up to the growth stage of BBCH 55, and a PHI of 42 days. Three trials in Germany were conducted at 160–

250 g ai/ha (80–125% GAP), with 2–3 applications and the last application at the growth stage of BBCH 55 (PHI of 58–63 days). Flusilazole residues, in ranked order, were: 0.12, 0.23 and 1.6 mg/kg.

The GAP of Spain specifies 200 g ai/ha, 1 application and the BBCH 61 (beginning of flowering) growth stage at the last application. The trials in Spain were conducted at approx. 200 g ai/ha, with 2 applications, but the growth stage at the last application was in the range of 73–85.

The critical GAPs of the United Kingdom specify 156–160 g ai/ha, 1–2 applications, and the BBCH 71 or 73 (watery ripe stage or early milk stage, respectively) growth stage at the last application. None of the submitted trials on wheat (straw) in the United Kingdom matched were conducted according to the GAP (growth stage at the last application was in the range of 39–65).

The Meeting noted that flusilazole residues obtained in barley and wheat straw in Germany appeared to be from similar populations and agreed to combine the results. Flusilazole residues, in ranked order, were: 0.11, 0.12, 0.23, 0.48, 0.62, 1.2, 1.4, 1.5, 1.6, 2.0 (2), 2.1 (2), 2.2, 2.3 and 2.5 mg/kg. The Meeting also agreed to extrapolate the results for barley and wheat straw to rye straw and estimated a maximum residue level of 5 mg/kg for flusilazole in barley, rye and wheat straw and fodder, dry (to replace the previous recommendation of 2 mg/kg), an STMR value of 1.6 mg/kg and a highest residue value of 2.5 mg/kg.

#### *Oat forage and fodder*

The Meeting received information on flusilazole residues in oat forage and dry foliage (dry fodder) from two supervised trials in South Africa. The GAP of South Africa for oat fodder specifies 75 g ai/ha, one application, and a PHI of 30 days. One trial was conducted at the GAP rate with a PHI of 29 days. Flusilazole residue in dry foliage (fodder) was < 0.1 mg/kg.

The Meeting considered one trial insufficient to estimate a maximum residue level for oat fodder.

#### *Sugar beet leaves or tops*

The Meeting received information on flusilazole residues in sugar beet leaves from supervised trials on sugar beet in southern Europe (Greece, Italy, and Spain) and in northern Europe (Belgium, Denmark, northern France, Germany, the Netherlands and the United Kingdom).

Six trials in southern Europe (two in Greece, three in Italy and one in Spain) were conducted according to the GAP of Greece (80 g ai/ha, 3 applications, and a PHI of 15 days). Flusilazole residues in sugar beet leaves, in ranked order, were: 0.10, 0.31, 0.45, 0.66, 0.89 and 1.0 mg/kg.

Sixteen trials in northern Europe (ten in Germany, two in the UK, and one in Belgium, Denmark, the Netherlands, and northern France) were conducted according to the GAP of Germany (150 g ai/ha, 2 applications, a PHI of 42 days). Flusilazole residues in sugar beet leaves, in ranked order, were: 0.11(2), 0.17, 0.19, 0.21, 0.22, 0.25(2), 0.26, 0.27, 0.33, 0.34, 0.37, 0.58, 0.84 and 0.88 mg/kg.

The Meeting noted that the residues obtained in southern and northern Europe were from similar populations and agreed to combine the results. Flusilazole residues in sugar beet leaves, in ranked order (median underlined), were: 0.10, 0.11 (2), 0.17, 0.19, 0.21, 0.22, 0.25 (2), 0.26, 0.27, 0.31, 0.33, 0.34, 0.37, 0.45, 0.58, 0.66, 0.84, 0.88, 0.89 and 1.0 mg/kg.

The Meeting estimated an STMR value of 0.29 mg/kg and a highest residue value of 1.0 mg/kg for flusilazole in sugar beet leaves.

#### *Rice hulls*

The Meeting received information on flusilazole residues in rice hulls (husks) from supervised trials in Spain. The GAP of Spain specifies 125 g ai/ha, 2 applications and a PHI of 30 days. Four trials were conducted at 129 g ai/ha (2 applications) with PHIs of 30 or 33 days. Flusilazole residues in rice hulls, in ranked order, were: 0.34, 0.39, 0.44, and 0.68 mg/kg.

The Meeting made no recommendation for rice hulls as none could be made for the primary commodity rice.

