5.7 CYAZOFAMID (281)

TOXICOLOGY

Cyazofamid is the ISO-approved common name for 4-chloro-2-cyano-*N*,*N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide (IUPAC), with CAS number 120116-88-3. It is a cyanoimidazol class fungicide.

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

All critical studies contained statements of compliance with GLP.

Biochemical aspects

Cyazofamid was dose-dependently absorbed in rats: up to 84% at a low dose (0.5 mg/kg bw) and up to 6% at a high dose (1000 mg/kg bw). Peak plasma and tissue concentrations of radiolabelled cyazofamid were achieved within 1 hour after oral administration at the low and high doses. The radioactive dose was distributed primarily to the kidney and liver at the low dose and was more widely distributed at the high dose. The metabolic pathway of cyazofamid is rapid hydrolysis to form dimethylsulfonamic acid and 4-chloro-5-*p*-tolylimidazole-2-carbonitrile (CCIM). CCIM is then either oxidized at the benzonyl methyl group, resulting in 4-(4-chloro-2-cyanoimidazole-5-yl) benzoic acid (CCBA), the major urinary metabolite, or conjugated with glutathione and further metabolized to form CH_3-SO-CCIM and CH_3SO_2-CCIM, which are also excreted in the urine. Cyazofamid and its metabolites are rapidly excreted in urine at the low dose: greater than 90% excretion within 24 hours.

Toxicological data

The oral LD_{50} for cyazofamid was greater than 5000 mg/kg bw in mice and rats. The dermal LD_{50} was greater than 5000 mg/kg bw in rats. The inhalation LC_{50} was greater than 5.5 mg/L in rats. Cyazofamid was slightly irritating to the skin and eyes of rabbits. Cyazofamid was not sensitizing in the guinea-pig maximization test.

The kidney was the main target organ of cyazofamid toxicity in short- and long-term studies in rats. Cyazofamid at higher doses also decreased body weight gain in rats.

In a 6-week toxicity study in mice administered cyazofamid in the diet at a concentration of 0, 40, 200, 1000, 3500 or 7000 ppm (equal to 0, 8, 38, 193, 653 and 1419 mg/kg bw per day for males and 0, 9, 47, 248, 854 and 1796 mg/kg bw per day for females, respectively), the NOAEL was 7000 ppm (equal to 1419 mg/kg bw per day), the highest dose tested.

In a 90-day toxicity study in rats administered cyazofamid in the diet at a concentration of 0, 10, 50, 500 or 5000 ppm (equal to 0, 0.597, 2.91, 29.5 and 295 mg/kg bw per day, respectively) for males and 0, 50, 500, 5000 or 20 000 ppm (equal to 0, 3.30, 33.3, 338 and 1360 mg/kg bw per day, respectively) for females, the NOAEL was 500 ppm (equal to 29.5 mg/kg bw per day), based on effects on the kidney (i.e. basophilic tubules, increased urinary protein and pH) in males at 5000 ppm (equal to 295 mg/kg bw per day).

In a 90-day toxicity study in dogs administered cyazofamid by capsule at 0, 40, 200 or 1000 mg/kg bw per day (both sexes), the NOAEL was 1000 mg/kg bw per day, the highest dose tested.

In a 1-year toxicity study in dogs administered cyazofamid by capsule at 0, 40, 200 or 1000 mg/kg bw per day (both sexes), the NOAEL was 200 mg/kg bw per day, based on decreased spleen weight in both sexes at 1000 mg/kg bw.

The Meeting concluded that the overall NOAEL for oral toxicity in dogs was 200 mg/kg bw per day, and the overall LOAEL was 1000 mg/kg bw per day.

In an 18-month toxicity and carcinogenicity study in mice administered cyazofamid in the diet at a concentration of 0, 70, 700 or 7000 ppm (equal to 0, 9.5, 94.8 and 985 mg/kg bw per day for

males and 0, 12.2, 124 and 1203 mg/kg bw per day for females, respectively), the NOAEL was 7000 ppm (equal to 985 mg/kg bw per day), the highest dose tested. No treatment-related tumours were observed in this study.

In a 2-year study of toxicity and carcinogenicity in rats administered cyazofamid in the diet at a concentration of 0, 10, 50, 500 or 5000 ppm (equal to 0, 0.336, 1.68, 17.1 and 171 mg/kg bw per day, respectively) for males and 0, 50, 500, 5000 or 20 000 ppm (equal to 0, 2.01, 20.2, 208 and 856 mg/kg bw per day, respectively) for females, the NOAEL was 500 ppm (equal to 17.1 mg/kg bw per day), based on kidney effects (i.e. increases in blood urea nitrogen and urinary volume) in males at 5000 ppm (equal to 171 mg/kg bw per day). No treatment-related tumours were observed in this study.

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

Cyazofamid was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. No evidence of genotoxicity was found.

The Meeting concluded that cyazofamid is unlikely to be genotoxic.

On the basis of the lack of genotoxicity and the absence of carcinogenicity in mice and rats, the Meeting concluded that cyazofamid is unlikely to pose a carcinogenic risk to humans.

In a single-generation reproductive toxicity study in rats administered cyazofamid in the diet at a concentration of 0, 1000, 3000, 7000 or 20 000 ppm (equal to 0, 66.5, 200, 450 and 1327 mg/kg bw per day for males and 0, 77.5, 252, 562 and 1613 mg/kg bw per day for females, respectively), the NOAELs for maternal, reproductive and offspring toxicity were 20 000 ppm (equal to 1327 mg/kg bw per day), the highest dose tested.

In a two-generation reproductive toxicity study in rats administered cyazofamid in the diet at a concentration of 0, 200, 2000 or 20 000 ppm (equal to 0, 9.5, 94.2 and 958 mg/kg bw per day for F_0 males; 0, 13.4, 134 and 1340 mg/kg bw per day for F_0 females; 0, 8.9, 89.2 and 936 mg/kg bw per day for F_1 males; and 0, 13.7, 138 and 1400 mg/kg bw per day for F_1 females, respectively), the NOAEL for parental toxicity was 2000 ppm (equal to 134 mg/kg bw per day), based on reduced body weights in F_0 females at 20 000 ppm (equal to 1340 mg/kg bw per day). The NOAEL for reproductive toxicity was 20 000 ppm (equal to 936 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 2000 ppm (equal to 138 mg/kg bw per day), based on reduced body weights in F_1 females at 20 000 ppm (equal to 1400 mg/kg bw per day).

In a developmental toxicity study in rats administered cyazofamid by gavage at a dose of 0, 20, 100, 500 or 1000 mg/kg bw per day, the NOAELs for maternal and embryo/fetal toxicity in rats were 1000 mg/kg bw per day, the highest dose tested.

In a developmental toxicity study in rabbits administered cyazofamid by gavage at a dose of 0, 20, 100, 500 or 1000 mg/kg bw per day, the NOAELs for maternal and embryo/fetal toxicity in rabbits were 1000 mg/kg bw per day, the highest dose tested.

The Meeting concluded that cyazofamid is not teratogenic.

In an acute neurotoxicity study in rats administered cyazofamid by a single gavage dose at 0, 80, 400 or 2000 mg/kg bw, the NOAEL for acute neurotoxicity was 2000 mg/kg bw, the highest dose tested.

In a 90-day neurotoxicity study in rats administered cyazofamid in the diet at a concentration of 0, 500, 2000 or 20 000 ppm (equal to 0, 34, 134 and 1356 mg/kg bw per day for males and 0, 39, 156 and 1539 mg/kg bw per day for females, respectively), the NOAEL for subchronic neurotoxicity was 1356 mg/kg bw per day, the highest dose tested.

The Meeting concluded that cyazofamid is not neurotoxic.

In an immunotoxicity study in mice administered cyazofamid in the diet at 0, 600, 3000 or 6000 ppm (equal to 0, 136, 599 and 1381 mg/kg bw per day), the NOAEL for immunotoxicity was 1381 mg/kg bw per day, the highest dose tested.

The Meeting concluded that cyazofamid is not immunotoxic.

Biochemical and toxicological data on metabolites and/or degradates

Studies with radiolabelled CCIM demonstrated that this metabolite was more rapidly absorbed than cyazofamid itself.

