CYAZOFAMID

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Explanation

Cyazofamid is the International Organization for Standardization (ISO)–approved common name for 4-chloro-2-cyano-*N*,*N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide (International Union of Pure and Applied Chemistry [IUPAC]), with Chemical Abstracts Service number 120116-88-3. It is a cyanoimidazole class fungicide.

Cyazofamid has not been evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) and was reviewed by the present Meeting at the request of the Codex Committee on Pesticide Residues.

All critical studies contained statements of compliance with good laboratory practice (GLP) and were conducted in accordance with regulated test guidelines. The Meeting considered that the database was adequate for the risk assessment.

Evaluation for acceptable intake

1. Biochemical aspects

Structures of the parent compound and metabolites and degradates of cyazofamid in various crops and animals are shown in Table 1.

1.1 Absorption, distribution and excretion

(a) Absorption

A single dose of radiolabelled cyazofamid – the benzene [^{14}C-Bz] cyazofamid (lot no. CP-1863-2; purity > 99.5%) or the imidazole [^{14}C-Im] cyazofamid (lot no. CP-1947; purity > 98.5%) (see Fig. 1) – suspended in a 0.75% aqueous methyl cellulose solution at 0.5 mg/kg body weight (bw) (low dose) or 1000 mg/kg bw (high dose) was administered by gavage to five male and five female Sprague-Dawley rats. One control animal of each sex received the vehicle. Blood, urine and faeces were collected. Blood samples were taken at 0.25, 0.5, 1, 1.5, 3, 6, 8, 24, 48 and 72 hours after dosing. Data from the radioassay were used to calculate various kinetic parameters.

The kinetics data are shown in Table 2. There were no statistically significant differences in kinetics between animals of the same sex that were treated with [^{14}C-Bz]cyazofamid or [^{14}C-Im]cyazofamid. The concentration of radiolabel in the blood rose rapidly, reaching a maximum (C_{max}) within 15–30 minutes (T_{max}), regardless of dose level, sex or label position. The concentration then decreased rapidly from this time to about 1.5 hours post-dosing. The elimination phase, from which the biological half-life is derived, began about 3 hours post-dosing. The limits of radiocarbon quantification – approximately 2 ng equivalents (eq)/g for the low dose and 1 µg eq/g for the high dose – were reached within 24 hours post-dosing (Huhtanen & Savides, 1998a).

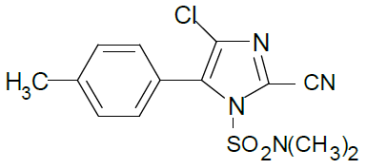
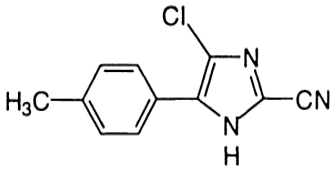
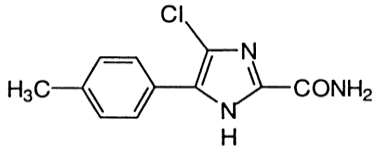
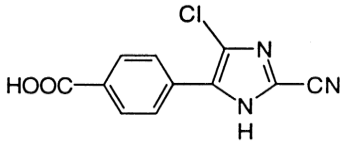
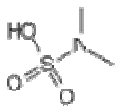
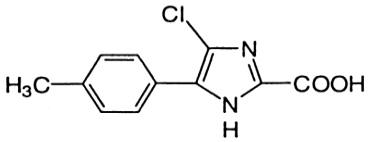
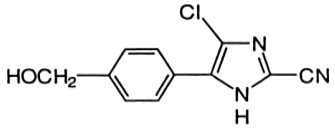
To compare the toxicokinetics of cyazofamid after different routes of administration, eight male Sprague-Dawley rats per group were treated orally or intravenously with [^{14}C]cyazofamid at a dose of 30 or 3 mg/kg bw, respectively. One animal in the intravenous treatment group was excluded from the analysis because the dose volume given and the resulting dose level were different from those for the other animals. Blood samples were collected at eight time points from 0.25 to 24 hours after each dosing.

After oral administration, absorption of total radioactivity was rapid, with maximum concentrations of radioactivity in blood attained at 0.5 hour after dosing. Absolute oral bioavailability (calculated as the area under the concentration–time curve [AUC] after a single dose from time of dosing until infinity normalized to 1 mg/kg bw in the intravenous group / the AUC after a single dose from time of dosing until infinity normalized to 1 mg/kg bw in the oral group) was 14%. The apparent terminal half-life of radioactivity in blood was slightly longer in the intravenous treatment group (5.73 hours) than in the oral dose group (4.90 hours) (Wenker, 2014).