### ***Fate of residues during processing***

The Meeting received information on the fate of flusilazole residues during processing of apples, grapes, soya beans, wheat and barley grain and on flusilazole fate under hydrolysis conditions simulating commercial food processing.

In a high-temperature hydrolysis study greater than 99% of flusilazole remained unchanged under conditions simulating industrial processing (temperatures ranging from 90–120°C; pH 5 and 7). Therefore, flusilazole can be considered stable to simulated pasteurization, baking, brewing, boiling and sterilization.

The STMR-P values calculated from the processing factors are summarized in the table below.

Raw agricultural commodity		Processed commodity		
Commodity	STMR (mg/kg)	Commodity	Processing factor*	STMR-P (mg/kg)
Apple	0.04	Apple juice	0.19(2)	0.008
		Apple pomace, wet	2.4(2)	0.094
		Apple pomace, dry	12(2)	0.48
Grapes	0.03	Grape juice	0.42(4)	0.012
		Wine	0.09(5)	0.003
		Dried Grapes (raisins)	1.8(3)	0.054
		Grape pomace, wet	3.6(2)	0.108
		Grape pomace, dry	11(2)	0.33
Soya beans	0.02	Soya bean meal	0.38	0.008
		Soya bean hulls	1.1	0.022
		Soya bean oil, refined	2.2	0.044
Wheat	0.04	Wheat bran	0.29	0.012
		Wheat flour, low-grade	< 0.91	< 0.036
		Wheat milled by products	0.59	0.024

\*mean value of (no. trials) except for soya beans where only one trial was performed

The Meeting estimated a maximum residue level of 2 mg/kg for *apple pomace, dry*, based on the highest residue of 0.13 mg/kg in pome fruits and the processing factor of 12.

Based on the HR value of 0.11 mg/kg in grapes and the processing factor of 1.8, the Meeting estimated a maximum residue level of 0.3 mg/kg for *dried grapes (including currants, raisins, and sultanas)* to replace its previous recommendation of 1 mg/kg.

Based on the highest residue of 0.03 mg/kg in soya beans and the processing factors of 1.1 and 2.2, the Meeting estimated a maximum residue level of 0.05 mg/kg for *soya bean hulls* and 0.1 mg/kg for *soya bean oil, refined*.

### ***Livestock dietary burden***

The Meeting estimated the dietary burden of flusilazole in farm animals on the basis of the diets listed in Annex 6 of the 2006 JMPR Report (OECD Feedstuffs Derived from Field Crops). Calculation from the highest residue, STMR (some bulk commodities) and STMR-P values provides the levels in feed suitable for estimating maximum residue levels, while calculation from STMR and STMR-P values for feed is suitable for estimating STMR values for animal commodities.

The table below shows estimated maximum and mean dietary burdens for beef cattle, dairy cattle, broilers and laying poultry based on the animal diets from the United States/Canada, the European Union, and Australia. The calculations are provided in Annex 6.

	Flusilazole, Animal dietary burden (mg/kg)					
	US-Canada		EU		Australia	
	Maximum	Mean	Maximum	Mean	Maximum	Mean
Beef cattle	7.5	2.25	6.3	2.9	18 <sup>1</sup>	8.0 <sup>2</sup>
Dairy cattle	7.5	3.4	6.7	2.9	11.5 <sup>3</sup>	5.3 <sup>4</sup>
Poultry - broiler	0.04	0.04	0.04	0.04	0.04	0.03
Poultry - layer	0.04	0.04	2.3 <sup>5</sup>	1.1 <sup>6</sup>	0.02	0.02

<sup>1</sup> Highest maximum beef cattle dietary burden suitable for MRL estimates for mammalian meat.

<sup>2</sup> Highest mean beef cattle dietary burden suitable for STMR estimates for mammalian meat.

<sup>3</sup> Highest maximum dairy cattle dietary burden suitable for MRL estimates for milk

<sup>4</sup> Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

<sup>5</sup> Highest maximum poultry dietary burden suitable for MRL estimates for poultry meat and eggs

<sup>6</sup> Highest mean poultry dietary burden suitable for STMR estimates for poultry meat and eggs.

### *Farm animal feeding studies*

The Meeting received information on lactating dairy cow and laying hen feeding studies.

Twelve lactating cows were randomly assigned among 4 dosing groups of 3 animals each: one control group and 3 groups dosed at one of 3 flusilazole feeding levels each (2, 10, and 50 mg/kg based on measured feed intake, corresponding). All groups were fed for 28 days. Residues in milk reached a plateau at about 7 days. During the withdrawal period, residues decreased significantly in all milk and tissue samples, indicating no bioaccumulation of flusilazole or its metabolites.