Acute toxicity studies of CCIM (the first metabolite in rodents and in muscle and fat of large animals such as goats) were conducted in rats. Results of an in vitro genotoxicity test on this compound were also provided.

CCIM was more acutely toxic than the parent, with an oral LD_{50} in rats of 324 mg/kg bw. In this study, no deaths were observed at 100 and 160 mg/kg bw. Clinical signs were seen at all doses, but these were slight and occurred in only some animals at 100 mg/kg bw. CCIM did not show evidence of genotoxicity in vitro.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.2 mg/kg bw on the basis of the NOAEL of 17.1 mg/kg bw per day in a 2-year study in rats, based on kidney effects in males at 171 mg/kg bw per day. A safety factor of 100 was applied.

The ADI also applies to CCIM, as CCIM is quickly formed and as plasma or liver concentrations are quickly decomposed to CCBA, the major urinary metabolite, or are conjugated with glutathione at low doses. The ADI for the sum of cyazofamid and CCIM is expressed as cyazofamid.

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

The Meeting established an ARfD for CCIM of 0.2 mg/kg bw, on the basis of a LOAEL of 100 mg/kg bw for clinical signs identified in an acute toxicity study in rats. A safety factor of 500 was applied, including an additional factor of 5 to account for the use of a LOAEL instead of a NOAEL.

A toxicological monograph was prepared.

Levels relevant to risk assessment of cyazofamid

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen-month study of toxicity and carcinogenicity[a]	Toxicity	7 000 ppm, equal to 985 mg/kg bw per day[b]	–
		Carcinogenicity	7 000 ppm, equal to 985 mg/kg bw per day[b]	–
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 17.1 mg/kg bw per day	5 000 ppm, equal to 171 mg/kg bw per day
		Carcinogenicity	5 000 ppm, equal to 171 mg/kg bw per day[b]	–

Species	Study	Effect	NOAEL	LOAEL
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	20 000 ppm, equal to 936 mg/kg bw per day[b]	–
		Parental toxicity	2 000 ppm, equal to 134 mg/kg bw per day	20 000 ppm, equal to 1 340 mg/kg bw per day
		Offspring toxicity	2 000 ppm, equal to 138 mg/kg bw per day	20 000 ppm, equal to 1 400 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	1 000 mg/kg bw per day[b]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[b]	–
Dog[e]	Thirteen-week and 1-year studies of toxicity[d,e]	Toxicity	200 mg/kg bw per day	1 000 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.
[d] Capsule administration.
[e] Two or more studies combined.

Estimate of acceptable daily intake (ADI) for sum of cyazofamid and CCIM, expressed as cyazofamid

0–0.2 mg/kg bw

Estimate of acute reference dose (ARfD) for cyazofamid

Unnecessary

Estimate of acute reference dose (ARfD) for CCIM

0.2 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 cyazofamid

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly but dose-dependently absorbed (T_{max} < 1 h; absorption < 84% at low dose and < 6% at high dose)
Dermal absorption	No data
Distribution	Liver and kidney at low dose; more widely distributed at high dose
Potential for accumulation	No significant tissue accumulation

Rate and extent of excretion	Rapidly excreted (> 90% within 24 h)
Metabolism in animals	Hydrolysis, oxidation and conjugation with glutathione
Toxicologically significant compounds in animals and plants	Cyazofamid, CCIM
Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 5 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.5 mg/L
Rabbit, dermal irritation	Slightly irritating to skin
Rabbit, ocular irritation	Slightly irritating to eye
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Kidney/basophilic tubule (rat)
Lowest relevant oral NOAEL	29.5 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	1 000 mg/kg bw per day (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Kidney/increased blood urea nitrogen, urine volume and kidney weight (rat)
Lowest relevant NOAEL	17.1 mg/kg bw per day (rat)
Carcinogenicity	Not carcinogenic in mice or rats[a]
Genotoxicity	
	No evidence of genotoxicity[a]
Reproductive toxicity	
Target/critical effect	Reduced body weight
Lowest relevant parental NOAEL	134 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	138 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	936 mg/kg bw per day (highest dose tested; rat)
Developmental toxicity	
Target/critical effect	No toxic effect
Lowest relevant maternal NOAEL	1 000 mg/kg bw per day (highest dose tested; rat, rabbit)
Lowest relevant embryo/fetal NOAEL	1 000 mg/kg bw per day (highest dose tested; rat, rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw (highest dose tested; rat)
Subchronic neurotoxicity NOAEL	1 356 mg/kg bw (highest dose tested; rat)
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	1 381 mg/kg bw (highest dose tested; mouse)

Studies on toxicologically relevant metabolites	*CCIM* Oral LD_{50}: 324 mg/kg bw (rat) LOAEL: 100 mg/kg bw on the basis of clinical signs (rat) Not genotoxic in vitro
Medical data	
	No adverse effects noted in medical surveillance reports on manufacturing plant personnel

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

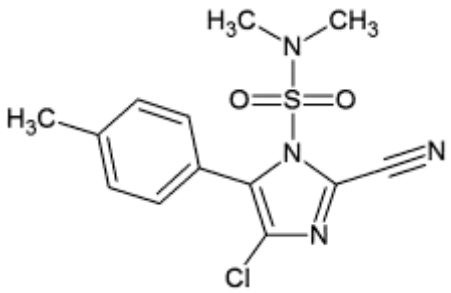
Summary

	Value	Study	Safety factor
Cyazofamid			
ADI	0–0.2 mg/kg bw	Two-year study of toxicity and carcinogenicity (rat)	100
ARfD	Unnecessary	–	–
CCIM			
ADI	Covered by ADI for parent		
ARfD	0.2 mg/kg bw	Acute toxicity study (rat)	500

RESIDUE AND ANALYTICAL ASPECTS

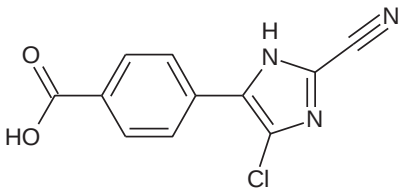
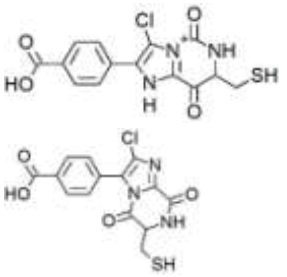
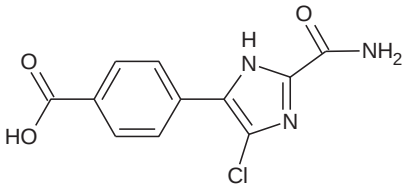
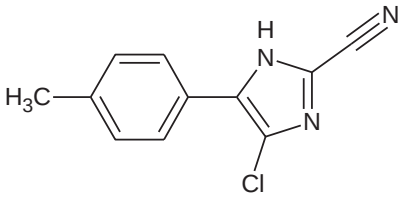
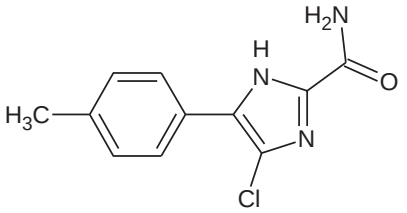
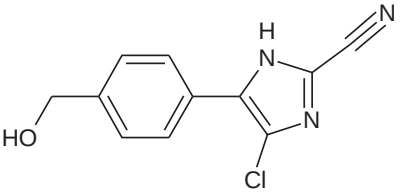
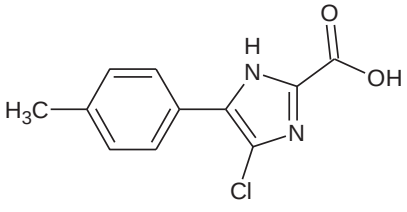
Cyazofamid (ISO common name, published) is a fungicide belonging to both the cyano-imidazole and sulphonamide classes of compounds. The biochemical mode of action is inhibition of all stages of fungal development. It was considered for the first time by the 2015 JMPR for toxicology and for residues.