(b) Distribution

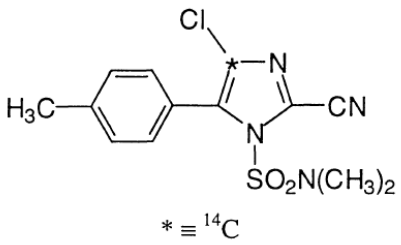
Sprague-Dawley rats (3–5 rats of each sex per group) were treated with a single dose of 0.5 mg/kg bw (low dose) or 1000 mg/kg bw (high dose) radiolabelled cyazofamid – [^{14}C-Bz]cyazofamid (lot no. CP-1863, purity ≥ 99.5%; lot no. CP-1863-2, purity ≥ 99.5%; lot no. 2074, purity ≥ 99.6%) or [^{14}C-Im]cyazofamid (lot no. CP-1864, purity ≥ 98.5%; lot no. CP-1947, purity ≥ 98.3%; lot no. CP-2073, purity ≥ 99.5%) – suspended in a 0.75% aqueous methyl cellulose solution by gavage. Two control animals of each sex received the vehicle. Experimental groups and their controls were terminated at times corresponding to 1) T_{max}, 2) the biological half-life, 3) 24 hours after dosing or 4) 168 hours after dosing. Blood and tissue samples were collected immediately following termination.

Table 1. Structures of the parent compound and metabolites and degradates of cyazofamid in various crops and animals

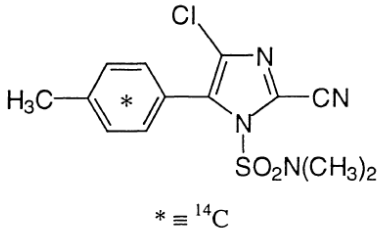
Code/common name (main sources)	Chemical name (IUPAC)	Structural formula
Cyazofamid	4-Chloro-2-cyano-*N*,*N*-dimethyl-5-*p*-tolylimidazole-1-sulfonamide	
CCIM (a first metabolite in rats, large animals, plants)	4-Chloro-5-*p*-tolylimidazole-2-carbonitrile	
CCIM-AM (large animals, plants)	4-Chloro-5-*p*-tolylimidazole-2-carboxamide	
CCBA (a major urinary metabolite in rats)	4-(4-Chloro-2-cyanoimidazol-5-yl)benzoic acid	
Dimethylsulfamic acid (proposed metabolite in rats)	Dimethylsulfamic acid	
CTCA (plants)	4-Chloro-5-*p*-tolylimidazole-2-carboxylic acid	
CHCN (rats)	4-Chloro-5-(4-hydroxymethylphenyl) imidazole-2-carbonitrile	

IUPAC: International Union of Pure and Applied Chemistry

Fig. 1. Positions of radiolabelling



Benzene [^{14}C-Bz] cyazofamid



Imidazole [^{14}C-Im] cyazofamid

Table 2. Kinetics data in rats

	[^{14}C-Bz]Cyazofamid				[^{14}C-Im]Cyazofamid			
	0.5 mg/kg bw		1 000 mg/kg bw		0.5 mg/kg bw		1 000 mg/kg bw	
Parameter	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
C_{max} (µg/g)	0.34	0.24	48.1	75.6	0.35	0.28	54.2	66.6
T_{max} (h)	0.50	0.50	0.25	0.25	0.50	0.50	0.25	0.25
$t_{½}$ (h)	4.37	4.63	7.58	9.72	4.84	5.82	10.40	11.6
AUC (h·µg/g)	0.67	0.48	103	104	0.81	0.63	96.2	102

AUC: area under the concentration–time curve; Bz: benzene; C_{max}: maximum concentration; Im: imidazole; $t_{½}$: biological half-life; T_{max}, time to reach maximum concentration
Source: Huhtanen & Savides (1998a)

In animals treated at the low and high doses and terminated at T_{max}, the mean concentration of radiolabel was highest in the kidneys, followed by the liver and the blood, at the low dose only; at the high dose, the radiolabel was diffusely distributed in various organs (Table 3). At the low dose, the concentration of radiolabel distributed in these tissues and organs was higher for [^{14}C-Bz]cyazofamid than for [^{14}C-Im]cyazofamid. The radioactivity distributed in these tissues at T_{max} was rapidly depleted from all tissues; in low-dose animals terminated at the biological half-life, mean total residues in liver and kidney were less than 0.2 µg/g in both sexes for both radiolabels. Mean total residues also decreased in tissues of high-dose animals terminated at the biological half-life, except in some dose groups in which residues in the carcass increased.

In animals terminated at 24 hours after dosing, levels of mean total radioactivity in blood were below the limit of quantification (LOQ) in three of the four low-dose groups and below 1 µg/g in three of the four high-dose groups. Mean total residues in all other tissues, including carcass, also decreased.

