5.8 CYPROCONAZOLE (239)

TOXICOLOGY

Cyproconazole is the International Organization for Standardization (ISO)–approved name for (2*RS*, 3*RS*, 2*SR*, 3*SR*)-2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)-butan-2-ol (International Union of Pure and Applied Chemistry [IUPAC]), for which the Chemical Abstracts Service (CAS) No. is 94361-06-5. The cyproconazole structure exists in four stereoisomeric forms as two diastereoisomeric pairs of enantiomers. Cyproconazole is a 1:1 mixture of the two diastereomeric pairs, each of which is a 1:1 mixture of the enantiomers (i.e., all four stereoisomers are present in similar amounts).

Cyproconazole is a broad-spectrum triazole fungicide. It acts by inhibiting sterol biosynthesis in fungi (demethylation inhibitor). Cyproconazole has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed at the present Meeting at the request of the Codex Committee on Pesticide Residues (CCPR). All pivotal studies with cyproconazole were certified as complying with good laboratory practice (GLP) unless otherwise stated.

Biochemical aspects

In a toxicokinetic study, male and female rats were given cyproconazole uniformly labelled with ^{14}C either in the phenyl ring or at the α-carbon position as a single dose at 10 or 130 mg/kg body weight (bw) or as 14 repeated doses of 10 mg/kg bw per day followed by a single oral dose of radioactive cyproconazole at 10 mg/kg bw. Cyproconazole was rapidly and extensively (86%) absorbed from the gastrointestinal tract and rapidly excreted from the body in urine and faeces (85% of the administered dose) within 168 h. The majority of excretion occurred in the first 48 h. The bile duct–cannulated rats excreted approximately 76% and 60% of the administered dose in the bile in males and females, respectively. Approximately 5% of the administered dose was recovered in the faeces in the cannulated rats. The absorption, distribution and excretion of cyproconazole were similar in rats administered repeated doses and single low and high doses. No significant radioactivity was detected in the exhaled air following a single oral dose of 10 mg/kg bw. Less than 0.42% of the administered dose was found in the carcass and tissues at 168 h. Tissue residues were highest in liver and adrenals (mainly cortex), followed by fat and kidney. There was no evidence of bioaccumulation in any tissues in rats. Cyproconazole was extensively metabolized, with a greater number of metabolites identified in the urine compared with the faeces. The metabolic profile revealed 35 metabolites, ranging from less than 0.1% to 4.9% and from less than 0.1% to 13.2% of the administered dose in the urine and faeces, respectively. Approximately 11% of the parent compound was detected in the faeces, and less than 0.4% in the urine. The predominant metabolic reactions of cyproconazole in the rat were 1) oxidative elimination of the triazole ring, 2) hydroxylation of the carbon bearing the methyl group, 3) oxidation of the methyl group to the carbinol and further to the carboxylic acid and 4) reductive elimination of the carbon bearing the methyl group, yielding a benzyl alcohol, which is further oxidized to the corresponding ketone.

Toxicological data

Cyproconazole has moderate acute toxicity when administered by the oral route to mice (median lethal dose [LD_{50}] of 200 mg/kg bw) and female rats (LD_{50} of 350 mg/kg bw). The LD_{50} in rats and rabbits treated dermally was greater than 2000 mg/kg bw. The median lethal concentration (LC_{50}) in rats treated by inhalation (nose only) was greater than 5.6 mg/L. Cyproconazole was slightly irritating

to the eyes and skin of rabbits. Cyproconazole was not a skin sensitizer in guinea-pigs as determined by the Magnusson and Kligman (maximization) test and the Buehler test.

The liver was the target organ for cyproconazole in short-term toxicity studies in mice, rats and dogs. Disturbances in lipid metabolism were also observed in all species studied. The no-observed-adverse-effect level (NOAEL) in a 90-day study of toxicity in mice was 15 ppm (equal to 2.2 mg/kg bw per day), based on decreased body weight gain in both sexes seen at 300 ppm (equal to 43.8 mg/kg bw per day).

The NOAEL in a 28-day study of toxicity in rats was 100 ppm (equal to 8.1 mg/kg bw per day), based on reduced body weight gain in females, changes in clinical chemistry, organ weight changes and histopathological findings in the liver seen at 300 ppm (equal to 25.3 mg/kg bw per day). Two 90-day dietary toxicity studies in rats were available, with a combined NOAEL of 80 ppm (equal to 6.4 mg/kg bw per day), based on reduced body weight gain seen at 320 ppm (equal to 23.8 mg/kg bw per day).

The NOAEL in the 90-day and 1-year toxicity studies in dogs was 100 ppm (equal to 3.2 mg/kg bw per day), based on retarded body weight gain seen at 350 ppm (equal to 12.1 mg/kg bw per day) and above.

The carcinogenic potential of cyproconazole was studied in mice and rats. In mice, the major changes following administration of cyproconazole occurred in the liver. There were a number of toxic effects (focal hepatocytic inflammation, single-cell hepatocytic necrosis and diffuse hepatocytic hypertrophy) at the two highest dose levels (100 and 200 ppm) in both sexes. The male mice were more severely affected than the females. The non-liver findings in mice were not considered treatment related. A treatment-related increase in the incidence of combined adenomas and carcinomas was found in males and females at 200 ppm and in males at 100 ppm. The NOAEL in this study was 15 ppm (equal to 1.8 mg/kg bw per day), and the lowest-observed-adverse-effect level (LOAEL) was 100 ppm (equal to 13.2 mg/kg bw per day).

To clarify the mode of action of mouse liver tumours, mechanistic studies were conducted in which the liver effects of cyproconazole and phenobarbital (PB) in various strains of mice were compared. The results of these studies indicated that cyproconazole as well as PB produced similar effects in a dose-related manner in mice. Studies using constitutive androstane receptor (CAR) null and wild-type mice treated with cyproconazole or PB for up to 7 days clearly indicated early gene expression changes in CAR regulation (*Cyp2b10, Gadd45β*), biochemical changes (induction of cytochrome P450 2B–dependent enzyme activities), hypertrophy, fat vacuolation and increased single-cell necrosis in the liver, indicating that these effects were a consequence of CAR activation by cyproconazole as well as PB. Based on these mechanistic studies, the Meeting concluded that development of liver tumours in mice administered cyproconazole depends upon CAR activation.

In the 2-year toxicity and carcinogenicity study in rats, the NOAEL was 50 ppm (equal to 2.2 mg/kg bw per day), on the basis of body weight depression at 350 ppm (equal to 15.6 mg/kg bw per day). There was no evidence of treatment-related tumorigenesis in the rat.

Cyproconazole gave a negative response in an adequate range of in vitro and in vivo genotoxicity tests.

The Meeting concluded that cyproconazole was unlikely to be genotoxic.

On the basis of the absence of genotoxicity, the absence of carcinogenicity in rats and no carcinogenicity in mice by a mode of action relevant to humans, the Meeting concluded that cyproconazole is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected at the highest dose tested (120 ppm, equal to 8.3 mg/kg bw per day). The NOAEL for parental systemic toxicity was 20 ppm (equal to 1.4 mg/kg bw per day), based on a significant increase in relative liver weight and an increased incidence of slight fatty changes (vacuolated hepatocytes) in the liver at 120 ppm (equal to 8.3 mg/kg bw per day). These effects in the liver cannot

be unequivocally attributed to the activation of CAR. Toxicity was less pronounced in the F_0 females and was not seen in F_1 males and females. The NOAEL for reproductive and offspring toxicity was 120 ppm (equal to 8.3 mg/kg bw per day), the highest dose tested.

There are three developmental toxicity studies in rats: the main study, a non-GLP range-finding study and a published study. Cyproconazole caused significantly diminished body weight gain during the early phase of treatment (days 6–11) as well as reduced food consumption in all three studies. In the dose range of 20–30 mg/kg bw per day, body weight gain was 29–37% below that of control groups. Major fetal malformations in these studies were cleft palate (also reported as palatoschisis) and internal hydrocephalus. These malformations occurred at dose levels of 20 mg/kg bw per day and above. The NOAEL for maternal toxicity in the main developmental study was 6 mg/kg bw per day, based on reduced maternal body weight gain during gestation days 6–11 and decreased food consumption seen at 12 mg/kg bw per day. The developmental NOAEL in the main study in rats was 12 mg/kg bw per day, based on decreased fetal body weights, post-implantation loss, increases in supernumerary ribs and increased fetal malformations (e.g., cleft palate) seen at 20 mg/kg bw per day.