The IUPAC name for cyazofamid is 4-chloro-2-cyano-*N,N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide and the CA name is 4-chloro-2-cyano-N,N-dimethyl-5-(4-methylphenyl)-1H-imidazole-1-sulfonamide, with registry number 120116-88-3.



Cyazofamid with ^{14}C radiolabelling in the benzene ring or in the imidazole ring was used in the metabolism and environmental fate studies. In this appraisal, these positions are referred to as the Bz and Im labels, respectively.

The following abbreviations, along with IUPAC names and structures, are used for the metabolites discussed in this appraisal:

CCBA	4-(4-chloro-2-cyanoimidazol-5-yl)benzoic acid	
CCBA (cysteine conjugates)		
CCBA-AM	4-(4-chloro-2-amidoimidazol-5-yl)benzoic acid	
CCIM	4-chloro-5-p-tolylimidazole-2-carbonitrile	
CCIM-AM	4-chloro-5-p-tolylimidazole-2-carboxamide	
CHCN	4-chloro-5-(4-hydroxymethylphenyl) imidazole-2-carbonitrile	
CTCA	4-chloro-5-p-tolylimidazole-2-carboxylic acid	

Plant metabolism

The Meeting received studies depicting the metabolism of cyazofamid in grapes, tomatoes, lettuce, and potatoes. All of the studies were conducted with cyazofamid which was radiolabelled, separately, in the benzene and imidazole rings.

Cyazofamid was applied five times, at ca. 100 g ai/ha at 21–25-day intervals, to grapevines growing in the field. Grapes were harvested 44 days after the last application (DALA). TRR in grapes was greater following treatment with Bz-labelled material (0.53 mg eq/kg, 0.89% of applied) than with Im-labelled material (0.31 mg eq/kg, 0.62% of applied). When processed into wine, radioactivity distributed primarily into the marc (wet pomace; 70% TRR, 3.7 mg eq/kg), with significantly lesser amounts in the vin de goutte (juice prior to pressing; 15% TRR, 0.21 mg eq/L) and vin de presse (juice after pressing; 10% TRR, 0.32 mg eq/L), indicating radioactivity may have been associated with surface residues. For grapes processed into juice, a similar trend was observed: 54% TRR (1.4mg eq/kg) in the marc and 33% TRR (0.3 mg eq/L) in the juice. Neither characterization nor identification of residues was reported in the study.

Metabolism of cyazofamid on tomatoes was investigated following treatment of field-grown plants with four foliar applications of cyazofamid at approximately 60, 95, 95, and 95 g ai/ha at 7-day intervals. In fruits harvested 1 DALA, TRR (surface rinses + juice + pulp) was 0.08 mg eq/kg from the Im treatment and 0.29 mg eq/kg from the Bz treatment. Of the total residue, the majority was contained in the surface rinse (54% and 83% for the Im and Bz labels, respectively). Of the radioactivity remaining in the fruits after rinsing, 71–81% TRR (ca. 0.033 mg eq/kg) was associated with the pulp and 13–29% TRR (ca. 5.5 mg eq/kg) was associated with the juice. Extraction of the pulp with, sequentially, hexane, ethyl acetate, and water released 75% of the radioactivity from the Bz-labelled sample and 90% from the Im-labelled sample. The principal residue from both labels was parent cyazofamid (ca. 78% TRR; 0.064 mg eq/kg Im, 0.22 mg/kg Bz), which is not unexpected given the short interval between application and harvest. The next-highest identified residue was CCIM (ca. 4–5% TRR, 0.004–0.13 mg/kg). A chromatographic fraction which was shown to consist primarily of radiolabelled sugars and citric acid accounted for 2.5–5.4% TRR (0.002–0.16 mg eq/kg), indicating breakdown of cyazofamid and incorporation into natural plant constituents.

Metabolism in lettuce was investigated following foliar treatment of glasshouse-grown plants. Three applications were made at a nominal rate of 100 g ai/ha on 14-day intervals. The test material was a mixture of cyazofamid labelled, separately, in the Im and Bz positions (in a 1:1 ratio). Lettuce leaves were harvested 14 DALA. Total radioactive residues were 0.85 mg eq/kg in the harvested leaves and 97% of the residues were extracted with ACN:H_2O (60:40, v/v with 0.1% acetic acid). Cyazofamid made up 89% of the TRR (0.76 mg/kg). No other compounds occurred at > 10% TRR. CCIM occurred at 3.7% TRR (0.031 mg/kg). Radioactivity in natural plant constituents occurred at 3.3% TRR (0.028 mg eq/kg). Based on analysis of the post-extraction solids (PES), those plant constituents consisted of starch and other water-soluble polysaccharides, protein, cellulose, and lignin.

Metabolism of cyazofamid was investigated in both field-grown and glasshouse-grown potatoes. In the field study, three foliar applications were made at rates of 100 or 400 g ai/ha. In the glasshouse study, five foliar applications were made at a rate of 400 g ai/ha. In both cases, applications were made on a 7-day interval and harvesting was done 7 DALA. In foliage, nearly all of the residue was cyazofamid. In tubers, the majority of the radioactivity was associated with the pulp. Sequential extractions of the pulp with ACN, ACN:H_2O (80:20, v/v), and ACN:H_2O (50:50, v/v) released 43 to 70% of the radioactivity, with the Bz-labelled samples generally being at the higher end of that range. In rinses of the tubers, the majority of the residue was cyazofamid (67–80% TRR, 0.0009–0.0018 mg/kg) and CCIM (14–20% TRR, 0.003 mg/kg); whereas in the tuber itself, the majority of the radioactivity was associated with starch (23–30% TRR, 0.005 mg/kg). Cyazofamid and CCIM were both < 5% TRR in tubers.

In plant metabolism studies with identification of residues, cyazofamid was the major residue in aerial portions of the plants and there was consistent demonstration of incorporation of radioactivity into natural plant components. The available data indicate that cyazofamid is translocated. The metabolite CCIM was consistently identified in these studies but never occurred at greater than 10% TRR.

Animal metabolism

The Meeting received studies elucidating the metabolism of cyazofamid in laboratory animals, lactating goats, and laying hens.

In rats, cyazofamid is well absorbed at doses relevant to dietary exposure, and rapidly metabolised, with the majority of excretion occurring via urine. In the plasma, there was no cyazofamid and the majority of radiolabel was CCIM. At 0.5 hours after a dose of [^{14}C-Bz]-CCIM, all of the radiolabel in the stomach contents was CCIM, and most of the radiolabel in liver (76.5%) and plasma (67.9%) was CCIM. CCBA, the main metabolite seen in these tissues 0.5 hours after dosing with CCIM, was also found in the blood and liver from the animals dosed with cyazofamid. Concentrations in blood and liver were greater in the CCIM-dosed animals than that in cyazofamid treated animals, suggesting that CCIM was much more rapidly absorbed than cyazofamid.

In goats dosed for five consecutive days at approximately 32 mg/animal/day (Im) or 25 mg/animal/day (Bz; both equivalent to 10 ppm in the diet), overall recovery of radioactivity was ca. 60% of the administered dose (AD). Most of the recovered radioactivity was in urine and faeces, with only 0.22% (Im) or 0.18% (Bz) of the AD accounted for in tissues. Despite the low retention of radioactivity, sufficient residues were present to characterize and identify specific compounds in all tissues. Total radioactive residues (TRR) in urine and faeces appeared to plateau by Day 3 of dosing. In milk from Bz-treated goats, TRR remained near the limit of quantification (LOQ, 0.005 mg eq/kg) for the duration of the dosing period. TRR did not plateau during the dosing period for the Im label, rising steadily from 0.005 mg eq/kg to 0.10 mg eq/kg. Aside from this difference in milk, there was little difference in the behaviour of cyazofamid based on the position of the radiolabel. Solvent (ACN or ACN:H_2O) extracted 74% TRR, 90% TRR, and 100% TRR in muscle, milk, and fat, respectively, and sequential extraction with ACN and ACN:H_2O extracted 92% TRR from kidney. For liver, the same sequential solvents used for kidney extracted only ca. 50% TRR. An additional 45% TRR was released from liver, in total, using HCl, NaOH, and protease treatments of the post-extraction solids (PES). Liver and kidney contained the highest levels of radioactivity (ca. 0.1 mg eq/kg). In other tissues and in milk, radioactivity was approximately an order of magnitude lower than in liver/kidney. Cyazofamid residues were < 0.001 mg/kg (0.1–0.3% TRR) in all tissues. The principal residues in tissues and milk were CCBA (free or cysteine-conjugated), CCIM, and their amide analogs. Total CCBA-related residues ranged from 12% TRR (< 0.002 mg/kg; muscle) to 85% TRR (0.090 mg/kg; kidney), and total CCIM-related residues ranged from 5.3% TRR (0.006 mg/kg; kidney) to 39% TRR (< 0.003 mg/kg; fat); the highest concentrations of CCIM-related residues was in liver, at 0.016 mg/kg (14% TRR). The chromatographic system used in the goat metabolism studies was generally not able to separate CCBA and its cysteine conjugate, and those residues were typically the main residues in all tissues.