In low-dose animals terminated at 168 hours after dosing, mean total residues in all tissues except liver, kidney and adrenals were less than 0.001 µg/g. The levels in the liver, kidneys and adrenals were less than 0.002 µg/g (McFadden, Yoshida & Huhtanen, 1999).

(c) Excretion

Sprague-Dawley rats (3–5 rats of each sex per group) were treated with a single dose of 0.5 mg/kg bw (low dose) or 1000 mg/kg bw (high dose) radiolabelled cyazofamid – [^{14}C-Bz]cyazofamid (lot no. CP-1863, purity ≥ 99.5%; lot no. CP-1863-2, purity ≥ 99.5%; lot no. 2074, purity ≥ 99.6%) or [^{14}C-Im]cyazofamid (lot no. CP-1864, purity ≥ 98.5%; lot no. CP-1947, purity ≥ 98.3%; lot no. CP-2073, purity ≥ 99.5%) – suspended in a 0.75% aqueous methyl cellulose solution by gavage. Two control animals of each sex received the vehicle. Urine and faeces were collected continuously from

Table 3. Main tissue distribution of cyazofamid in rats

	Concentration of cyazofamid (µg/g)							
	[^{14}C-Bz]Cyazofamid				[^{14}C-Im]Cyazofamid			
	0.5 mg/kg bw		1 000 mg/kg bw		0.5 mg/kg bw		1 000 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
T_{max}								
Kidneys	1.72	1.23	64.9	69.9	0.715	0.535	35.7	57.1
Liver	0.455	0.776	25.1	41.2	0.182	0.310	23.8	31.3
Blood	0.424	0.334	28.9	34.2	0.179	0.152	22.1	29.4
Thyroids	< 0.2[a]	< 0.2[a]	22.4	28.0	< 0.2[a]	< 0.2[a]	< 10.0[a]	< 10.0[a]
Adrenals	< 0.2[a]	< 0.2[a]	13.4	< 10.0[a]	< 0.2[a]	< 0.2[a]	< 10.0[a]	15.3
Fat	< 0.2[a]	< 0.2[a]	11.0	62.4	< 0.2[a]	< 0.2[a]	10.3	10.0
Testes/ovaries	< 0.2[a]	< 0.2[a]	< 10.0[a]	21.7	< 0.2[a]	< 0.2[a]	< 10.0[a]	18.4
Lung	< 0.2[a]	< 0.2[a]	< 10.0[a]	14.6	< 0.2[a]	< 0.2[a]	< 10.0[a]	< 10.0[a]
Uterus	–	< 0.2[a]	–	12.7	–	< 0.2[a]	–	10.7
Heart	< 0.2[a]	< 0.2[a]	< 10.0[a]	10.5	< 0.2[a]	< 0.2[a]	< 10.0[a]	< 10.0[a]
168 h after dosing								
Kidneys	0.001 2	0.001 7	< 0.5[a]	< 0.5[a]	< 0.001[a]	0.001 3	< 0.5[a]	< 0.5[a]
Liver	0.001 4	0.001 7	< 0.5[a]	< 0.5[a]	< 0.001[a]	< 0.001[a]	< 0.5[a]	< 0.5[a]
Adrenals	< 0.001[a]	0.001 1	< 0.5[a]	< 0.5[a]	< 0.001[a]	< 0.001[a]	< 0.5[a]	< 0.5[a]

bw: body weight; Bz: benzene; Im: imidazole; LOQ: limit of quantification; T_{max}: time to reach maximum concentration
[a] Below the LOQ.
Source: McFadden, Yoshida & Huhtanen (1999)

the animals after the administration. As previously, each experimental group was associated with a termination time corresponding to 1) T_{max}, 2) the biological half-life, 3) 24 hours after dosing or 4) 168 hours after dosing. Urine, faeces, cage rinses, blood, tissues and carcasses were analysed for radiolabel content. Samples collected from control animals were used to determine background radioactivity.

The mean recovery of radioactivity was 97.7% for all [^{14}C-Bz]cyazofamid dose groups and 96.8% for all [^{14}C-Im]cyazofamid dose groups. Dosed radioactivity was rapidly excreted from both sexes for both radiolabels, and greater than 90% of the administered dose was excreted in 24 hours. In rats at 168 hours, less than 0.5% of the administered dose remained in the tissues in all dose groups, indicating rapid removal of cyazofamid and its metabolites from rat tissues (Table 4). At the low dose, both radiolabelled cyazofamids were excreted more in urine than in faeces in both sexes, whereas more than 95% of the radiolabelled cyazofamids administered to both sexes were excreted in faeces at the high dose (Table 4) (McFadden, Yoshida & Huhtanen, 1999).