There are two developmental toxicity studies available in rabbits. In the first study, treatment of pregnant Chinchilla rabbits with cyproconazole resulted in maternal body weight loss and reduced food consumption early during treatment at the highest dose level of 50 mg/kg bw per day. A slightly increased number of post-implantation losses were also noted in this group. No treatment-related fetal abnormalities were observed. The NOAEL for maternal and developmental toxicity in Chinchilla rabbits was 10 mg/kg bw per day. In the second study, New Zealand White rabbits were treated at the same dose levels. As in the previous study, the high dose resulted in maternal toxicity in the form of body weight loss and reduced food consumption early during treatment. The incidence of post-implantation losses was not affected. In contrast to the first study, the second one revealed an increased number of fetal malformations, mainly affecting sternebrae, ribs, vertebral column, hind limbs and tail. The NOAEL for maternal and developmental toxicity was 10 mg/kg bw per day.

The Meeting concluded that cyproconazole can cause developmental toxicity, including malformations, but only at doses that are maternally toxic.

In a 90-day dietary study of toxicity in rats, five rats of each sex per dose were subjected to neuropathological examination and were also evaluated in the functional observational battery (FOB) and for the assessment of motor activity. No effects on FOB parameters, motor activity or neuropathology were observed at doses up to 1400 ppm (equal to 106.8 mg/kg bw per day).

No adverse effects due to occupational exposure to cyproconazole were reported in workers working in the production, formulation and packaging plant or in research laboratories.

The Meeting concluded that the existing database on cyproconazole was adequate to characterize the potential risk to fetuses, infants and children.

Toxicological data on metabolites

Several toxicological studies were conducted on cyproconazole metabolites M21/M21a and M36 (also named NOA 405870 and NOA 405872, respectively). The IUPAC name for metabolite M21/M21a is 5-(4-chlorophenyl)-5-hydroxy-4-methyl-6-(1H-1,2,4-triazol-1-yl)-2-hexanoic acid. The IUPAC name for metabolite M36 is 5-(4-chlorophenyl)-3,5-dihydroxy-4-methyl-6-[1,2,4]triazol-1-yl-hexanoic acid. These metabolites were found in the rat at minor amounts only (0.02–0.06% of applied dose in urine). They were found in the milk and in the urine of lactating goats. Therefore, the toxicological profile of these metabolites was investigated.

The acute oral LD_{50} for metabolite M21/M21a (NOA 405870) was greater than 2000 mg/kg bw. The metabolite was negative for mutagenicity in a bacterial reverse mutation assay (Ames test). For metabolite M36 (NOA 405872), an oral LD_{50} value of greater than 2000 mg/kg bw was observed in mice and rats. Based on the results from three genotoxicity studies, it is concluded that M36 is

unlikely to be genotoxic in vivo. A 28-day feeding study in rats resulted in deaths at the test limit dose of 20 000 ppm and significant reductions in body weight at 5000 ppm and above. The NOAEL in this study was 1500 ppm (equal to 155 mg/kg bw per day), based on reduced body weights seen at 5000 ppm (equal to 527 mg/kg bw per day).

Toxicological evaluation

The Meeting established an acceptable daily intake (ADI) of 0–0.02 mg/kg bw on the basis of the overall NOAEL of 2.2 mg/kg bw per day from the 2-year study of toxicity and carcinogenicity and the multigeneration reproduction study in rats based on reduced body weight gain and liver toxicity seen at higher doses. A safety factor of 100 was applied. This ADI was supported by the NOAEL of 15 ppm (equal to 2.2 mg/kg bw per day) observed in a 90-day toxicity study in mice on the basis of reduced body weight gain observed at 300 ppm (equal to 43.8 mg/kg bw per day).

The Meeting established an acute reference dose (ARfD) of 0.06 mg/kg bw on the basis of a maternal toxicity NOAEL of 6 mg/kg bw per day in studies of developmental toxicity in rats, based on body weight loss during the early treatment period (gestation days 6–11) and reduced food consumption seen at 12 mg/kg bw per day. The ARfD is protective of developmental toxicity seen at a slightly higher dose in rabbits.

A toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mice	Ninety-day study of toxicity[a]	Toxicity	15 ppm, equal to 2.2 mg/kg bw per day	300 ppm, equal to 43.8 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 2.2 mg/kg bw per day	350 ppm, equal to 15.6 mg/kg bw per day[b]
		Carcinogenicity	350 ppm, equal to 15.6 mg/kg bw per day[b]	—
	Multigeneration study of reproductive toxicity[a]	Parental toxicity	20 ppm, equal to 1.4 mg/kg bw per day[b]	120 ppm, equal to 8.3 mg/kg bw per day[b]
		Reproductive toxicity	120 ppm, equal to 8.3 mg/kg bw per day[b]	—
		Offspring toxicity	120 ppm, equal to 8.3 mg/kg bw per day[b]	—
	Developmental toxicity study [c,d]	Maternal toxicity	6 mg/kg bw per day	12 mg/kg bw per day
		Embryo and fetal toxicity	12 mg/kg bw per day	20 mg/kg bw per day[b]
Rabbit	Developmental toxicity study[c,d]	Maternal toxicity	10 mg/kg bw per day	50 mg/kg bw per day[b]
		Embryo and fetal toxicity	10 mg/kg bw per day	50 mg/kg bw per day[b]
Dog	Ninety-day and 1-year studies of toxicity[a,d]	Toxicity	100 ppm, equal to 3.2 mg/kg bw per day	350 ppm, equal to 12.1 mg/kg bw per day[b]

[a] Dietary administration.

[b] Highest dose tested.

[c] Gavage administration.

[d] Two or more studies combined.

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.06 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

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

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly absorbed, > 86% within 144 h
Dermal absorption	Not available
Distribution	Widely distributed in tissues; highest residues in liver, adrenal, fat and kidney
Potential for accumulation	None
Rate and extent of excretion	Rapid and extensive
Metabolism in animals	Extensively metabolized (35 metabolites identified)
Toxicologically significant compounds (animals, plants and environment)	Cyproconazole and 1,2,4-triazole
Acute toxicity	
Rat, LD_{50}, oral	350 mg/kg bw (rats)
Rat, LD_{50}, dermal	> 2000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.6 mg/L (4 h exposure, nose only)
Rabbit, dermal irritation	Slightly irritating
Rabbit, ocular irritation	Slightly irritating
Guinea-pig, dermal sensitization	Not a sensitizer (Magnusson and Kligman test, Buehler test)
Short-term studies of toxicity	
Target/critical effect	Reduced body weights in mice and rats
Lowest relevant oral NOAEL	2.2 mg/kg bw per day (90-day study of toxicity in mice)
Lowest relevant dermal NOAEL	100 mg/kg bw per day (rat, highest dose tested)
Lowest relevant inhalation NOAEL	0.017 mg/L, equal to 4.6 mg/kg bw per day (16-day inhalation study in rats)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver
Lowest relevant NOAEL	2.2 mg/kg bw per day (2-year carcinogenicity study in rats)
Carcinogenicity	Not carcinogenic in rats; carcinogenic in mice by mode of action not relevant to humans