In hens dosed for five consecutive days at 1.1 mg/bird/day (10 ppm in the diet), total radioactive residues (TRR) in excreta accounted for approximately 85–90% of the dosed material, and < 0.1% of the AD was retained in tissues/eggs. Total radioactive residues were < 0.006 mg eq/kg in all samples of eggs, muscle, blood, fat, and skin. Residue plateau in eggs could not be assessed. Acetonitrile + ACN:H_2O extraction was not efficient at solubilizing residues in kidney (ca. 50% TRR) and liver (ca. 30% TRR); however, chemical and enzymatic treatment of the resulting PES was able to release the unextracted residues, resulting in 100% recovery of TRR. In kidney, the only identified compounds occurring at > 10% TRR were CCBA (solvent-extracted; 12% TRR, 0.0035–0.0064 mg/kg), and CHCN conjugates (not further identified; solvent-extracted; 17% TRR, 0.005–0.010 mg/kg and PES acid hydrolysate; 30–67% TRR, 0.003–0.010 mg/kg). Two unidentified fractions from the acid-hydrolysate treatment, CM-2 and CM-3, accounted for ca. 15% TRR (0.001 mg eq/kg) each. Residue profiles in liver were similar to those in kidney, consisting of CCBA (acid hydrolysate only, 14% TRR, 0.002 mg/kg), CHCN conjugates (solvent extract, 12% TRR, 0.011 mg eq/kg; acid hydrolysate, 47% TRR, 0.0073 mg eq/kg), and CM-2/CM-3 (acid hydrolysate, 13% TRR, 0.002 mg eq/kg).

Overall, the animal metabolism studies show that the majority (99+%) of the dosed radioactivity is excreted. In goat, the principal terminal residues are CCBA, CCIM, and their related

conjugates and amides. In hens, the principal terminal residues are CCBA, CHCN, and their conjugates. Although CCBA is common to both species, the formation of that compound appears to occur through different pathways.

Environmental fate

Cyazofamid is prone to hydrolysis (25 °C, pH 4, 7, 9). The main product of hydrolysis at 25 °C at all pH levels was CCIM, which represented ca. 82% of the radioactivity at pHs of 4, 5, and 7, and 77% at pH 9. At pH 9, CCIM-AM was found at level of ca. 10% of the radioactivity. CCIM itself is stable to hydrolysis. Cyazofamid is also prone to photolysis in aqueous systems [DT_{50} of 30 minutes], forming CCIM and CCTS; both of which undergo further photolysis. In soil, photolysis does not appear to be a significant pathway for degradation since dissipation was similar in both irradiated and dark samples.

In an aerobic soil metabolism study, cyazofamid had DT_{50} estimates of ca. five days and DT_{90} estimates ranging from 16 to 25 days. The major residues following treatment with cyazofamid were CCIM (peak on Day 3, ca. 20% AD, ca 0.025 mg eq/kg), CCIM-AM (peak on Day 7, 13% AD, 0.016 mg eq/kg), and CTCA (peak ca. Day 20 at ca. 20% of the applied dose, 0.025 mg eq/kg). The aerobic soil metabolism study also showed an increase in unextracted residues over time (up to 64% at study termination) as well as production of $^{14}CO_2$ (14% of applied material by study termination). In unextracted residues, radioactivity was associated predominantly with fulvic acid as well as humin and humic acid.

In a study with confined rotational crops, bare soil was treated with 5× 100 g/ha (for both radiolabel positions on a 7-day interval). Crops of lettuce, carrot, and wheat were put into the treated soil at plant-back intervals (PBIs) of 31, 120, and 360 days. For all PBIs, residues in lettuce, carrot root, carrot tops (Days 120 and 360), and wheat grain were too low to allow residue identification/characterization. In carrot tops (Day 31 only), residues of CCBA (2.2% TRR), CCIM (10.4% TRR), CCIM-AM (39.5% TRR, 0.001 mg/kg), and cyazofamid (20.1% TRR, 0.003 mg/kg) were identified. In wheat chaff, forage and straw, residues were associated primarily with carbohydrates (0.01–0.20 mg eq/kg). Residues of cyazofamid and metabolites were ≤ 0.003 mg eq/kg in those matrices. No field rotational crop or field dissipation studies were provided. The Meeting concluded that the confined rotational crop study adequately reflects critical gap conditions and that residues are not expected in rotational crops following treatments according to the GAPs under consideration.

Overall, there are no indications that cyazofamid or any of its degradation products are expected to accumulate in soils. Significant dissipation pathways in an agricultural system appear to be hydrolysis and potentially photolysis. The DT_{90} estimates for cyazofamid in the aerobic soil metabolism study indicate that applications made more than ca. 1 month prior to harvest will not contribute significantly to the residue levels in harvested crops.

Methods of residue analysis

The Meeting received analytical methods for the analysis of cyazofamid and CCIM in plant matrices. Method validation recoveries were reported for grapes, cucurbit vegetables, root crops, Brassica vegetables, leafy vegetables, beans, peppers, and hops. Three methods for plant matrices underwent independent laboratory validation. No methods were submitted for analysis of animal materials or soil (aside from the techniques used in the studies with radiolabelled material).

In summary, extraction of residues in field trial samples was accomplished with ACN, ACN:H_2O (80:20, v/v), ACN:H_2O w/ 2% acetic acid (50:50, v/v), ACN:acetone (80:20, v/v) or acetone. Extracted residues were then generally cleaned up by partitioning into a non-polar organic solvent, with additional clean-up by solid-phase extraction (or in one case gel-permeation chromatography). Analysis of residues was by LC-MS/MS, HPLC-UV, or GC-NPD. Three methods underwent independent laboratory validation. For those methods, extraction of cyazofamid and CCIM is by ACN:acetone, H_2O followed by acetonitrile, or acetonitrile only. Clean-up varies across the three methods, consisting of traditional solid-phase extraction (C_{-18}), dispersive solid-phase extraction (magnesium sulphate, sodium chloride, sodium citrate dibasic sesquihydrate, and sodium citrate

tribasic dehydrate), or liquid/liquid partitioning (hexane and methylene chloride, sequentially) with Florisil® solid-phase extraction. For the validated methods, residue separation and quantitation is by LC-MS/MS in positive ionisation mode or by HPLC-UV (280 nm). For LC-MS/MS, evaluated ion transitions [M+H+] for quantification were 325.1 m/z→108.0 m/z for cyazofamid and 218.3 m/z→183.2 m/z for CCIM. Confirmation of cyazofamid is made using the same ion transitions, but with a cyano column on a gradient mobile phase. Confirmation of CCIM is based on a mass transition of 218.3 m/z→139.2 m/z. Based on results from other submitted studies, a confirmatory transition for cyazofamid is available (325.1 m/z→261.2 m/z). Method validation testing resulted in percent recoveries for cyazofamid ranging from 70 to 111% (except for raisins at 67%) and for CCIM ranging from 74 to 120% (except for potato chips at 68%). For both analytes in all matrices, relative standard deviations of recovery were less than 21%. An LOQ of 0.01 mg/kg was achieved for all matrices and analytes.