In a repeated-dose study, an oral dose of cyazofamid at 0.5 mg/kg bw was administered once daily for 14 days to Sprague-Dawley rats. On the 15th day, a single dose of [^{14}C-Bz]cyazofamid (purity 98.9%) suspended in 0.75% methyl cellulose at 0.5 mg/kg bw was administered orally, and the distribution and elimination of the dose were determined in two rats of each sex at 0.5, 24 and 168 hours after the last treatment.

Table 4. Excretion of cyazofamid in urine and faeces of rats

	% of administered dose							
	[^{14}C-Bz]Cyazofamid				[^{14}C-Im]Cyazofamid			
	0.5 mg/kg bw		1 000 mg/kg bw		0.5 mg/kg bw		1 000 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
24 h after dosing								
Urine[a]	60.5	50.9	2.1	2.1	60.8	44.7	2.7	1.9
Faeces	39.1	47.4	99.0	89.1	32.0	50.2	92.2	95.0
Tissues	0.70	3.77	2.50	10.17	1.27	2.08	4.09	4.55
168 h after dosing								
Urine[a]	64.8	50.8	2.6	2.6	68.2	49.0	3.6	2.1
Faeces	30.4	44.8	94.2	95.7	29.7	46.7	96.9	97.5
Tissues	0.07	0.04	0.00	0.00	0.27	0.23	0.03	0.02

bw: body weight; Bz: benzene; Im: imidazole

[a] Including the recovery from cage wash.

Source: McFadden, Yoshida & Huhtanen (1999)

Repeated administration of cyazofamid resulted in a somewhat higher excretion in urine than was found after a single dose. At 168 hours after the treatment with radiolabelled cyazofamid, 62.8–72.8% of the administered dose was excreted in urine, and 20.8–31.6% of the administered dose was excreted in faeces (McFadden & Savides, 1999).

The biliary excretion of ^{14}C-labelled cyazofamid was determined following administration of [^{14}C-Bz]cyazofamid or [^{14}C-Im]cyazofamid suspended in a 0.75% aqueous methyl cellulose solution by oral gavage to male and female bile duct–cannulated Sprague-Dawley rats at 0.5 mg/kg bw (low dose) or 1000 mg/kg bw (high dose). Three rats of each sex per dose level and label group were compared, except for [^{14}C-Im]cyazofamid, which had four rats of each sex at the low dose. The animals were terminated 72 hours after dosing.

At the low dose, both radiolabelled cyazofamids were mainly excreted in the urine, with lesser amounts found in faeces and bile (Table 5). At the high dose, the radiolabelled cyazofamids were mainly excreted in the faeces. For each group, the faecal radiocarbon accounted for more than 94.7% of the administered dose. However, the biliary radiocarbon collected over the 72-hour period accounted for approximately 1% of the administered dose for the males and 1.3% for the females. The biliary excretion was rapid, with the maximum levels of radiocarbon collected within the first 2 hours after dosing (Huhtanen & Savides, 1998b).

1.2 Biotransformation

(a) In vivo

[^{14}C-Bz]Cyazofamid (lot no. CP-1863-2; purity 99.5%) at 0.5 mg/kg bw or [^{14}C-Bz]CCIM (lot no. CP-1982; purity 97.6%) at 0.33 mg/kg bw was administered by oral gavage to male Sprague-Dawley rats, and the rats were terminated 0.5 hour later. Each of the two groups consisted of five animals. Blood was collected by exsanguination at termination. Liver and stomach (with contents) were also collected at termination. Blood, liver and stomach contents were analysed for radiolabel content and were used for the characterization of metabolites to identify the pathway of metabolism.

At 0.5 hour after dosing with [^{14}C-Bz]cyazofamid, most (97.2%) of the radiolabel in the stomach contents was the parent, and a small fraction was CCIM. Analysis of the liver from this

Table 5. Excretion of radiolabel in bile, urine and faeces of rats

	% of administered dose							
	[^{14}C-Bz]Cyazofamid				[^{14}C-Im]Cyazofamid			
	0.5 mg/kg bw		1 000 mg/kg bw		0.5 mg/kg bw		1 000 mg/kg bw	
	Males	Females	Males	Females	Males	Females	Males	Females
Bile	22.1	38.8	0.8	1.4	12.2	28.9	1.1	1.3
Urine[a]	61.6	40.5	5.2	3.6	41.0	43.6	4.1	2.7
Faeces	9.8	18.6	95.0	96.0	42.3	22.4	94.7	94.7

bw: body weight; Bz: benzene; Im: imidazole
[a] Including the recovery from cage wash.
Source: Huhtanen & Savides (1998b)

group showed that only 6.1% of the radiolabel was cyazofamid, whereas 24.2% was CCIM, and 41.9% was CCBA. In the plasma, there was no cyazofamid, and the majority of radiolabel was CCIM. These data demonstrate that a dose of cyazofamid is rapidly metabolized by hydroxylation and that CCIM is the first metabolite of cyazofamid.