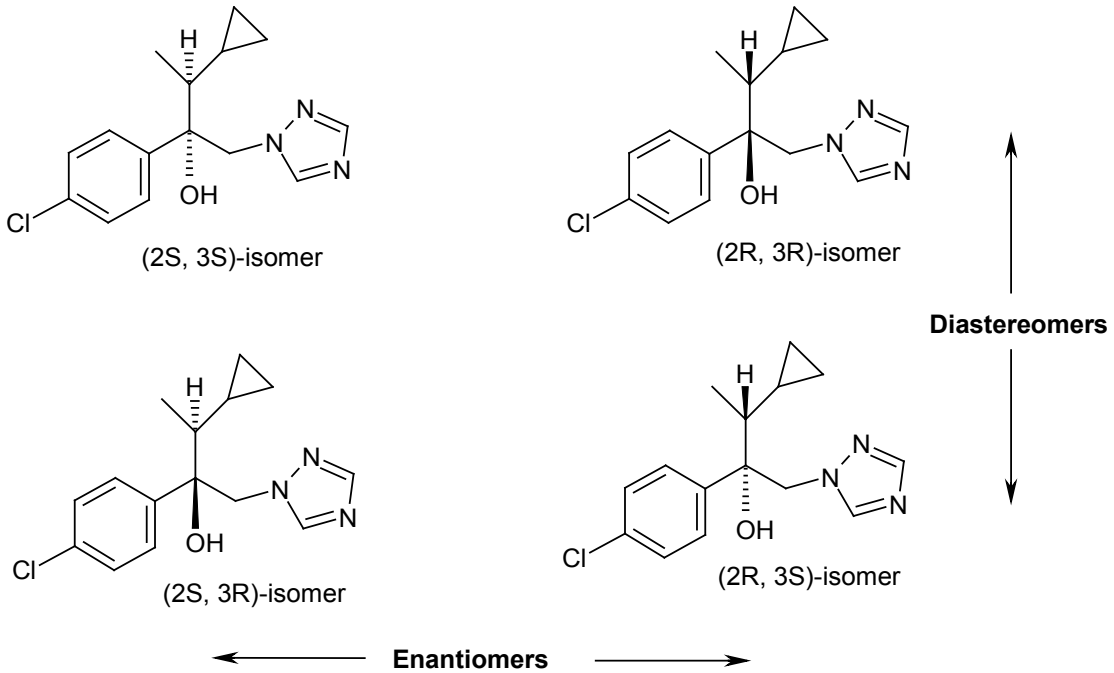
Genotoxicity	
	Not genotoxic
Reproductive toxicity	
Reproduction target/critical effect	None
Lowest relevant reproductive NOAEL	8.3 mg/kg bw per day (rats; highest dose tested)
Developmental target/critical effect	Developmental toxicity, including teratogenicity, only at maternally toxic dose in rats and rabbits
Lowest relevant developmental NOAEL	10 mg/kg bw per day (rats and rabbits)
Neurotoxicity/delayed neurotoxicity	
Subchronic neurotoxicity, 90-day rat	Not neurotoxic
Mechanistic data	
	Mechanistic studies supporting CAR-mediated liver toxicity and tumours in mice
Medical data	
	No adverse effects reported

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.02 mg/kg bw	2-year study of toxicity and carcinogenicity and multigeneration reproduction study in rats	100
ARfD	0.06 mg/kg bw	Developmental studies of toxicity in rats	100

RESIDUE AND ANALYTICAL ASPECTS

Cyproconazole is an azole fungicide used to control a wide range of fungi on cereal crops, coffee, sugar beet, fruit trees, grapes, including rust on cereal crops, powdery mildew on cereal crops, fruit tree and grapes, and scab on apple. It was considered for the first time by the 2010 JMPR. Cyproconazole is an approximately 1:1 mixture of two diastereomers, each of which is exactly a 1:1 mixture of the enantiomers. All four stereoisomers are present in similar amounts.



The manufacturer submitted studies on physical and chemical properties, animal and plant metabolism, environmental fate in soil, rotational crops, analytical methods, freezer storage stability, use patterns, supervised field trials on plants, processing, and residues in animal commodities (livestock feeding). Japan and the Netherlands also submitted use pattern information.

List of metabolites and degradation products

CODE OR COMMON NAME	CHEMICAL NAME
Cyproconazole (CGA 221949) M1/M2	α-(4-chlorophenyl)-α-(1-cyclopropylethyl)-1*H*-1,2,4-triazole-1-ethanol
M9/M14 NOA 421153	2-(4-chlorophenyl)-3-cyclopropyl-1-[1,2,4]triazol-1-yl-butane-2,3-diol
M11/M18 NOA 421154	3-(4-chlorophenyl)-2-cyclopropyl-4-[1,2,4]triazol-1-yl-butane-1,3-diol
M10/M10a NOA 452154	3-(4-chlorophenyl)-2-cyclopropyl-3-hydroxy-4-[1,2,4]triazol-1-yl-butyric acid
M15 NOA 408616	1-(4-chlorophenyl)-2-[1,2,4]triazol-1-yl-ethanol
M16 CGA 123420	1-(4-chlorophenyl)-2-[1,2,4]triazol-1-yl-ethanone
M21/M21a NOA 405870	5-(4-chlorophenyl)-5-hydroxy-4-methyl-6-[1,2,4]triazol-1-yl-hex-2-enoic acid
M36 NOA 405872	δ-(4-chlorophenyl)-β,δ-dihydroxy-γ-methyl-1H-1,2,4-triazole-1-hexenoic acid
M31/M48 NOA 410714	2-chloro-5-(2-cyclopropyl-1-hydroxy-1-[1,2,4]triazol-1-ylmethyl-propyl)-phenol
M38 NOA 421155	1-[2-(4-chlorophenyl)-3-cyclopropyl-but-1-enyl]-1H-[1,2,4]triazole

CODE OR COMMON NAME	CHEMICAL NAME
M39 CGA 131013	3-(1H-1,2,4-triazol-1-yl)-alanine
M41 (C3/C5)	glucoside of 3-(4-chlorophenyl)-2-cyclopropyl)-4-(1H-1,2,4-triazol-1-yl)-1,3-butanediol
M42	glucoside of 2-(4-chlorophenyl)-3-cyclopropyl-1-(1H-1,2,4-triazol-1-yl)-2,3-butanediol
M43	glucoside of α-(4-chlorophenyl)-α-[1-(2-hydroxycyclopropyl)ethyl]-1H-1,2,4-triazole-1-ethanol
M44/M45 (C4)	glucosides of α-(4-chloro3-hydroxyphenyl)-α-(1-cyclopropylethyl)-1H-1,2,4-triazole-1-ethanol
M46	malonic acid conjugate of M42
M47	malonic acid conjugate of M41
M50	Sulfuric acid mono-[1-(4-chlorophenyl)-2-[1,2,4]triazol-1-yl-ethyl] ester
M51	Sulfuric acid mono-[3-(4-chlorophenyl)-2-cyclopropyl-2,3-dihydroxy-4-[1,2,4]triazol-1-yl-butyl] ester
M52 (M54) [stereoisomer of either M52 or M53]	Sulfuric acid mono-[3-(4-chlorophenyl)-2-cyclopropyl-3-hydroxy-4-[1,2,4]triazol-1-yl-butyl] ester
M53 (M54) [stereoisomer of either M52 or M53]	Sulfuric acid mono-[3-(4-chlorophenyl)-2-cyclopropyl-3-hydroxy-4-[1,2,4]triazol-1-yl-butyl] ester
M55 SYN 533911/SYN 533912	5-chloro-2-(1-hydroxy-2-4-[1,2,4]triazol-1-yl-ethyl) phenol or 2-chloro-5-(1-hydroxy-2-4-[1,2,4]triazol-1-yl-ethyl) phenol
M56 SYN 533921	5-[1-(4-chlorophenyl)-1-hydroxy-2-[1,2,4]triazol-1-yl-ethyl]-4-hydroxy-5-methyl-dihydro-furan-2-one
M57 NOA 405870	(E)-5-(4-chlorophenyl)-4,5-dihydroxy-4-methyl-6-[1,2,4]triazol-1-yl-hex-2-enoic acid
M58 CGA 155705	4-chlorobenozic acid
M59	2-(4-chlorophenyl)-3-methyl-1-[1,2,4]triazol-1-yl-pentane-2,4-diol
CGA71019 1,2,4-Triazole	1H-1,2,4-triazole
CGA142856 Triazole acetic acid	1,2,4-triazol-1-yl-acetic acid

Animal metabolism

The Meeting received animal metabolism studies with cyproconazole in rats, lactating goats and laying hens. The metabolism and distribution of cyproconazole in animals was investigated using the [α-carbon^{14}C]-cyproconazole in goats, hens, and rats and the [U-^{14}C-phenyl]-cyproconazole in hens. The rat studies are addressed in the Toxicology section of the Report.

Three lactating goat metabolism studies were provided in which goats were dosed with [α-carbon^{14}C]-Cyproconazole for 12 consecutive days at 1 ppm in the diet, for three consecutive days at 30 ppm in the diet, or for four consecutive days at 10 ppm in the diet. Most (> 85% TRR) of the radioactivity was extractable in milk and tissues. The TRR levels were low in muscle (about

0.01 mg/kg). Cyproconazole was a major component of the residue in liver (20% TRR), fat (27–47% TRR), kidney (24–32% TRR, of which up to 24% conjugated), and muscle (11% TRR), but minor in milk (0–9% TRR). The major metabolites in milk were NOA405872 (M36) (47–68% TRR) and NOA405870 (M21) (17–30% TRR), both of which are carboxylic acid derivatives. NOA452154 was a minor metabolite (8% TRR) in milk. Significant metabolites in liver were NOA421153/M9/M14 (27–27% TRR), NOA421155/M38 (4–16% TRR), and NOA421154/M10 (9–12% TRR). A significant metabolite in fat was NOA421155/M38 (11–36% TRR). In kidney, NOA405872/M36 was significant (12% TRR) in one study. Trace amounts of NOA408616/M15 (about 1% TRR) were found in liver, kidney, fat, and muscle, and slightly higher levels of the corresponding ketone CGA123420/M16 (1–4% TRR) were found in the same tissues.