The solvent used for extraction is similar to that used in the metabolism studies with lettuce and potato (the first two extraction solvents in the tomato metabolism study were much less polar). On that basis, the methods are expected to have adequate extraction efficiency of incurred residues.

Testing of cyazofamid and CCIM through the FDA PAM multi-residue method protocols demonstrated that for most protocols, the test compounds showed poor sensitivity, poor recovery, and/or poor chromatography. An open-literature study [1] demonstrated good recovery of both cyazofamid (80% to 105%) and CCIM (75% to 99%) from fortified crop samples using the QuEChERS method, with relative standard deviations of ≤ 16%.

Analytical methods are available for analysis of cyazofamid and CCIM in plant commodities. Analytical methods for the analysis of cyazofamid residues in animal commodities were not provided.

Stability of residues in stored analytical samples

The Meeting received data indicating that residues of cyazofamid and CCIM are stable under frozen conditions as follows:

Matrix	Cyazofamid	CCIM
Grape (homogenized)	Up to 8 days	No Data
Grape (unhomogenized)	At least 365 days	No Data
Basil (fresh)	At least 284 days	Not stable [a] (less than 284 days)
Basil (dried)	At least 297 days	Not stable [a] (less than 297 days)
Hops cones	At least 509 days	At least 509 days
Cabbage	At least 860 days	At least 860 days
Tomato	Up to 365 days	At least 1093 days
Lettuce	At least 634 days	At least 634 days
Mustard greens	At least 977 days	At least 977 days
Spinach	At least 949 days	At least 949 days
Bean plants with pods	At least 889 days	At least 889 days
Bean pods with seeds	At least 887 days	At least 887 days
Been seeds without pods	At least 140 days	At least 140 days
Dry beans	At least 400 days	At least 400 days
Carrot	Not stable [a] (less than 374 days)	Not stable [a] (less than 374 days)
Potato	Up to 181 days	Up to 181 days

[a] Residues were measured only at the indicated storage period, and the amount remaining was < 70%. Basil and carrot samples were analysed on the same day as extraction.

Cyazofamid and CCIM were demonstrated to be stable in extracts of oilseed rape and dry beans for at least four days. Stability of these analytes in extracts from other matrices was not reported.

[1] Lee, H. Kim, E, Lee, JH. Sung, JH, Choi, H, and Kim, JH. 2014. Bull Environ Contam Toxicol 93(5):586-90. Analysis of cyazofamid and its metabolite in the environmental and crop samples using LC-MS/MS.

Definition of the residue

In plants, parent cyazofamid was the only compound to occur as a major residue in metabolism studies, and suitable methods are available for analysis. CCIM was consistently identified in metabolism studies as a minor residue and occurred at levels that were typically at least five-fold lower than cyazofamid, and typically < 0.01 mg/kg, in supervised residue trials. The Meeting considered residues of cyazofamid in rotational crops and concluded that uptake of residues from soil into rotational crops will be insignificant. Cyazofamid is expected to degrade during the production of processed products; especially those in which heating and/or hydrolysis occurs, resulting in the formation of CCIM. Nevertheless, levels of CCIM in processed commodities are generally low.

Cyazofamid exhibited low acute oral toxicity, and there was an absence of developmental toxicity and any other toxicological effects that would be likely to be elicited by a single dose. The primary plant metabolite, CCIM, however, was more acutely toxic than the parent compound and resulted in clinical signs at all doses tested in acute toxicity studies. For long-term exposures, the toxicity of CCIM is adequately addressed by parent cyazofamid.

The Meeting concluded that the residue definition for enforcement of MRLs in plant commodities is the parent compound, cyazofamid, only. Furthermore, the Meeting concluded that the residue definition for assessing long-term dietary intake from plant commodities is the combined residues of cyazofamid and CCIM, expressed as cyazofamid. An ARfD is not necessary for cyazofamid; however, the current Meeting established an ARfD for CCIM, and the residue definition for assessing short-term dietary intake from plant commodities is CCIM.

Studies depicting the nature of the residues in animals show generally low transfer of residues to tissues, milk, and eggs. Metabolism studies indicate that of the amount retained, residues are expected to be highest in offal and lower by approximately an order of magnitude in other matrices. Cyazofamid was not detected in any livestock matrix. The metabolite CCBA (free and as cysteine conjugates) was consistently found as a major residue (> 10% TRR, ranging from 0.002 mg/kg to 0.09 mg/kg) in goat and hen commodities. Data from goat kidney indicate that the cysteine conjugates form the majority of the CCBA residues (separate free/conjugated residue data were not reported for other matrices). The Meeting was uncertain about the relative amounts of free and cysteine-conjugated CCBA in tissues other than liver and about the availability of reference standards for cysteine-conjugated CCBA. The Meeting agreed not to establish residue definitions for livestock commodities.

Definition of the residue for compliance with the MRLs for plant commodities: *Cyazofamid.*

Definition of the residue for long-term dietary intake from plant commodities: *Cyazofamid and CCIM, expressed as cyazofamid.*

Noting that the current meeting established an ARfD for CCIM (in the absence of an ARfD for cyazofamid), the definition of the residue for short-term dietary intake from plant commodities is *CCIM*.

Definition of the residue for compliance with the MRLs and for dietary intake for animal commodities: *Not defined.*

Results of supervised residue trials on crops

The Meeting received supervised residue trial data for grapes, basil, hops, broccoli, cabbage, cucumber, summer squash, muskmelon, peppers, tomatoes, head and leaf lettuce, mustard greens, spinach, snap beans, lima beans, carrots, and potatoes. The trials were conducted in the USA for all crops, as well as Argentina, Europe (north and south), and Mexico for grapes; Germany for hops; Canada for lettuces; and Brazil and Canada for potatoes. For basil, residue data reflect both field and glasshouse growing conditions. All residue results are supported by adequate method and storage stability data unless otherwise noted.

For field trials with cabbage, all cabbage heads were cut in the field in order to reduce the size/weight of the sample; for lettuce and muskmelon, some samples were cut in the field. A

comparison of the residue levels in field-cut and uncut samples indicates that field-cutting did not compromise the quality of the residue data obtained from field-cut samples.

For estimating dietary intake, combined residues (cyazofamid + CCIM) were calculated by multiplying the individual sample results from field trials of CCIM by the molecular weight factor of 1.49 (cyazofamid molecular weight = 324.8, CCIM molecular weight = 217.7) and adding the result to the corresponding residue of cyazofamid. For residues below the LOQ, the residue was assumed to be at the LOQ for calculation purposes; the "less than" designation was retained only if both residues were below the LOQ. Examples are shown below:

Cyazofamid	CCIM	Combined (expressed to two significant figures)
0.5 mg/kg	0.06 mg/kg	0.5 mg/kg + (0.06 mg/kg × 1.49) = 0.59 mg/kg
0.5 mg/kg	< 0.01 mg/kg	0.5 mg/kg + (0.01 mg/kg × 1.49) = 0.51 mg/kg
< 0.01 mg/kg	0.06 mg/kg	0.01 mg/kg + (0.06 mg/kg × 1.49) = 0.099 mg/kg
< 0.01 mg/kg	< 0.01 mg/kg	< 0.01 mg/kg + (< 0.01 mg/kg × 1.49) = < 0.025 mg/kg

Grapes

In grapes, the critical GAP based on highest application rate and shortest PHI is from the registration in Germany (eight foliar applications at 0.1 kg ai/ha on a 12- to 14-day interval with a 21-day PHI). Only a single field trial is available from Germany; however, additional residue trials matching the critical GAP are available from France, Italy, Spain, and Portugal. The Meeting noted that in all of these trials, grapes were stored as whole berries and, therefore, the residue levels are supported by the available storage stability data.

Mean field trial residues of cyazofamid from independent field trials matching the critical GAP (n=7) were: 0.01, 0.03, 0.04, 0.04, 0.06, 0.09, and 0.66 mg/kg.

Based on those data, the Meeting estimated a maximum residue level for grapes of 1.5 mg/kg.

From the trials cited above, residues of CCIM were (n=7): < 0.01 (7) mg/kg. For assessing short-term dietary intake from grapes, the HR, from a single sample, is 0.01 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=7): 0.02, 0.04, 0.05, 0.06, 0.08, 0.1, and 0.67 mg/kg. For assessing long-term dietary intake from grapes, the STMR from that data set is 0.06 mg/kg.