At 0.5 hour 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 hour after dosing with CCIM, was also found in the blood and liver from the animals dosed with cyazofamid. Concentrations of CCIM in blood and liver were similar to those found after dosing with cyazofamid, suggesting that CCIM was absorbed similarly to cyazofamid (Table 6).

The metabolism of ^{14}C-labelled cyazofamid was determined following administration of [^{14}C-Bz]cyazofamid or [^{14}C-Im]cyazofamid suspended in a 0.75% aqueous methyl cellulose solution by oral gavage to male and female bile duct–cannulated Sprague-Dawley rats at 0.5 mg/kg bw (low dose) or 1000 mg/kg bw (high dose). Study details are as described in section 1.1 (Huhtanen & Savides, 1998b).

Table 6. Summary of tissue residues 0.5 hour after a single dose treatment with [^{14}C-Bz]cyazofamid and [^{14}C-Bz]CCIM in rats[a]

	% of dose in region of interest					
	[^{14}C-Bz]Cyazofamid			[^{14}C-Bz]CCIM		
	Stomach contents	Liver	Plasma	Stomach contents	Liver	Plasma
Cyazofamid	97.2	6.1	ND	ND	ND	ND
CCIM	2.8	24.2	61.7	100.0	76.5	67.9
CHCN	ND	ND	4.0	ND	3.8	5.6
CCBA	ND	41.9	34.4	ND	18.2	26.6
Unknown	ND	27.2	ND	ND	ND	ND
Total	100.0	99.9	100.1	100.0	98.9	100.1

Bz: benzene; Im: imidazole; ND: not detected
[a] For full names of metabolites, see Table 1.
Source: Murray & Savides (1999)

Table 7. Characterization of metabolites in bile, urine and faeces of rats 72 hours after dosing at 0.5 mg/kg bw[a]

	% of administered dose							
	Females				Males			
	Bile	**Urine**	**Faeces**	**Total**	**Bile**	**Urine**	**Faeces**	**Total**
Cyazofamid	ND	ND	15.0	15.0	ND	ND	21.4	21.4
CCBA	4.5	37.9	ND	42.4	4.1	49.8	ND	53.9
CCIM	ND	ND	< 0.4	< 0.4	ND	ND	< 0.5	< 0.5
Conjugates	29.4	4.2	ND	33.6	13.3	1.5	ND	14.8
Non-extractables	ND	ND	3.2	3.2	ND	ND	2.2	2.2

bw: body weight; ND: not detected

[a] For full names of metabolites, see Table 1.

Source: Huhtanen & Savides (1998b)

As shown in Table 7, the main metabolites were CCBA, at 4.1–4.5% of the administered dose in bile and 37.9–49.8% of the administered dose in urine; and the conjugates of CCBA, CCIM and CHCN, at 13.3–29.4% of the administered dose in bile and 1.5–4.2% of the administered dose in urine. Unchanged cyazofamid was detected in faeces at 15.0–21.4% of the administered dose (Huhtanen & Savides, 1998b).

Sprague-Dawley rats (3–5 rats of each sex per group) were treated by gavage with a single dose of 0.5 mg/kg bw (low dose) or 1000 mg/kg bw (high dose) radiolabelled cyazofamid – [^{14}C-Bz]cyazofamid (lot no. CP-1863, purity ≥ 99.5%; lot no. CP-1863-2, purity ≥ 99.5%; lot no. 2074, purity ≥ 99.6%) or [^{14}C-Im]cyazofamid (lot no. CP-1864, purity ≥ 98.5%; lot no. CP-1947, purity ≥ 98.3%; lot no. CP-2073, purity ≥ 99.5%) – suspended in a 0.75% aqueous methyl cellulose solution. Two control animals of each sex received the vehicle. Urine and faeces were collected continuously from the animals after the administration. Selected urinary and faecal samples were used for the characterization and/or isolation of metabolites by high-performance liquid chromatography. This study was the same as the excretion study described above.

CCBA, CH_3SO-CCIM and CH_3SO_2-CCIM accounted for more than 90% of the urinary radiocarbon in urine from animals at 168 hours. CCBA was the major metabolite in the urine in males at the low and high doses and in females at the high dose. In the urine from females in both low-dose groups, the average CCBA content accounted for over 50% of the urinary radiocarbon. The total percentage of the metabolites CH_3SO-CCIM and CH_3SO_2-CCIM accounted for approximately 30% of the urinary radiocarbon from low-dose female rats (McFadden, Yoshida & Huhtanen, 1999).