Taken together, the studies show that cyproconazole is metabolized in goats via:

- Oxidation of the carbon bearing the methyl and cyclopropyl rings to form M14 (NOA421153);
- Oxidation of the methyl group to form M18/M11 (NOA421154) and to M10 (NOA452154);
- Elimination-reduction or removal of the cyclopropyl side chain to form M15 (NOA408616) and subsequent oxidation to the corresponding ketone M16C (GA123420) (minor);
- Elimination of water to form M38 (NOA421155);
- Oxidative opening of the cyclopropyl ring to form M36 (NOA405872) and subsequent dehydration to form M21 (NOA405870) and elimination into milk;
- Glucuronide and/or sulfate conjugation of cyproconazole (minor, except kidney, where it is 5× cyproconazole concentration).

The metabolic fate of cyproconazole was investigated in laying hens using [α-^{14}C]-cyproconazole (1 ppm for 3 days) and [U-^{14}C-phenyl]-cyproconazole (114 ppm for 4 days). Cyproconazole was a major part of the TRR in all matrices: 4% (TRR 0.07 mg/kg)–40% (TRR 3 mg/kg), muscle, 41%–67% fat, 4%–38% liver, 10–30% egg whites, 22–50% egg yolks. Conjugated cyproconazole was about 12% of the free cyproconazole concentration in eggs. NOA421153 (M9/M14) was a major metabolite in muscle (20–31% TRR), fat (15–37% TRR), liver (20–38% TRR), egg whites (35–44% TRR), and egg yolks (14–28% TRR). NOA408616 (M15) was significant in muscle (14–46% TRR), liver (10–22% TRR), egg whites (18–36% TRR), and egg yolks (4–10% TRR).

The metabolism of cyproconazole in poultry proceeds predominantly via hydroxylation, oxidation and elimination reactions. Parent compound was a major component in eggs and all tissues. The major metabolites in eggs and tissues resulted from either (i) hydroxylation of the carbon bearing the cyclopropyl group (M9 and M14) or (ii) elimination of the methyl-cyclopropyl side chain (M16) followed by reduction (M15). Hydroxylation of the methyl group (M11 and M18) was also a route of metabolism.

The metabolism of cyproconazole is qualitatively similar in ruminants and poultry. The major routes of metabolism involved either hydroxylation of the carbon bearing the cyclopropyl group to form M9 /M14 or elimination of the methyl-cyclopropyl side chain (M16) followed by reduction (M15). Hydroxylation of the methyl group (M11 and M18) was also a major route of metabolism, as was opening and modification of the cyclopropyl ring (M21, M36, M56, M57, and M59). The data (ruminant faces with NOA 421152 or M3/M4)) indicate that there is only limited cleavage of the triazole ring and that the majority of residues retain the intact phenyl and triazole rings.

Metabolites found in the ruminant and poultry metabolism studies in edible tissues, eggs, and milk were also found in the rat metabolism study. Among the more prominent fractions in urine were NOA421152 (M3 & M4), NOA408616, NOA421154 (M18) and NOA452669 (M30/33). In faeces, NOA421152 (M3 & M4) and NOA421153 (M14) were the major metabolites beside parent. Further

metabolites at significant amounts were NOA421152 (M4), NOA421153 (M9), NOA452154, NOA451353, NOA421154 (M18), and NOA452668.

Cyproconazole plant metabolism studies were considered for peanut, grape, apples, sugar beet, and wheat. The peanut study does not meet the needs of a metabolism study. Peanut vines in a glasshouse were painted with an EC formulation of [α-carbon^{14}C]-Cyproconazole and harvested 3 to 6 weeks later. The foliage contained cyproconazole (30–40% applied radioactivity) and very small amounts (1–2%) of M9/M14 and M18.

Grapes vines were treated with [α-carbon^{14}C]-Cyproconazole, and grape fruits were harvested 29 days after the last application. A portion (28% TRR) of the residue was removed by surface wash, and an additional 56% TRR was solvent extractable. The major component of the residue was cyproconazole (63% TRR). Identified metabolites were all < 2% TRR, e.g., M9/M14 and M13.

Apple trees were foliarly treated 4 times at 2 week intervals with [α-carbon^{14}C]-Cyproconazole. Apples were harvested 28 days after the last treatment. Surface residue was 17% TRR. About 60% TRR was associated with the washed fruit. Cyproconazole made up 76% of the whole fruit TRR. No metabolite exceeded 3% TRR, e.g., M9/M14 and M13 at 2.8% TRR.

[Phenyl(U)-^{14}C]-cyproconazole, and [U-triazole^{14}C]-cyproconazole were applied in separate studies in two applications at rates of 160 – 200 g ai/ha/application to wheat plants. Cyproconazole was the major component of the TRR for both labels for livestock commodities: 44% straw; 60–72% forage. Cyproconazole composed 5–45% TRR in grain. M39 (triazole alanine) was a significant grain metabolite for the [U-triazole^{14}C]cyproconazole (63% TRR), as was M9/M14 for the [Phenyl(U)-^{14}C]-cyproconazole (14% TRR).

The metabolism of [U-triazole^{14}C]-cyproconazole in sugar beets revealed that cyproconazole is the major portion of the TRR (80% roots; 76% leaves). M9/M14 comprised 2.5% TRR in leaves and 4% TRR in roots.

In all studies, levels of cyproconazole conjugates, as released by acid hydrolysis, were generally ≤ 5% TRR.

The metabolism of cyproconazole in the various plants studied is qualitatively similar. Generally cyproconazole is the major portion of the residue. The metabolism of cyproconazole in plants involves (i) hydroxylation of the methyl- and cyclopropyl-substituted carbon to form Metabolites M9/M14; (ii) oxidation of the methyl group to form Metabolites M11/M18; (iii) elimination of the cyclopropyl-substituted carbon to form the benzylic alcohol (M15) and further oxidation to the ketone (M16); (iv) hydroxylation of the cyclopropyl ring and the phenyl ring; (v) conjugation of parent and hydroxylated metabolites to form various glycosides; and (vi) oxidative elimination of the triazole ring and its subsequent conversion to triazole alanine.

Plant metabolites were also metabolites of the rat metabolism, with the exception of M39/CGA 131013 (triazolyl-alanine).

Environmental fate

Under aerobic conditions in soil cyproconazole is moderately stable. Cyproconazole ([U-triazole^{14}C]-cyproconazole) has a half-life of about 150 days, with 1,2,4-triazole and 1,2,4-tgriazol-1-yl acetic acid at about 25% of the applied radioactivity at 140 days. The half-life (first order kinetics) varied from 100 days to > 1 year with ^{14}C-benzyl cyproconazole and is about 100 days with [Phenyl(U)-^{14}C]-cyproconazole. Mineralization to carbon dioxide varied from 2% to 33% over 112 days. No other degradates were identified.

Cyproconazole is photolytically stable in soil, with no loss after 30 days of irradiation.

Cyproconazole is hydrolytically stable at pHs of 4, 5, 7, and 9 for 5 days at 50 ºC.

Residues in succeeding crops

Residues of cyproconazole are found in succeeding crops in confined rotational crop situations. Studies were reported for the application of [α-^{14}C]cyproconazole to soil followed by the planting of representative crops. [U-triazole^{14}C]cyproconazole was not studied. Cyproconazole is the major component of the residue, and TRRs typically range from < 0.01 mg/kg to 0.44 mg/kg with a 30 day plantback interval (PBI). Following a soil application of [α-^{14}C]cyproconazole at 010 kg ai/ha, rotational crops were planted at intervals (PBI) of 30 and 90 days. Cyproconazole ranged from 33% TRR (0.003 mg/kg) in wheat grain and 39% TRR (0.036 mg/kg) in sugar beet tops to 72% TRR (0.029 mg/kg) in lettuce leaves at a 30 day PBI. Metabolites detected (2–12% TRR) included M9/M14 and M18.

In field crop rotational studies following 7 applications of cyproconazole, each about 0.10 kg ai/ha (0.7 kg ai/ha total), cyproconazole residues at a 30 day PBI were < 0.01 mg/kg in wheat grain and carrot tops, 0.034 in collard greens, 0.081mg/kg in wheat straw, 0.021 mg/kg in radish root and carrot root, 0.062 mg/kg in radish tops, and 0.13 mg/kg in mustard greens. All residues were < 0.01 mg/kg at 1 year PBI. In trials following a single application at 0.082 kg ai/ha (typical 1× for primary crops), cyproconazole was < 0.01 mg/kg in spinach, radish (root and top), and wheat at a 60 day PBI.