Brassica (Cole or Cabbage) Vegetables, Head Cabbage, Flowerhead Brassicas

The critical GAP is from the registration of cyazofamid on the Brassica (Cole) leafy vegetables crop group in the USA (one soil application at 0.753 kg ai/ha followed by five foliar applications at 0.08 kg ai/ha on a 7-10-day interval with a zero-day PHI). Supervised residue trials matching this GAP are available from the USA.

Mean field trial residues of cyazofamid in broccoli from independent field trials matching the critical GAP (n=5) were: 0.23, 0.34, 0.37, 0.46, and 0.84 mg/kg.

Mean field trial residues of cyazofamid in cabbage (with wrapper leaves) from independent field trials matching the critical GAP (n=9) were: 0.13, 0.15, 0.20, 0.25, 0.28, 0.30, 0.32, 0.56, and 0.75 mg/kg.

Noting that the residue trials address crops in the Codex commodity designation Brassica (Cole or cabbage) vegetables, head cabbage, flowerhead Brassicas and that the median residues from each crop are within a 5-fold range, the Meeting determined that a group MRL is appropriate. The cyazofamid residue data across the test crops are not significantly different by the Kruskal-Wallis test; therefore, the Meeting grouped the data together and is estimating a group maximum residue level for Brassica (Cole or cabbage) vegetables, head cabbage, flowerhead Brassicas based on the following cyazofamid residue data set (n=14): 0.13, 0.15, 0.20, 0.23, 0.25, 0.28, 0.30, 0.32, 0.34, 0.37, 0.46, 0.56, 0.75, and 0.84 mg/kg.

Based on those data, the Meeting estimated a maximum residue level for Brassica (Cole or cabbage) vegetables, head cabbage, and flowerhead Brassicas of 1.5 mg/kg.

From the trials cited above, residues of CCIM were (n=14): < 0.01 (11), 0.012, 0.014, and 0.023 mg/kg. For assessing short-term dietary intake from Brassica (Cole or cabbage) vegetables, head cabbage, flowerhead Brassicas, the HR, from a single sample, is 0.025 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=14): 0.14, 0.16, 0.21, 0.24, 0.26, 0.3, 0.31, 0.33, 0.35, 0.38, 0.47, 0.58, 0.78, and 0.85 mg/kg. For assessing long-term dietary intake from Brassica (Cole or cabbage) vegetables, head cabbage, flowerhead Brassicas, the STMR from that data set is 0.31 mg/kg.

Fruiting vegetables, Cucurbits

The critical GAP is from the registration of cyazofamid on the cucurbit vegetables crop group in the USA (six foliar applications at 0.08 kg ai/ha on a 7–10-day interval with a zero-day PHI). Supervised residue trials matching this GAP are available from the USA.

Mean field trial residues of cyazofamid in cucumber from independent field trials matching the critical GAP (n=4) were: 0.01 and 0.02 (3) mg/kg.

Mean field trial residues of cyazofamid in summer squash from independent field trials matching the critical GAP (n=4) were: 0.02 (2) and 0.04 (2) mg/kg.

Mean field trial residues of cyazofamid in muskmelon from independent field trials matching the critical GAP (n=6) were: < 0.01, 0.02 (3), 0.03 (2), and 0.06 mg/kg.

Noting that the residue trials address crops in the Codex commodity designation Fruiting Vegetables, Cucurbits and that the median residues from each crop are within a 5-fold range, the Meeting determined that a group MRL is appropriate. The cyazofamid residue data across the test crops are not significantly different by the Kruskal-Wallis test; therefore, the Meeting grouped the data together and is estimating a group maximum residue level for Fruiting Vegetables, Cucurbits based on the following cyazofamid residue data set (n=14): < 0.01, 0.01, 0.02 (5), 0.03 (2), 0.04 (2), and 0.06 mg/kg..

Based on those data, the Meeting estimated a maximum residue level for Fruiting Vegetables, Cucurbits of 0.09 mg/kg.

From the trials cited above, residues of CCIM were (n=14): < 0.01 (12) and 0.01 (2) mg/kg. For assessing short-term dietary intake from Fruiting Vegetables, Cucurbits, the HR, from a single sample, is 0.01 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=14): 0.02 (2), 0.03, 0.04 (7), 0.04, 0.06 (2), and 0.08 mg/kg. For assessing long-term dietary intake from Fruiting Vegetables, Cucurbits, the STMR from that data set is 0.04 mg/kg.

Fruiting vegetables, other than Cucurbits (except Sweet Corn and Mushroom)

The critical GAP is from the registration of cyazofamid on the fruiting vegetables crop group in the USA (six foliar applications at 0.08 kg ai/ha on a 7–10-day interval with a zero-day PHI). Supervised residue trials matching this GAP are available from the USA.

Mean field trial residues of cyazofamid in peppers, sweet (including pimento or pimiento) from independent field trials matching the critical GAP (n=6) were: 0.038, 0.055, 0.058, 0.072, 0.098, and 0.22 mg/kg.

Mean field trial residues of cyazofamid in peppers, chili from independent field trials matching the critical GAP (n=3) were: 0.24, 0.25, and 0.31 mg/kg.

Mean field trial residues of cyazofamid in tomatoes from independent field trials matching the critical GAP (n=14) were: < 0.010, 0.025, 0.030 (2), 0.035, 0.040, 0.050 (4), 0.065, 0.075, 0.11, and 0.15 mg/kg.

Noting that the residue trials in the USA address crops in the Codex commodity designation Fruiting Vegetables, Other Than Cucurbits (except Sweet Corn and Mushrooms), the Meeting considered whether a group MRL is appropriate. Based on the five-fold difference in the median residue values, The Meeting concluded that a group recommendation is appropriate. Analysis of the data set by the Kruskal-Wallis test indicated that the residues are not from the same populations and should not be combined when estimating the maximum residue level. Of the crops in this category, field trials with chilli pepper resulted in the greatest median residue level and greatest overall single-sample residue; however, the number of trials on chilli pepper is insufficient for making a group recommendation and the Meeting decided to make recommendations for the individual crops.

The Meeting estimated a maximum residue levels for sweet peppers at 0.4 mg/kg, for chilli peppers at 0.8 mg/kg, and for tomato at 0.2 mg/kg. Furthermore, the Meeting extrapolated the tomato data to eggplant and estimated a maximum residue level for eggplant at 0.2 mg/kg.

From the trials cited above, residues of CCIM and their associated HRs (from single samples) for assessing short-term dietary intake were as follows:

Sweet pepper (n=5): < 0.01 (4) and 0.012 mg/kg [HR = 0.014 mg/kg]

Chili pepper (n=3): 0.012 and 0.014 (2) mg/kg [HR = 0.017 mg/kg]

Tomato (n=15): < 0.01 (13), 0.01, and 0.015 mg/kg [HR = 0.02 mg/kg]; and by extension,

Eggplant: [HR = 0.02 mg/kg].

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, and their associated STMRs for assessing long-term dietary intake were as follows:

Sweet pepper (n=5): 0.05, 0.07, 0.07, 0.09, and 0.24 mg/kg [STMR = 0.072 mg/kg]

Chilli pepper (n=3): 0.27 (2) and 0.33 mg/kg [STMR = 0.027 mg/kg];

Tomato (n=15): 0.02 (2), 0.04 (3), 0.05, 0.06 (5), 0.08, 0.09, 0.13, and 0.16 mg/kg [STMR = 0.06 mg/kg]; and by extension, Eggplant: [STMR = 0.06 mg/kg].

Leafy Vegetables (Including Brassica Leafy Vegetables)

The critical GAPs are from the registration of cyazofamid on the leafy greens crop subgroup in the USA (six foliar applications at 0.08 kg ai/ha on a 7–10-day interval with a zero-day PHI) and *Brassica* (Cole) leafy vegetables crop group in the USA (for mustard greens; one soil application at 0.753 kg ai/ha followed by five foliar applications at 0.08 kg ai/ha on a 7–10-day interval with a zero-day PHI). Supervised residue trials matching this GAP are available from Canada (head lettuce only) and the USA.