(b) In vitro

To examine the metabolism of cyazofamid in vitro, blood from male Sprague-Dawley rats was fortified with 0.40 µg/mL of [^{14}C-Bz]cyazofamid (lot no. CP-1863-2-P; purity 97.5%) or with 0.27 µg/mL of [^{14}C-Bz]CCIM (lot no. CP-1982; purity 97.6%). After the fortification, the samples were incubated in a water bath at 37 °C and analysed at 0, 15, 30 and 60 minutes for cyazofamid and at 0 or 60 minutes for CCIM. Stomach content samples from male Sprague-Dawley rats were fortified with [^{14}C-Bz]cyazofamid (lot no. CP-1863-2-P; purity 97.5%) or with [^{14}C-Bz]CCIM (lot no. CP-1982; purity 97.6%). After the fortification, the samples with cyazofamid or CCIM were analysed 0 and 60 minutes after incubation in a water bath at 37 °C.

The in vitro metabolism of cyazofamid and CCIM in blood and stomach is shown in Table 8.

Table 8. In vitro metabolism of cyazofamid in blood and stomach

Sampling time (min)	Average % of applied dose						
	Cyazofamid				CCIM		
	Cyazofamid	CCIM	Others	Total	CCIM	Others	Total
Blood							
0	94.0	3.0	1.6	98.6	95.2	3.1	98.3
15	79.6	14.1	1.9	95.6	NE	NE	NE
30	74.5	17.6	0.9	93.1	NE	NE	NE
60	62.1	31.3	1.7	95.1	96.1	2.6	98.7
Stomach							
0	101.4	ND	2.2	103.6	103.6	ND	106.1
60	104.7	ND	2.1	106.8	106.8	ND	98.8

ND: not detected; NE: not examined
Source: Sakai, Tada & Kanza (1999)

Cyazofamid was metabolized rapidly in blood. After 60 minutes, approximately 30% of the cyazofamid was metabolized, mainly to CCIM. CCIM was stable in blood. Both cyazofamid and CCIM were stable in the stomach contents. CCIM was a major first metabolite of cyazofamid in rats.

Based on the results of these studies, it can be concluded that the metabolism of cyazofamid proceeds through formation of CCIM as the main pathway. A proposed metabolic scheme in rats is presented in Fig. 2 (Sakai, Tada & Kanza, 1999).

2. Toxicological studies

2.1 Acute toxicity

Studies on the acute toxicity, skin or eye irritation, and skin sensitization potential of cyazofamid are summarized in Table 9.

(a) Lethal doses

Oral administration

Cyazofamid was dispersed in 0.5% aqueous methyl cellulose and administered at 5000 mg/kg bw to five CD-1 mice of each sex per group.

No deaths were observed. No treatment-related clinical findings and no effects on body weight were observed in the mice. The oral median lethal dose (LD_{50}) of cyazofamid in mice was greater than 5000 mg/kg bw (Yoshida & Watson, 1999).

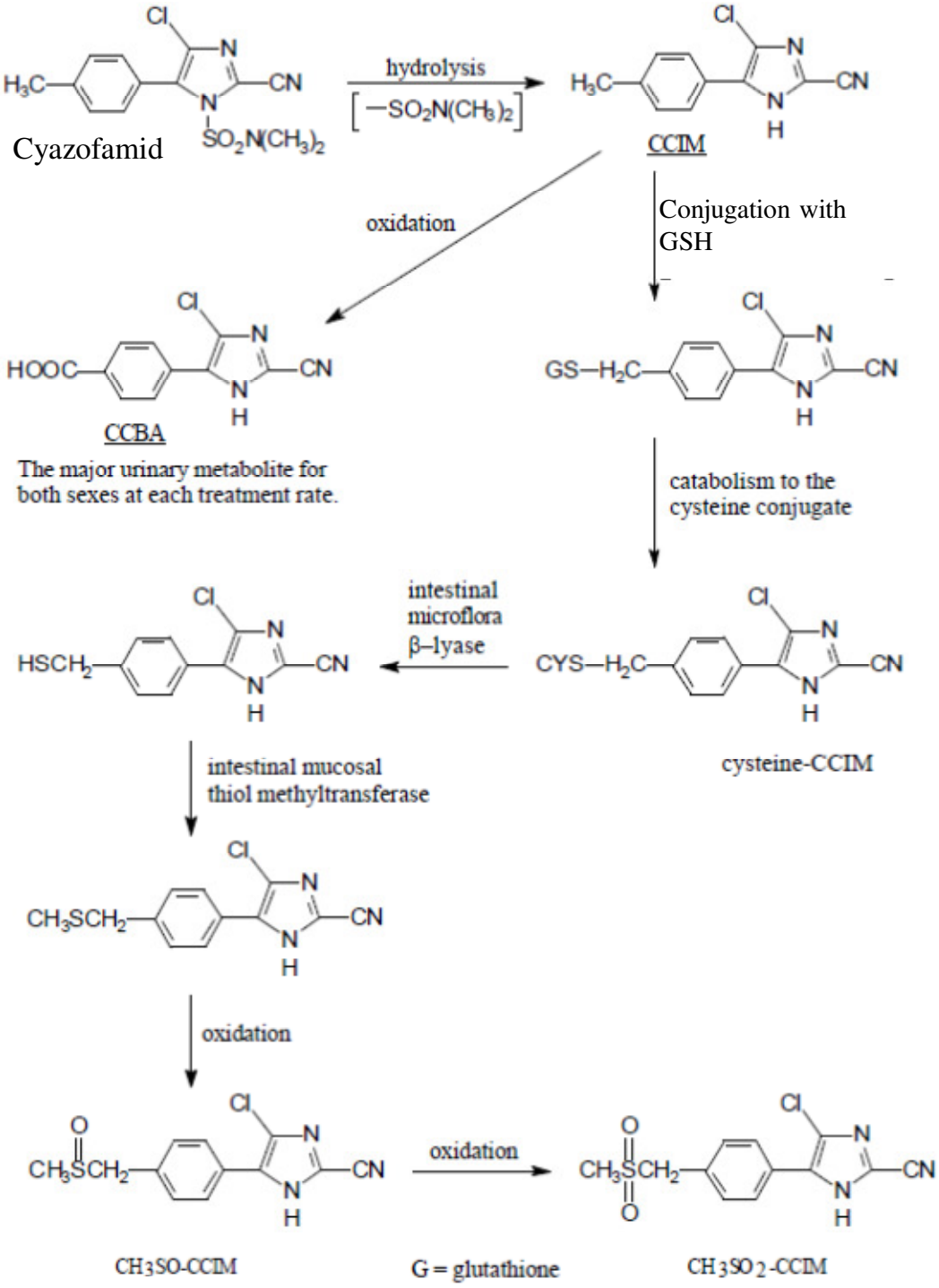
Cyazofamid was dispersed in 0.5% aqueous methyl cellulose and administered at 5000 mg/kg bw to five Sprague-Dawley rats of each sex per group.