The Meeting concluded that residues of cyproconazole in rotational crops with a minimum plantback interval of 30 days may be possible, but residues would be at or near the LOQ of the analytical method, 0.01 mg/kg. This is based on primary crop use patterns under consideration.

Methods of analysis

Adequate analytical methods exist for the determination of cyproconazole residues for data collection and enforcement purposes in both plant and livestock matrices. Early methods for crop matrices involved an optional acid hydrolysis (1 N HCl) to release cyproconazole conjugates, extraction, clean-up, and analysis by GC with NPD, ECD, or MSD. The methods determined cyproconazole only with an LOQ of 0.01–0.04 mg/kg. These methods have been validated via the analysis of spiked samples and include an independent laboratory validation for the MSD variation.

An HPLC/UV method was described for data collection for wheat commodities. Samples are extracted with aqueous methanol, cleaned-up with SPE, and analysed by HPLC with UV detection. The limit of quantitation was 0.01–0.02 mg/kg.

More recently an HPLC-MS/MS method has been developed for plant matrices. Homogenized samples are extracted with aqueous acetonitrile, filtered, and monitored for m/z 292 (Q1) and m/z 70 (Q3) for cyproconazole. The demonstrated LOQ is 0.01 mg/kg.

Analytical residue enforcement method DFG S19 has been developed (HPLC- MS/MS) and validated for a dry crop, a high-fat crop, a high-water crop, and an acidic crop. The LOQ is 0.01 mg/kg.

An HPLC-MS/MS method (RAM 499/01) has been developed and validated for the determination of cyproconazole only in livestock commodities. Major metabolites such as M36 in milk and M14 in liver are not determined currently by the method. For this method, free and conjugated cyproconazole residues are extracted with acetonitrile (ACN):water and hydrolysed using either concentrated ammonia (eggs and tissues) or 2M HCl (milk). Cyproconazole residues are then determined by LC-MS/MS using external standards. The method LOQ is 0.01 ppm for cyproconazole in each livestock commodity. The method has also undergone a successful independent laboratory validation (ILV) trial and was radiovalidated using samples from a goat dosed with [^{14}C]cyproconazole.

Analytical residue enforcement method DFG S19 has been developed (HPLC-MS/MS) for cyproconazole in livestock matrices and validated by an independent laboratory.

Stability of residues in stored analytical samples

Cyproconazole has been shown to be stable in numerous plant commodities stored frozen at ≤-12 ºC. Cyproconazole is stable (≥ 80% recovery) for at least 40–42 months in grapes, raisins, nectarines, peaches, peanut nutmeat, peanut hay, and wheat hay. It is stable (≥ 80% recovery) for at least 36 –39 months in wheat grain, wheat forage, and peanut hulls. Likewise, cyproconazole was stable in most livestock commodities fortified with cyproconazole at 0.01–10 mg/kg and stored frozen at -20 ºC. Cyproconazole was stable in milk for at least 12 months and in kidney and liver for at least 9 months. However, the percent cyproconazole remaining in fat at all fortification levels and storage intervals was variable and may be more a reflection of analytical method difficulties than actual storage stability. Some stability was indicated for up to one month in fat (60–90% remaining).

Definition of the residue

The livestock commodity analytical methods used for data collection (livestock feeding) determine cyproconazole and the metabolites M36 and M21. No hydrolyses were used to free potential conjugates. The analytical method validated for enforcement purposes determines only cyproconazole. This method uses an acid hydrolysis step (milk) or ammonia hydrolysis (eggs and tissues) to free conjugates.

Cyproconazole was the major component of the residue in all poultry commodities and all ruminant commodities except milk. Conjugated cyproconazole was found in eggs and ruminant kidney. Cyproconazole was a minor component in milk (10% TRR), whereas the major metabolites were M36 (NOA405872) and M21 (NOA405890). M36 and M21 comprised up to 80% of the TRR in milk. These two metabolites are carboxylic acids resulting from transformation of the cyclopropyl ring. Various toxicity studies with M36 reveal that this metabolite is very significantly less toxic than cyproconazole. Moreover, a feeding study with ruminants shows that M36/M21 residues are near the limit of quantitation of the analytical method at current livestock dietary burden levels. Therefore, M36/M21 need not be considered in the dietary risk assessment for milk.

While cyproconazole was a major component of the TRR in hen and goat liver, there were significant amounts of the hydroxylated cyproconazole metabolite M14, 30% TRR goat liver and 20 – 38% TRR hen liver. Cyproconazole was 27% TRR and 27%, respectively. In the feeding studies, M14 was 0.2–0.5 ppm in cow liver at a 3 ppm dietary burden and not determined in poultry liver. Cyproconazole was about 0.2 ppm in cow liver and < 0.01 mg/kg in poultry liver at a 3 ppm feeding level. M14 is considered to be less toxic than cyproconazole. Given the significant percentage of cyproconazole in the liver residue, the lower relative toxicity of M14, and the small contribution of liver to the diet, metabolite M14 need not be included in the residue definition for dietary intake.

Triazolyl-alanine was 63% TRR (0.13–0.20 mg/kg) on wheat grain in the wheat metabolism study. In livestock feeding studies, concentrations of triazole, triazolyl-alanine, and triazole acetic acid were < 0.01 mg/kg, except cattle liver (triazolyl-alanine 0.04 mg/kg).

The 2007 JMPR addressed the issue of triazole metabolites (JMPR 2007 Report, General Consideration 2.3). It was noted that 1,2,4-triazole, triazolyl-acetic acid and triazolyl-alanine may be derived from several sources. In a situation in which the metabolites arise from multiple triazole fungicides, they cannot be included in the residue definition. Since the metabolites cannot be linked to a specific triazole fungicide, they would have to be evaluated on their own. The 2007 Meeting further concluded that they did not have sufficient information to judge levels that would be without potential effect in consumers.

Cyproconazole was the major component of the residue in plant metabolism studies conducted with grape, apples, sugar beet, and wheat (except grain, 15% TRR). Concentrations of cyproconazole conjugates generally were < 5% TRR. No metabolite exceeded 10% TRR, except for M39 (triazole alanine) in wheat grain (63% TRR) and M9/M14 in wheat grain (14% TRR). Anaerobic soil metabolism studies showed that cyproconazole is relatively stable and does not form metabolites in significant concentrations. Confined rotational crop studies revealed that

cyproconazole is the major quantifiable residue in follow-on crops; metabolites were < 12% TRR. A limited rotational crop field trial (conduced at 1× for the primary crop) indicated that cyproconazole residues in follow-on crops would most likely be near the LOQ (0.01 mg/kg).

The plant commodity analytical methods used for data collection and the methods validated for enforcement of MRLs determine only cyproconazole.

The Meeting recommended that the residue definition for plant and animal commodities for compliance with MRLs should be cyproconazole. While cyproconazole is a minor component of the residue in milk, there is sufficient cyproconazole present to monitor compliance.

The Meeting recommended that the residue definition for dietary risk assessment for plant commodities should be cyproconazole.

The Meeting recommended that the residue definition for dietary risk assessment for animal commodities should be cyproconazole, free and conjugated.

The log Kow of cyproconazole (log K_{ow} 3.1) suggests that cyproconazole will show no clear preference for distribution in fat versus water. The ratio of cyproconazole residues (TRR) in muscle and fat observed in the livestock metabolism studies (lactating cow 1 muscle: 4–6 fat) indicates a slight preference for fat solubility. In the cow feeding study, cyproconazole had a slight preference for cream over skim milk (0.008 ppm vs 0.003 ppm) and a more indicative preference for fat over meat (0.052 mg/kg fat versus 0.005 mg/kg meat, or about 10 to 1).

Proposed definition of the residue (for compliance with MRL for plant commodities): *cyproconazole*.

Proposed definition of the residue (for compliance with MRL for animal commodities): *cyproconazole*.

The residue is considered fat-soluble.

Proposed definition of the residue (for estimation of dietary intake for plant commodities): *cyproconazole*.

Proposed definition of the residue (for estimation of dietary intake for animal commodities): *cyproconazole, free and conjugated*.