Mean field trial residues of cyazofamid in head lettuce from independent field trials matching the critical GAP (n=11) were: 0.070, 0.20, 0.26, 0.46, 0.63 (2), 0.73, 1.2, 1.5, 1.7, and 1.8 mg/kg.

Mean field trial residues of cyazofamid in leaf lettuce from independent field trials matching the critical GAP (n=11) were: 0.53, 0.76, 0.87, 0.89, 1.4, 1.8, 2.7, 2.8, 3.0, 4.0, and 4.4 mg/kg.

Mean field trial residues of cyazofamid in mustard greens from independent field trials matching the critical GAP (n=9) were: 1.4, 1.9, 3.3, 3.4, 3.5, 3.7, 5.5, 6.0, and 6.3 mg/kg.

Mean field trial residues of cyazofamid in spinach from independent field trials matching the critical GAP (n=10) were: 1.6, 2.0 (2), 2.2, 2.9, 3.3, 3.4, 3.6, 4.6, and 6.4 mg/kg.

Noting that the residue trials address crops in the Codex commodity designation Leafy Vegetables, the Meeting considered whether a group MRL is appropriate. The differences in median residue values across all four crops is greater than five-fold, indicating that a crop group recommendation is not appropriate. As median residue values for head lettuce, leaf lettuce, and spinach are within a five-fold range, the Meeting decided to make a recommendation for leafy vegetables, except Brassica leafy vegetables and to use data from mustard greens to make a recommendation for Brassica leafy vegetables.

Analysis of the residue data for lettuces and spinach by Kruskal-Wallis indicates that the residues are not from the same population and should not be combined when estimating the maximum residue level. Of these crops, the data from spinach has the highest median and highest residue.

On the basis of the data from spinach, the Meeting estimated a maximum residue level for Leafy Vegetables, except Brassica Leafy Vegetables at 10 mg/kg.

From the trials cited above, residues of CCIM and their associated HRs (from single samples) for assessing short-term dietary intake were as follows:

Head lettuce (n=11): < 0.010 (4), 0.01, 0.011, 0.013, 0.017 (2), 0.022, and 0.026 mg/kg [HR = 0.029 mg/kg];

Leaf lettuce (n=11): 0.011, 0.012, 0.016, 0.021, 0.025, 0.027, 0.037, 0.041 (2), 0.042, and 0.044 mg/kg [HR = 0.05 mg/kg];

Spinach (n=10): 0.029, 0.034, 0.045, 0.049, 0.05, 0.059, 0.088, 0.093, 0.12, and 0.14 mg/kg [HR = 0.15 mg/kg].

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, and their associated STMRs for assessing long-term dietary intake were as follows:

Head lettuce (n=11): 0.08, 0.21, 0.27, 0.47, 0.64, 0.65, 0.74, 1.2, 1.5, 1.7, and 1.8 mg/kg [STMR = 0.65 mg/kg]

Leaf lettuce (n=11): 0.55, 0.80, 0.89, 0.93, 1.4, 1.8, 2.8, 2.9, 3.0, 4.1, and 4.5 mg/kg [STMR = 1.8 mg/kg];

Spinach (n=10): 1.6, 2.0, 2.1, 2.2, 3.0, 3.4, 3.5, 3.7, 4.8, and 6.6 mg/kg [STMR = 3.2 mg/kg].

For estimating dietary intake of the combined residues of cyazofamid and CCIM from leafy vegetables, except Brassica leafy vegetables, the data from spinach provide the highest residue estimate, with an STMR of 3.2 mg/kg.

For Brassica leafy vegetables, the Meeting estimated a maximum residue level of 15 mg/kg based on the data from mustard greens.

Residues of CCIM were (n=9): 0.032, 0.035 (2), 0.05, 0.053, 0.092, 0.11, 0.15, and 0.18 mg/kg. For assessing short-term dietary intake from Brassica leafy vegetables, the HR, from a single sample, is 0.19 mg/kg.

Combined residues of cyazofamid and CCIM in Mustard Greens were (n=9): 1.6, 2.0, 3.3, 3.5, 3.7, 4.0, 5.6, 6.1, and 6.4 mg/kg. For assessing long-term dietary intake from Brassica leafy vegetables, the STMR from that data set is 3.7 mg/kg.

Beans and beans, shelled

The critical GAP is from the registration of cyazofamid on beans (succulent podded and succulent shelled) in the USA (six foliar applications at 0.08 kg ai/ha on a 7–14-day interval with a zero-day PHI). Supervised residue trials in lima beans matching this GAP are available from the USA.

Mean field trial residues of cyazofamid in lima beans from independent field trials matching the critical GAP (n=6) were: < 0.010 (5) and 0.040 mg/kg.

Mean field trial residues of cyazofamid in snap beans from independent field trials matching the critical GAP (n=8) were: 0.018, 0.046, 0.059, 0.10, 0.12, 0.19, and 0.20 (2) mg/kg.

Noting that the residue trials in the USA address crops in the Codex commodity designation Legume Vegetables, the Meeting considered whether a group MRL is appropriate. Based on the spread in the median residue values, the Meeting determined that the residues from the trials are too dissimilar and that a group MRL is not appropriate.

The Meeting used the residue data from lima beans to estimate a maximum residue level for beans, shelled of 0.07 mg/kg.

From the trials cited above, residues of CCIM were (n=6): < 0.01 (6) mg/kg. For assessing short-term dietary intake from beans, shelled, the HR, from a single sample, is 0.01 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=6): 0.025 (5), and 0.06 mg/kg. For assessing long-term dietary intake from beans, shelled, the STMR from that data set is 0.025 mg/kg.

The Meeting used the residue data from snap beans to estimate a maximum residue level for beans, except broad bean and soya bean of 0.4 mg/kg.

From the trials cited above, residues of CCIM were (n=8): < 0.01 (8) mg/kg. For assessing short-term dietary intake from beans, except broad bean and soya bean, the HR, from a single sample, is 0.01 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=8): 0.04, 0.06, 0.07, 0.12, 0.13, 0.20, and 0.21 (2) mg/kg. For assessing long-term dietary intake from beans, except broad bean and soya bean the STMR from that data set is 0.125 mg/kg.

Carrot and Potato

The critical GAP for carrots is from the registration of cyazofamid on carrots in the USA (five foliar applications at 0.175 kg ai/ha on a 14–21-day interval with a 14-day PHI). Supervised residue trials matching this GAP are available from Canada and the USA.

Mean field trial residues of cyazofamid in carrots from independent field trials matching the critical GAP (n=15) were: < 0.010 (9), 0.022 (2), 0.027, 0.029, 0.034, and 0.039 mg/kg. Carrot samples were stored frozen for 91 to 443 days prior to analysis. Stability of cyazofamid in carrots during frozen storage was not demonstrated (58% remaining at 374 days, no other time points sampled). As a result, the Meeting did not estimate a maximum residue level, HR, or STMR for carrot.

The critical GAP for potatoes is from the registration of cyazofamid on potatoes in Brazil (six foliar applications at 0.1 kg ai/ha on a 7–10-day interval with a seven-day PHI). The submitted residue trials conducted in Brazil did not match the critical GAP. However, supervised residue trials matching this GAP are available from the USA.

Mean field trial residues of cyazofamid in potatoes from independent field trials matching the critical GAP (n=23) were: < 0.010 (23). A single sample from an exaggerated rate (10-fold for the final application only) had a quantifiable residue of cyazofamid (0.02 mg/kg)

Based on those data and the results from the metabolism study, the Meeting estimated a maximum residue level for potato of 0.01* mg/kg.

From the trials cited above, residues of CCIM were: < 0.01 mg/kg. For assessing short-term dietary intake from potato, the HR, from a single sample, is 0.01 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=23): < 0.025 (23) mg/kg. Noting the low residue at the exaggerated rate, the Meeting decided to set the STMR at 0.01 mg/kg for assessing long-term dietary intake from potato.