No deaths were observed. No treatment-related clinical findings and no effects on body weight were noted in the rats. The oral LD_{50} of cyazofamid in rats was greater than 5000 mg/kg bw (Yoshida, Brock & Watson, 1998b).

Dermal application

Slightly moistened (distilled water) cyazofamid was applied to the shaved skin of five Sprague-Dawley rats of each sex at 2000 mg/kg bw. The test material was covered with a gauze patch

Fig. 2. Proposed metabolic pathway of cyazofamid



GSH: glutathione

Source: Huhtanen & Savides (1998c)

Table 9. The acute toxicity, skin or eye irritation, and skin sensitization potential of cyazofamid

Route (method)	Species/ strain	Lot no.; purity	Result	Reference
Oral (gavage)	CD-1 mice	9506; 95.7%	LD_{50}: > 5 000 mg/kg bw in both sexes	Yoshida & Watson (1999)
Oral (gavage)	Sprague-Dawley rats	9506; 95.5%	LD_{50}: > 5 000 mg/kg bw in both sexes	Yoshida, Brock & Watson (1998b)
Dermal	Sprague-Dawley rats	9506; 95.5%	LD_{50}: >2 000 mg/kg bw in both sexes	Yoshida, Brock & Watson (1998a)
Inhalation (nose only)	Sprague-Dawley rats	9506; 95.5%	LC_{50}: > 5.5 mg/L in both sexes	Ulrich (1998)
Skin irritation	NZW rabbits	9506; 95.5%	Slightly irritating	Yoshida, Brock & Watson (1998c)
Eye irritation	NZW rabbits	9506; 95.5%	Slightly irritating	Yoshida, Brock & Watson (1998d)
Dermal sensitization (maximization method)	Hartley guinea-pig	9506; 95.5%	Not sensitizing	Yoshida, Brock & Watson (1998e)

bw: body weight; LC_{50}: median lethal concentration; LD_{50}: median lethal dose; NZW: New Zealand White

backed with hypoallergenic tape and was secured in place by an elastic wrapping. After a 24-hour exposure period, the cyazofamid was removed, and all rats were euthanized 14 days after the treatment.

No deaths were observed. Very slight erythema at the application site and incidences of dried red material around the eyes and/or nose and of anogenital staining were observed within several days after the treatment. These dermal changes were considered to be indicative of the slight irritant potential of cyazofamid. The dermal LD_{50} of cyazofamid in rats was greater than 2000 mg/kg bw (Yoshida, Brock & Watson, 1998a).

Inhalation

Five Sprague-Dawley rats of each sex per group were given a single inhaled exposure to a dust aerosol of cyazofamid at a concentration of 5.5 mg/L via whole-body exposure for 4 hours. The aerosol was characterized by a mass median aerodynamic diameter of 2.9 µm, with a geometric standard deviation of 2.2. Fourteen days after the inhalation, all rats were euthanized.