Results of supervised trials on crops

The NAFTA calculator was used as a tool in the estimation of the maximum residue levels from the selected residue data sets obtained from trials conducted according to GAP. As a first step, the Meeting reviewed all relevant factors related to each data set in arriving at a best estimate of the maximum residue level with the calculator using expert judgment. Then, the NAFTA calculator was employed. If the statistical calculation spreadsheet suggested a different value than that recommended by the JMPR, a brief explanation of the deviation was supplied. Some common factors that may lead to rejection of the statistical estimate include when the number of data points in a data set is < 15 or when there are a large number of values < LOQ.

Pome fruits

Supervised residue trials on apples were provided from Spain and Brazil, but no GAP (label) was available for Brazil. Using the GAP of Italy (0.02 kg ai/hL, 7 day PHI), the trial results from Spain in ranked order are: 0.03 (2), 0.05 mg/kg.

The Meeting considered three trials insufficient for the estimation of an MRL and STMR.

Legume vegetables

Supervised field trials on succulent peas were provided from France (North and South) and the UK. A GAP was provided for France (0.06 kg ai/ha, 2 applications, 21 day PHI). The trial results for Europe in ranked order are: < 0.01 (7), 0.01 mg/kg.

The Meeting estimated a maximum residue level of 0.01 mg/kg an HR of 0.01 mg/kg, and an STMR of 0.01 mg/kg. Statistical calculation is not useful for cases with highly censored data.

Pulses

Field trials for dried pea and dried bean were reported from France (19) and the UK (10). A GAP was provided for pea and bean in the UK (0.08 kg as/ha or 0.04 kg ai/hL, 2 applications, 42 day PHI). Using this GAP, the ranked order of residues on peas (dry) in Europe for dry pea is: < 0.01 (14), 0.01 (2), < 0.02 (5). The ranked order of residues on beans (dry) in Europe were: < 0.01 (4) mg/kg. Additional bean trials at shorter PHIs (29–30) had residues of 0.01–0.05 mg/kg.

The Meeting used the dry pea and dry bean data for mutual support. The Meeting estimates a maximum residue level of 0.02 (*) mg/kg, an HR of 0.02 mg/kg, and an STMR estimate of 0.02 mg/kg for peas (dry) and beans (dry). Statistical calculation is not useful for cases with highly censored data.

Field trials on soya beans were reported from the USA. The GAP is 0.04 kg ai/ha, 2 applications, and a 30 day PHI. Nineteen trials were conducted at this GAP, and the residue results (n = 19) in ranked order are: < 0.01 (4), 0.01 (3), < 0.02 (2), 0.02 (4), 0.03 (4), 0.04, 0.05 mg/kg.

The Meeting estimated an STMR of 0.02 mg/kg, and HR of 0.05 mg/kg, and a maximum residue level of 0.07 mg/kg.

The NAFTA statistical method estimated a maximum residue level of 0.06 mg/kg, based on the mean plus three standard deviations. The statistical method is unreliable with multiple LOQ values (2) and 13 non-censored data points.

Root and tuber vegetables

Field trials for sugar beets were reported from Europe. A GAP was provided for Italy (0.08 kg ai/ha, 2 applications, 21 day PHI) and for the Netherlands (0.06 kg ai/ha, 2 applications, 45 day PHI). One trial in Switzerland, four trials in France North, and four trials in the UK meet the GAP of the Netherlands, and the results (n = 9) in ranked order are: < 0.01 (4), < 0.02 (4), and 0.02 mg/kg. Six trials in Italy, two trials in Spain, and one trial in France South meet the GAP of Italy, and the results of the trials (n = 9) in ranked order were: < 0.01, < 0.02 (5), 0.02, 0.03, 0.04 mg/kg. Using the trials matching the GAP of Italy, the Meeting estimated an STMR of 0.02 mg/kg, an HR of 0.04 mg/kg, and a maximum residue level of 0.05 mg/kg.

Statistical calculation is not useful for cases with highly censored data.

Cereal grains

Field trials for wheat were reported from Europe (12 France, 26 Germany, 2 Switzerland). A GAP for wheat was provided for Germany (0.096 kg ai/ha or 0.047 kg ai/hl, 2 applications, 35 day PHI or until BBCH 61). Three trials in France, 23 trials in Germany, and two trials in Switzerland meet the GAP of Germany, and the results of the trials (n = 28) in ranked order are: < 0.01 (8), 0.01 (4), < 0.02 (4), 0.02 (6), 0.04 (2), 0.05 (4) mg/kg.

Field trials for rye were reported from Europe (Germany 5). A GAP for rye was provided for Germany (0.096 kg ai/ha, 2 applications, 35 day PHI or application until BBCH 61) and for the Netherlands (0.08 kg ai/ha, 1 application, PHI 42 days). Note that the GAPs for wheat and rye are

identical in Germany. Using the GAP of Germany, the residue results from the German trials in ranked order are: 0.01 (2) and 0.03 mg/kg.

Field trials for barley were reported from Europe (France (12), Switzerland (5), Germany (9) and the UK (10)). A GAP for barley was provided for Germany (0.096 kg ai/ha or 0.048 kg ai/hL, 2 applications, 35 day PHI or until BBCH stage 61). Note that this GAP is identical to the GAP for wheat and rye in Germany. One trial in France (North), three trials in Switzerland, and nine trials in Germany were at the GAP of Germany. The residue results in ranked order are: 0.01, 0.02 (4), 0.03 (4), 0.04 (3), 0.07 mg/kg.

The Meeting determined that the data sets for wheat, rye, and barley are from similar populations and combined the sets. The residues (n = 44) in ranked order are < 0.01 (8), 0.01 (7), < 0.02 (4), 0.02 (10), 0.03 (5), 0.04 (5), 0.05 (4), 0.07 mg/kg. The GAPs for the various grains were identical. No cereal grain group GAP was provided, but GAPs were provided for wheat, rye, triticale, barley, and oats, which represent all major small cereal grains except rice and which justify the extension. The Meeting estimated for cereal grains except rice and maize an STMR of 0.02, an HR of 0.07 mg/kg, and a maximum residue level of 0.08 mg/kg.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.07 m/kg based on the mean plus 3 standard deviations, but the Meeting considered that the estimate should be above the highest residue.

Field trials for maize (field corn) were reported from the USA, where the GAP is 0.04 kg ai/ha, 2 applications, and 30 day PHI. Twenty-two trials were conducted at this GAP, and the residue results in ranked order were: < 0.01 (22) mg/kg. In three trials, samples were collected at a PHI of 7 days, and residues were < 0.01 mg/kg.

The Meeting estimated an STMR of 0.01 mg/kg, an HR of 0.01 mg/kg, and a maximum residue level of 0.01 (*) mg/kg for maize.

Statistical method for maximum residue level estimation are not applicable where all data are < LOQ.

Oilseeds

Field trials on rape (canola) were reported from Europe. A GAP was provided for the UK (0.08 kg ai/ha, 2 applications, and a PHI of 30 days or BBCH 79, whichever occurs first). One trial from Switzerland, ten trials from France, and one trial from Germany complied with the UK GAP. The residue results (n = 12) in ranked order are: 0.01, 0.03 (3), 0.04, 0.05, 0.08 (2), 0.09, 0.10, 0.21, 0.23 mg/kg.

The Meeting estimated an STMR of 0.065, an HR of 0.23, and a maximum residue level of 0.4 mg/kg for rape seed.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 0.4 mg/kg, based on the UCL median 95th, which was in agreement with the Meeting's estimation.

Field trials on peanut were reported from Australia (3), Brazil (1), and the USA (4). GAP was provided for Australia (0.06 kg ai/ha, 5 applications, 14 day PHI). There is no registered use on peanuts in the USA. No GAP was provided for Brazil. The ranked orders of residues from Australian trials, corresponding to the Australian GAP, were: < 0.02 (3) mg/kg.

The Meeting considered three trials insufficient for the estimation of an STMR, HR, and maximum residue level for peanuts.

Primary animal feed commodities

Legume animal feed

Field trials on soya beans were reported from the USA. The GAP is 0.04 kg ai/ha, 2 applications, and a 30 day PHI. The PHI for forage is 14 days. Fifteen trials were at GAP for soya bean forage, and the results in ranked order were: 0.11, 0.21, 0.22, 0.31 (2), 0.33, 0.35, 0.37, 0.40, 0.41, 0.48, 0.50, 0.52, 0.80, 0.82 mg/kg.

The Meeting estimated an STMR of 0.37 mg/kg and a highest residue of 0.82 mg/kg for soya bean forage.