Total residues of flusilazole and [bis(4-fluorophenyl)methyl]silanol (IN-F7321) in whole milk (on days 7–28, i.e., at the plateau) and tissues obtained at the 2, 10, and 50 mg/kg dosing levels in the diet are summarized in the table below.

Matrix	Dose (mg/kg)	Residue	
		Highest residue	Mean residue
Whole milk	2	< 0.01	< 0.01
	10	0.05	0.02
	50	0.10	0.06
Muscle	2	< 0.01	< 0.01
	10	0.06	0.05
	50	0.19	0.19
Kidney	2	0.21	0.21
	10	0.85	0.77
	50	5.0	3.9
Liver	2	0.18	0.15
	10	0.65	0.55
	50	1.6	1.4
Fat <sup>a</sup>	2	0.06	0.06
	10	0.56	0.36
	50	1.4	1.35

<sup>a</sup>Residues for omental, renal, and subcutaneous fat for the 2, 10 and 50 mg/kg dose, respectively. These were the highest residues at the respective dose levels for the three kinds of analysed fat samples.

In a hen feeding study, eighty laying hens were divided into 4 groups and each group was divided into 4 subgroups of 5 hens each. Each subgroup of a group was dosed for 28 days at 0, 2, 10, or 50 mg/kg of flusilazole in the diet. Residues in eggs reached a plateau at about 7 days. During the withdrawal period, residues decreased significantly in all egg and tissue samples, indicating no bioaccumulation of flusilazole or its metabolites.

Total residues of flusilazole and [bis(4-fluorophenyl)methyl]silanol (IN-F7321) in eggs (on days 7–28, i.e., at the plateau) and tissues (on day 28) obtained at the 2, 10, and 50 mg/kg dosing levels in the diet are summarized in the table below.

Matrix	Dose (mg/kg)	Highest residue, mg/kg	Mean residue, mg/kg
Whole egg	2	0.05	0.03
	10	0.40	0.16
	50	1.8	0.85
Muscle	2	< 0.02	< 0.02
	10	0.10	0.05
	50	0.37	0.19
Liver <sup>a</sup>	2	0.08	0.04
	10	0.13	0.10
	50	0.58	0.41
Fat	2	0.10	0.09
	10	0.54	0.45
	50	3.7	3.2

<sup>a</sup> Flusilazole was not analysed in liver for the 2 and 10 mg/kg dosing levels, but the residues can be assumed to be < 0.01 mg/kg because < 0.01 mg/kg was obtained for the 50 mg/kg dosing level.

In both the cattle and poultry feeding studies, the flusilazole residues in muscle were significantly lower than in fat and confirms that the residue (sum of flusilazole and [bis(4-fluorophenyl)methyl]silanol) is fat-soluble and that fat is the target tissue.

#### ***Animal commodity maximum residue levels***

The dietary burdens for the estimation of maximum residue levels for animal commodities are 18 mg/kg for beef cattle, 11.5 mg/kg for dairy cattle and 2.3 mg/kg for poultry. The dietary burdens for the estimation of STMR values for animal commodities are 8.0 mg/kg for beef cattle, 5.3 mg/kg for dairy cattle and 1.1 mg/kg for poultry. The sum of flusilazole and [bis(4-fluorophenyl)methyl]silanol (IN-F7321) residues was used for the estimation of “flusilazole residue” levels in animal commodities.

The maximum dietary burden of 18 mg/kg for beef cattle fell between the 10 and 50 mg/kg dosing levels in the cattle feeding study. The residues in muscle were significantly lower than in fat. The target tissue for flusilazole residues is fat. Using the highest residues of 0.56 and 1.4 mg/kg in fat for 10 and 50 mg/kg dosing levels, respectively, the interpolated highest residue in fat for the dietary burden of 18 mg/kg was 0.73 mg/kg. Similarly, for beef liver and kidney the highest residues were 0.84 and 1.68 mg/kg, respectively.

The mean dietary burden was 8.0 mg/kg for beef cattle. By interpolation, the mean residues obtained in fat, liver and kidney were 0.285, 0.45 and 0.65 mg/kg, respectively.