Basil and Hops

In basil, the critical GAP is from the registration in the USA (nine foliar applications at 0.088 kg ai/ha on a 10–14-day interval with a zero-day PHI). Mean field trial residues of cyazofamid in basil (sweet) from independent field trials conducted in the USA and matching the critical GAP (n=4) were: 2.5, 2.9, 7.2, and 9.4 mg/kg.

Stability of CCIM in sweet basil was not demonstrated (47% remaining at 284 days, the only time point analysed). As the data are insufficient for evaluating dietary intake, the Meeting is not making a recommendation for residues of cyazofamid in sweet basil.

In hops, the critical GAP is from the registration in the USA (six foliar applications at 0.06–0.08 kg ai/ha on a 7–10-day interval with a 3-day PHI). Mean field trial residues of cyazofamid in dried cones from independent field trials conducted in Canada and the USA and matching the critical GAP (with DAT ranging from 2 to 4 days; n=5) were: 2.5, 2.9, 3.2, 6.3, and 7.4 mg/kg.

Based on those data, the Meeting estimated a maximum residue level for hops (dried cones) of 15 mg/kg.

From the trials cited above, residues of CCIM were (n=5): 0.13, 0.17, 0.18, 0.24, and 0.44 mg/kg. For assessing short-term dietary intake from hops (dried cones), the HR, from a single sample, is 0.45 mg/kg.

From the trials cited above, the combined residues of cyazofamid and CCIM, expressed as cyazofamid, were (n=5): 3.1, 3.2, 3.6, 6.5, and 7.5 mg/kg. For assessing long-term dietary intake from hops (dried cones), the STMR from that data set is 3.6 mg/kg.

Fate of residues during processing

High-temperature hydrolysis

The Meeting received a study investigating the high-temperature hydrolysis of cyazofamid. Samples of aqueous buffered solutions were spiked with cyazofamid at ca. 1 mg/L and put under conditions simulating pasteurisation (90 °C, pH 4, 20 min.); baking, brewing, boiling (100 °C, pH 5, 60 min); and sterilisation (120 °C, pH 6, 20 min.). Solutions were analysed by HPLC-MS/MS prior to and after processing. Cyazofamid was readily hydrolysed to CCIM (ca. 80% for pasteurisation and 100% for both baking/brewing/boiling and sterilisation).

Based on the results of the high-temperature hydrolysis study, the Meeting assumed 100% yield in the conversion of cyazofamid to CCIM in all foods other than those specified as "raw" when conducting the short-term intake assessment for CCIM.

Residues after processing

In basil, the critical GAP is from the registration in the USA (nine foliar applications at 0.088 kg ai/ha on a 10–14-day interval with a zero-day PHI). Mean field trial residues of cyazofamid in dried basil from independent field trials conducted in the USA and matching the critical GAP (n=6) were: 9.7, 13, 14 (3), and 40 mg/kg

Stability of CCIM in basil (dry) was not demonstrated (59% remaining at 297 days, the only time point analysed). As the data are insufficient for evaluating dietary intake, the Meeting is not making a recommendation for residues of cyazofamid in basil (dry).

The Meeting received data depicting the concentration/dilution of residues during processing of grapes into raisins, must and wine; tomato into paste and puree; and potatoes into wet peel, chip, and flake commodities. Processed commodities were derived using simulated commercial practices. The residue data are supported by adequate analytical methods. Storage stability data demonstrate that residues of cyazofamid and CCIM are stable in those commodities under the conditions and storage periods used in the processing studies. Residues in raw and processed commodities are supported by adequate concurrent recovery data, with the exception of cyazofamid in raisins (67±10%) and CCIM in potato chips (68±4%).

Cyazofamid did not concentrate in any processed commodity. As no concentration of residues was observed, recommendations for maximum residue levels for grapes, tomatoes, or potatoes processed commodities are not necessary. The Meeting noted that for the potato commodities, residues were < LOQ in all samples and processing factors could not be calculated; however, the tubers used in the processing study were treated at an exaggerated rate such that quantifiable residues are not expected in processed commodities even if concentration is occurring upon processing.

For estimating short-term dietary intake, the Meeting based processing factors on the combined residues of cyazofamid (as CCIM equivalents) and CCIM in raw commodities and residues

of CCIM only in processed commodities. When residues were < 0.01 in a sample, they were assumed to be 0.01 for purposes of deriving a processing factor.

For grapes, the combined residues of cyazofamid (as CCIM equivalents) and CCIM from field trials at the critical GAP were: 0.017, 0.033, 0.037, 0.050, 0.070, and 0.45 mg/kg, with an STMR of 0.044 mg/kg and an HR, from a single sample, of 0.47 mg/kg.

For tomatoes, the combined residues of cyazofamid (as CCIM equivalents) and CCIM from field trials at the critical GAP were: 0.017, 0.027, 0.030 (2), 0.033, 0.037, 0.044 (4), 0.054, 0.060, 0.075, and 0.11 mg/kg, with an STMR of 0.044 mg/kg and an HR, from a single sample, of 0.12 mg/kg.

For dried hops, the combined residues of cyazofamid (as CCIM equivalents) and CCIM from field trials at the critical GAP were: 2.1 (2), 2.4, 4.4, 5.0, and 5.1 mg/kg, with an STMR of 3.4 mg/kg and an HR, from a single sample, of 5.4 mg/kg.

For estimating long-term dietary intake, the Meeting based processing factors on the combined residues of cyazofamid and CCIM, expressed as cyazofamid, in raw and processed commodities. For all raw and processed commodities except potato, residues of parent or CCIM were quantifiable and processing factors could be derived. When residues were < 0.01 in a sample, they were assumed to be 0.01 for purposes of deriving a processing factor.

Crop	Processed commodity	Long-term processing factor [a]	Short-term yield factor [b]	Long-term processing factor [a]	Short-term yield factor [b]	STMR-P (Cyazofamid + CCIM), mg/kg	STMR-P (CCIM), mg/kg	HR-P (CCIM), mg/kg
Grape	Fruit (RAC)	–	–	–	–	STMR [c] = 0.06	STMR [d] = 0.044	HR [d] = 0.47
	Dried	0.22	0.064	0.22	0.064	0.013	0.0028	0.030
	Must	0.3, 0.5 (2), 0.59, 1.3, 1.8, 1.9	0.11, 0.25, 0.3 (3), 0.33	0.59	0.3	0.035	0.013	0.14
	Wine	0.18, 0.5 (7), 0.55, 0.66	0.11, 0.3 (7), 0.33, 0.5	0.5	0.3	0.03	0.013	0.14
Tomato	Fruit (RAC)	–	–	–	–	STMR = 0.06	STMR [d] = 0.044	HR [d] = 0.12
	Paste	0.72	0.54	0.72	0.54	0.043	0.024	0.069
	Puree	0.45	0.27	0.45	0.27	0.027	0.012	0.034
Potato	Tuber (RAC)	–	–	–	–	STMR [c] = 0.01	STMR [d] = 0.01	HR [d] = 0.01
	Chips	Not calculated		Not calculated		0.01	0.01	0.01
	Flakes	Not calculated		Not calculated		0.01	0.01	0.01
	Wet peel	Not calculated		Not calculated		0.01	0.01	0.01
Hops	Dried cones (RAC)	–	–	–	–	STMR [c] = 3.6	STMR [d] = 3.4	HR [d] = 5.4
	Beer	0.002	0.0014	0.002	0.0014	0.0072	0.0048	0.0076

[a] [Cyazofamid + CCIM (cyazofamid equivalents) in the processed commodity] ÷ [cyazofamid + CCIM (cyazofamid equivalents) in the raw commodity].

[b] CCIM in the processed commodity ÷ [cyazofamid (CCIM equivalents) + CCIM in the raw commodity].

[c] Cyazofamid + CCIM (cyazofamid equivalents)

[d] Cyazofamid (CCIM equivalents) + CCIM

Residues in animal commodities

The Meeting has not made a determination as to the residue definitions for compliance and dietary intake for animal commodities. Furthermore, the Meeting did not receive animal feeding studies or residue data for livestock feedstuffs from some crops considered in this appraisal (grape: grape pomace, beans: vines). The Meeting did not make a recommendation for animal commodities.