Fifteen trials were at GAP for soya bean fodder (hay), and the results in ranked order were: 0.17, 0.32, 0.33 (2), 0.41, 0.43, 0.44, 0.66, 0.67, 0.71, 0.75 (2), 1.3, 1.5, 1.9 mg/kg.

The Meeting estimated an STMR of 0.66 mg/kg and a highest residue of 1.9 mg/kg. The Meeting also estimated a maximum residue level of 3 mg/kg for soya bean hay.

The maximum residue level estimate derived from use of the NAFTA statistical calculator was 3.0 mg/kg, based on the 99th percentile of a log normal distribution, which was in agreement with the Meeting's estimation.

Pea Vines (Green)

Supervised field trials on succulent peas were provided from France. A GAP was provided for France (0.07 kg ai/ha, 2 applications, 21 day PHI). All trials were conducted at about 125% of the GAP maximum application rate. The residue results in ranked order for pea vines (n = 6) were: 0.02, 0.07, 0.34, 0.35, 0.43, 0.83 mg/kg.

The Meeting estimated an STMR of 0.345 and a highest residue of 0.83 mg/kg.

Pea Hay or Fodder (dry)

Field trials for dried pea fodder were reported from France and the UK. A GAP was provided for pea in the UK (0.08 kg as/ha or 0.04 kg ai/hL, 2 applications, 42 day PHI). Only two of the 17 trials were conducted at GAP, and residues in ranked order for pea fodder are: 0.12, 0.24 mg/kg.

Bean Fodder

Field trials for dried bean fodder were reported from France and the UK. A GAP was provided for bean in the UK (0.08 kg as/ha or 0.04 kg ai/hL, 2 applications, 42 day PHI). Only 1 of 8 trials was at GAP, and residues in ranked order for bean fodder are: 0.09 mg/kg.

The Meeting combined pea fodder and bean fodder results in mutual support, but decided that 3 trials were not sufficient to estimate a highest residue, STMR, and/or maximum residue level.

Sugar Beet Tops (Leaves)

Field trials for sugar beets were reported from Europe. A GAP was provided for Italy (0.08 kg ai/ha, 2 applications, 21 day PHI) and for the Netherlands (0.06 kg ai/ha, 2 applications, 45 day PHI). Using the GAP of the Netherlands to evaluate the trials of the UK, France, Germany, and Switzerland residue values (n = 5; Switzerland and France) for sugar beet tops in ranked order are: 0.07, 0.15, 0.17, 0.23, 0.35 mg/kg. Using the GAP of Italy to evaluate the trials in Spain and Italy, residue values (n = 4; Italy) for sugar beet tops in ranked order were: 0.06, 0.29, 0.34, 0.54 mg/kg.

Using the trials evaluated against the GAP of Italy, the Meeting estimated an STMR of 0.315 mg/kg and a highest residue of 0.54 mg/kg for sugar beet tops.

Cereal Grain Straw, Forage, Fodder, Silage

Field trials for wheat were reported from Europe (12 France, 26 Germany, 2 Switzerland). A GAP was provided for Germany (0.096 kg ai/ha or 0.047 kg ai/hL, 2 applications, 35 day PHI or until BBCH 61). The residue results for wheat straw (n = 30; France – (n = 3) 0.39, 1.6, 1.7; Switzerland (n = 2) – 0.09, 0.11; Germany (n = 25) – 0.15, 0.22, 0.23, 0.24, 0.36, 0.37, 0.42, 0.43, 0.76, 0.77, 0.78 (2), 0.79, 0.85 (2), 0.92 (3), 1.1, 1.3, 1.4, 1.7, 2.1, 2.4, 3.6, in ranked order were: 0.09, 0.11. 0.15, 0.22, 0.23, 0.24, 0.36, 0.37, 0.39, 0.42, 0.43, 0.76, 0.77, 0.78 (2), 0.79, 0.85 (2), 0.92 (3), 1.1, 1.3, 1.4, 1.6, 1.7 (2), 2.1, 2.4. 3.6 mg/kg.

Field trials in rye were reported from Europe (Germany n = 5). A GAP was provided for Germany (0.096 kg ai/ha, 2 applications, 35 day PHI or application until BBCH 61) and for the Netherlands (0.08 kg ai/ha, 1 application, PHI 42 days). Note that the GAPs for wheat and rye are identical in Germany. Using the GAP of Germany, trial results at GAP for rye straw in ranked order were: 0.64, 0.68, 1.2 mg/kg.

Field trials for barley were reported from Europe (France 12, Switzerland 5, Germany 9, and the UK 10). A GAP was provided for Germany (0.096 kg ai/ha or 0.048 kg ai/hL, 2 applications, 35 day PHI or until BBCH stage 61). Note that this GAP is identical to the GAP for wheat and rye in Germany. For barley straw, One trial in France North (0.16), three trials in Switzerland (0.24, 0.34, 0.42), and 13 trials in Germany were at the GAP. The results (n = 17) in ranked order were: 0.01, 0.14, 0.15, 0.16, 0.20, 0.22, 0.24, 0.28, 0.34, 0.42, 0.52 (3), 0.53, 0.56, 0.63, 0.67 mg/kg.

Noting that the GAPs for wheat, rye, and barley were identical and that the residue values were similar, the Meeting decided to estimate values for the cereal grain straws group (except rice and maize). The residue values for wheat straw were used, as this set is the largest and contains the highest high residue.

The Meeting estimated an STMR of 0.785 mg/kg, and a highest residue of 3.6 mg/kg. The Meeting also estimated a maximum residue level of 5 mg/kg for cereal grain straws (except rice and maize).

The NAFTA statistical spreadsheet provided a maximum residue level estimate of 5.1 mg/kg, based on the 95th percentile at the 99th UCL.

Field trials for maize (field corn) were reported from the USA, where the GAP is 0.04 kg ai/ha, 2 applications, and 30 day PHI for grain and fodder/straw and a 21 day PHI for forage/silage. Twenty-three trials for maize fodder were at the GAP, and the results in ranked order were: < 0.01 (2), 0.08 (2), 0.12 (2), 0.21, 0.22, 0.23, 0.24, 0.27, 0.28 (2), 0.33, 0.34, 0.35 (3), 0.45, 0.46, 0.74, 0.80, 1.5 mg/kg.

The Meeting estimated an STMR of 0.28 and a highest residue of 1.5 mg/kg. The Meeting also estimates a maximum residue level of 2 mg/kg for maize fodder.

The NAFTA statistical calculation estimates a maximum residue level of 1.4 mg/kg. However, JMPR (FAO) guidance specifies that an estimate shall not be below the highest result (1.5 mg/kg).

Twenty-two trials for maize forage are at the GAP, and the results in ranked order were: < 0.01, 0.03, 0.05, 0.06 (2), 0.07, 0.08 (2), 0.09, 0.10 (2), 0.12, 0.13, 0.14, 0.16, 0.19, 0.20, 0.23, 0.24, 0.29, 0.31, 0.44 mg/kg.

The Meeting estimated an STMR of 0.11 and a highest residue of 0.44 mg/kg.

Oilseed forages and fodders

Field trials on rape (canola) were reported from Europe. A GAP was provided for the UK (0.08 kg ai/ha, 2 applications, and a PHI of 30 days). Six trials for rape forage from France were at GAP, and the results in ranked order were: 0.24, 0.28, 0.48, 0.52, 1.2, 1.9 mg/kg.

The Meeting estimated an STMR of 0.50 and a highest residue of 1.9 mg/kg for rape forage.

Field trials on peanut were reported from Australia (3), Brazil (1), and the USA (4). GAP was provided for Australia (0.06 kg ai/ha, 5 applications, 14 day PHI). There is no registered use on peanuts in the USA. For two trials at GAP in Australia, residues on peanut fodder were 5.3 and 14 mg/kg. For two trials at GAP in Australia, residues on peanut forage (green) were 1.3 and 5.3 mg/kg.

The Meeting considered two trials insufficient for the estimation of an STMR or highest residue for peanut fodder or peanut forage.

Fate of residue during processing

The effects of processing on the nature of residues in processed commodities were investigated in buffer solutions under conditions simulating pasteurization, boiling, and sterilization. Radio-labelled cyproconazole was demonstrated to be stable under these conditions.

The fate of cyproconazole residues has been studied in processing studies for apples, maize, rape seed (canola), soya bean, and peanuts. Estimated relevant processing factors and STMR-Ps are summarized below.