On the fat basis, the Meeting estimated a maximum residue level of 1.0 mg/kg for meat (fat) from mammals (other than marine mammals), an STMR value of 0.285 mg/kg and an HR value of 0.73 mg/kg. Based on the liver and kidney results, the Meeting estimated a maximum residue level of 2 mg/kg for mammalian edible offal and, based on the kidney data, an STMR value of 0.65 mg/kg and an HR value of 1.68 mg/kg.

The mean dietary burden of 5.3 mg/kg for dairy cattle fell between the 2 and 10 mg/kg dosing levels in the feeding study. The interpolated highest residue in whole milk, using the mean residues in the feeding study, was 0.03 mg/kg. Similarly, the mean residue based upon a dietary burden of 5.3 mg/kg, in whole milk was 0.01 mg/kg. The Meeting estimated a maximum residue level of 0.05 mg/kg for whole milk, an STMR value of 0.01 mg/kg and an HR value of 0.03 mg/kg.

Maximum and mean dietary burdens for poultry (2.3 and 1.1 mg/kg, respectively) were near the lowest dosing level of 2 mg/kg. By interpolation, the highest residues obtained in fat, liver and eggs between the 2 and 10 mg/kg feeding level were 0.13, 0.09 and 0.07 mg/kg, respectively. Extrapolating the mean residues gave 0.05 mg/kg for fat, 0.02 mg/kg for liver and 0.02 mg/kg for eggs.

On the fat basis, the Meeting estimated a maximum residue level of 0.2 mg/kg for poultry meat (fat), an STMR value of 0.05 mg/kg and an HR value of 0.13 mg/kg. Based on the liver results, the Meeting estimated a maximum residue level of 0.2 mg/kg for poultry edible offal, an STMR value of 0.02 mg/kg and an HR value of 0.09 mg/kg. The Meeting estimated a maximum residue level of 0.1 mg/kg for eggs, an STMR value of 0.02 and HR value of 0.07 mg/kg.

The Meeting agreed to withdraw its previous recommendations of maximum residue levels of 0.01\* mg/kg for cattle fat, cattle meat, cattle milk, chicken meat, chicken eggs, and chicken edible offal; and 0.02\* mg/kg for cattle edible offal.

## DIETARY RISK ASSESSMENT

### *Long-term intake*

The International Estimated Daily Intakes (IEDIs) of flusilazole based on STMR and STMR-P values estimated for 22 commodities for the thirteen GEMS/Food regional diets were 2–10% of the maximum ADI (0.007 mg/kg bw). The results are shown in Annex 3 of the Report. The Meeting concluded that the long-term dietary intake of flusilazole residues is unlikely to present a public health concern.

### *Short-term intake*

The International Estimated Short Term Intake (IESTI) of flusilazole calculated on the basis of the recommendations made by the JMPR represented for the general population 0–40% and for children 0–100% of the ARfD (0.02 mg/kg bw). The results are shown in Annex 4 of the Report. The Meeting concluded that the short-term intake of residues of flusilazole resulting from uses that have been considered by the JMPR is unlikely to present a public health concern.

## 5.15 FOLPET (041)

### TOXICOLOGY

#### Evaluation for an acute reference dose

Folpet, the ISO approved name for *N*-(trichloromethylthio)phthalimide, is registered for the control of fungal diseases in crops (CAS No. 133-07-3). The toxicology of folpet was evaluated by the JMPR in 1969 and 1995 and addenda to the monograph were prepared in 1973, 1984, 1986, 1990 and 2004. In 1995, an ADI of 0–0.1 mg/kg bw was established based on a NOAEL of 10 mg/kg bw per day in a 2-year study of toxicity and carcinogenicity in rats, a 1-year study of toxicity in dogs, and studies of reproductive toxicity in rats and rabbits, and using a safety factor of 100. In 2004, the Meeting established an ARfD for folpet of 0.2 mg/kg bw for women of childbearing age only, based on a NOAEL of 20 mg/kg bw per day for increased incidences of hydrocephalus at 60 mg/kg bw per day in rabbits and using a safety factor of 100.

The Meeting concluded that the database was insufficient (particularly with regard to information about the possible developmental effects of the metabolite phthalimide) to establish the mode of action by which the increased incidence of hydrocephalus was induced.

The sponsor conducted a study of developmental toxicity with phthalimide, and studies to evaluate the potential effects of folpet and phthalimide on the intestinal flora of the rabbit. It is known that the rabbit is dependent on the presence of caecotrophs for adequate nutrition. The sponsor suggested that disruption of the intestinal flora might result in maternal malnutrition, with possible consequent adverse effects on foetal development.