Commodity	Number of Studies (n)	Median Cyproconazole Transfer Factors	Cyproconazole RAC-STMR (mg/kg)	Cyproconazole STMR-P (mg/kg)
Oilseed rape – press cake	1	0.83	0.065	0.054
Oilseed rape – crude oil	4	0.86	0.065	0.056
Oilseed rape – solvent extracted meal	1	0.25	0.065	0.016
Oilseed rape – refined oil	4	0.08	0.065	0.0052
Soya bean – meal	4	0.64	0.02	0.013
Soya bean – hulls	4	0.75	0.02	0.015
Soya bean – refined oil	4	1.8	0.02	0.036

The Meeting decided to estimate a maximum residue level of 0.1 mg/kg for refined soya bean oil based on a highest residue of 0.05 mg/kg for soya beans and a processing factor of 1.8 (0.05 mg/kg × 1.8 = 0.09 mg/kg).

Residues in animal commodities

Farm animal dietary burden

The Meeting estimated the dietary burden of cyproconazole in farm animals on the basis of the diets listed in Appendix IX of the FAO Manual (2009 Edition). Calculation from highest residues, STMR (some bulk blended commodities), and STMR-P values provides the levels in feed suitable for estimating MRLs, while calculation from STMR and STMR-P values for feed is suitable foe estimating STMR values for animal commodities. The percentage dry matter is assumed to be 100% when the highest residue levels and STMRs are expressed on a dry weight basis.

Estimated maximum and mean dietary burdens of farm animals

Dietary burden calculations for beef cattle, dairy cattle, chicken broilers, and laying poultry are provided in Annex 6 of the 2010 JMPR Report. The calculations were made according to the animal diets from the US/CAN, EU, and Australia in Appendix IX of the FAO Manual (2009 Edition).

Commodity	Level	Animal Dietary Burden, Cyproconazole, ppm of dry matter diet.			
		US/CAN	EU	Australia	Japan
Beef cattle	Max	0.644	3.08	6.33[a]	0.022
	Mean	0.153	0.929	1.67[c]	0.022
Dairy cattle	Max	2.88	3.21	5.05[b]	0.711
	Mean	0.764	1.04	1.40[d]	0.180
Poultry – broiler	Max	0.022	0.022	0.022	0.014
	Mean	0.022	0.022	0.022	0.014
Poultry – layer	Max	0.022	1.40 [e,g]	0.022	0.013
	Mean	0.022	0.413 [f,h]	0.022	0.013

[a] Highest maximum beef or dairy cattle dietary burden suitable for MRL estimates for mammalian tissues

[b] Highest maximum dairy cattle dietary burden suitable for MRL estimates for mammalian milk

[c] Highest mean beef or dairy cattle dietary burden suitable for STMR estimates for mammalian tissues.

[d] Highest mean dairy cattle dietary burden suitable for STMR estimates for milk.

[e] Highest maximum poultry dietary burden suitable for MRL estimates for poultry tissues.

[f] Highest mean poultry dietary burden suitable for STMR estimates for poultry tissues.

[g] Highest maximum poultry dietary burden suitable for MRL estimates for poultry eggs.

[h] Highest mean poultry dietary burden suitable for STMR estimates for poultry eggs.

Farm animal feeding studies

A cow feeding study involved Friesian dairy cows dosed orally with cyproconazole for 35 days at levels equivalent to 1, 3, 10, and 30 ppm in the diet. Aside for one usually high value (0.025 ppm) from the 1-ppm dose group on day 14, cyproconazole residues in milk were ≤ 0.006 ppm for the 1, 3 and 10 ppm dose groups and were < 0.003–0.014 ppm for the 30 ppm dose group, with the maximum values occurring on days 7 or 14. At intervals in excess of 14 days, cyproconazole was found (> 0.003 ppm) in the 30 ppm dosing level only.

In tissues, cyproconazole was found at the following levels at dosing levels of 1, 2, 10, and 30 ppm, respectively: liver 0.090 (avg 0.082), 0.218 (avg 0.214), 0.604 (avg 0.514), 0.930 (avg 0.748); fat < 0.003; 0.003; 0.024 (avg 0.017), 0.052 (avg 0.022); kidney < 0.003, 0.009 (avg 0.007), 0.031 (avg 0.016, 0.038 (avg 0.028); muscle < 0.003, < 0.003, 0.003, 0.005 mg/kg.

In the table below, dietary burdens are shown in round brackets (), feeding levels and residue concentrations from the feeding study are shown in square brackets [], and estimated concentrations related to the dietary burdens are shown without brackets.

Cattle Dietary Burden [a] (ppm)						
Feeding Level [ppm]	Cream	Milk	Muscle	Liver	Kidney	Fat
MAXIMUM RESIDUE LEVEL	Mean	Mean	Highest	Highest	Highest	Highest
MAXIMUM RESIDUE LEVEL beef cattle (6.33) [3/10]			0.003 [< 0.003/0.003]	0.46 [0.218/0.604]	0.115 [0.054/0.1861]	0.015 [0.003/0.024]
MAXIMUM RESIDUE LEVEL dairy cattle (5.05) [3/10]	- -	0.006 [0.005/0.006]	0.003 [< 0.003/0.003]	0.36 [0.218/0.604]	0.092 [0.054/0.186]	0.012 [0.003/0.024]
STMR	Mean	Mean	Mean	Mean	Mean	Mean
STMR beef cattle			0.003	0.14	0.026	0.003

Cattle Dietary Burden [a] (ppm)						
Feeding Level [ppm]	Cream	Milk	Muscle	Liver	Kidney	Fat
(1.67) [1/3]			[< 0.003/< 0.003]	[0.082/0.214]	[< 0.018/0.042]	[< 0.003/ 0.003]
STMR dairy Cattle (1.40) [1/3]	- -	0.009 [0.009/0.004]	0.003 [< 0.003/< 0.003]	0.11 [0.082/0.214]]	0.023 [< 0.018/0.042]	0.003 [< 0.003/0.003]

[a] Data from the first cattle feeding study (Oakes, 1994, T021566-04).

The data from the lactating dairy cow feeding study was used to support mammalian (except marine) milk and meat maximum residue levels. In this study only free cyproconazole was determined. The ruminant metabolism study showed that conjugated cyproconazole was about 5× the free cyproconazole concentration in kidney. Therefore, the measured cyproconazole concentration in kidney was multiplied by 6 for the dietary intake calculation.

Insufficient data were provided in the dairy cow feeding study to allow estimation of milk fat levels.

The Meeting estimated the following STMR values: milk 0.009 mg/kg; muscle 0.003 mg/kg; edible offal 0.14 mg/kg; fat 0.003 mg/kg. The HR values are: muscle 0.003 mg/kg, edible offal 0.46 mg/kg, fat 0.020 mg/kg.

The Meeting estimated the following maximum residue levels for mammalian commodities (except marine): milk at 0.01 mg/kg; meat (fat) at 0.02 mg/kg and edible offal at 0.5 mg/kg.

A poultry feeding study was also available, in which 15 hens/treatment group were dosed for 29 days with cyproconazole at feed concentrations of 0.12, 0.45, and 1.87 ppm. Cyproconazole was < 0.01 mg/kg in eggs and all tissues at all feeding levels. Noting that the mean and maximum dietary burden for poultry for meat and eggs are 0.44 ppm and 1.6 ppm, respectively, the Meeting concluded that cyproconazole residues are unlikely in poultry commodities.

The Meeting estimated the following STMR values: eggs, 0.01 mg/kg; muscle, 0.01 mg/kg fat, 0.01 mg/kg; edible offal, 0.01 mg/kg. The Meeting estimated the following maximum residue levels: eggs, 0.01 mg/kg meat, 0.01 (*) mg/kg; edible offal, 0.01 (*) mg/kg. The HR values are eggs, 0.01 mg/kg; muscle, 0.01 mg/kg; fat, 0.01 mg/kg; edible offal, 0.01 mg/kg.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDIs) of cyproconazole were calculated for the 13 GEMS/Food Consumption Cluster Diets using STMRs and STMR-Ps estimated by the current Meeting (Annex 3). The ADI is 0–0.02 mg/kg bw and the calculated IEDIs were 0.5–2% of the maximum ADI. The Meeting concluded that the long-term intake of residues of cyproconazole resulting from the uses considered by the current JMPR is unlikely to present a public health concern.

Short-term intake

The International Estimated Short-Term Intakes (IESTI) of cyproconazole were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting (see Annex 4). The ARfD is 0.06 mg/kg and the calculated IESTIs were 0–5% of the ARfD for the general population and 0–4% of the ARfD for children. The Meeting concluded that

the short-term intake of residues of cyproconazole, when used in ways that have been considered by the JMPR, is unlikely to present a public health concern.