No deaths were observed. After the exposure, rales were noted in two animals. There were no other treatment-related clinical signs. Slight body weights were decreased in four female rats from day 0 to day 3; however, mean body weights for the female rats increased thereafter. No other differences were noted in body weights or body weight gains. There were no treated-related changes at necropsy. The inhalation median lethal concentration (LC_{50}) of cyazofamid in rats was greater than 5.5 mg/L in both sexes (Ulrich, 1998).

(b) Dermal irritation

Cyazofamid was administered to a shaved dorsal skin site on each of three male and three female New Zealand White (young adult) rabbits. Cyazofamid was applied at each site over a 4-hour exposure period by the use of a gauze patch and bandage.

Slight erythema (scores 1–2) was observed in all animals 30–60 minutes after exposure. This change was continued in three animals at the same severity as observed at 30–60 minutes up to 72 hours after the exposure, but disappeared in the remaining rabbits. No findings were noted in any of

the rabbits thereafter. Cyazofamid was considered slightly irritating to the skin of rabbits (Yoshida, Brock & Watson, 1998c).

(c) Ocular irritation

A dose of 0.09 g of cyazofamid was administered to the conjunctival sac of the right eye of each of nine New Zealand White rabbits. In the washed group (three animals), both the treated and untreated eyes of each animal were flushed with deionized water for approximately 30 seconds approximately 30 seconds after instillation.

Conjunctival redness, chemosis and/or discharge were observed in all animals of the unwashed group at 1 hour after the treatment. Most of these effects disappeared within 24 hours, although mild redness and/or discharges lasted through 48 hours. Conjunctival redness, chemosis and discharge were also noted in all animals of the washed group at 1 hour after administration. These effects disappeared 24 hours after the administration. Cyazofamid was considered slightly irritating to the eyes of rabbits (Yoshida, Brock & Watson, 1998d).

(d) Dermal sensitization

The method of Magnusson and Kligman (maximization design) was utilized. Cyazofamid was injected intradermally into sites on the scapular region of each of 10 male and 10 female Hartley guinea-pigs at a 1% (weight per volume [w/v]) concentration in sterile distilled water at the study initiation (day 0) and applied topically at a 75% (w/v) concentration in distilled water on day 7. Two weeks after the application (day 21), the test material was topically administered on the left flank of each of the animals at a 25% (w/v) concentration in distilled water. One week after the initial challenge dose (day 28), the animals were rechallenged at the same concentration. Dinitrochlorobenzene was used as a positive control.

The sensitization rates for cyazofamid were less than 25% at 48 hours in both the initial challenge and rechallenge phases. The results indicate that cyazofamid is not sensitizing in the maximization test (Yoshida, Brock & Watson, 1998e).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

Cyazofamid (lot no. 9506; purity 95.5%) was administered in the diet to 10 CD-1 mice of each sex per group at 0, 40, 200, 1000, 3500 or 7000 parts per million (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) for 6 weeks. Feed and water were available ad libitum. The mice were checked for viability twice a day. Physical examinations, body weights and feed consumption were recorded weekly. After 6 weeks of treatment, surviving animals had blood withdrawn from the orbital sinus under anaesthesia with ether and were necropsied. The adrenals, brain, liver with gallbladder, kidneys and testes were weighed. Haematology and blood chemistry examinations were performed. Histopathological examination was conducted for adrenals, bone marrow (sternum), kidneys, lungs, heart, liver, spleen, and ovaries or testes in the control and 7000 ppm groups and for any gross lesions.

There were no treatment-related deaths or clinical signs in any treated group. Although slightly depressed body weights and lower body weight gains were observed at weeks 5 and 6 in males and at weeks 0–6 in females at 1000 ppm and higher, the decreases were less than 10% and were not statistically significant. Therefore, they were considered not to be toxicologically significant.

There were no treatment-related changes in feed consumption, haematology or blood biochemistry. Liver weights relative to body weight were statistically significantly increased at 7000 ppm in both sexes, but the increases in both sexes were less than 10% and were not accompanied by any histopathological findings indicating hepatotoxicity. The increases were therefore considered not

to be toxicologically significant. Microscopically, no treatment-related changes were observed in any treated groups.

The no-observed-adverse-effect level (NOAEL) for 6-week oral toxicity in mice was 7000 ppm (equal to 1419 mg/kg bw per day), the highest dose tested (O'Meara, Serrone & Lorence, 1996).

Rats

In a 28-day oral toxicity study, cyazofamid (lot no. 9506; purity 97.3%) was administered to six F344/DuCrj rats of each sex per group at a dietary concentration of 0, 50, 500, 5000 or 20 000 ppm (equal to 0, 3.77, 38.5, 370 and 1490 mg/kg bw per day for males and 0, 3.64, 37.1, 389 and 1540 mg/kg bw per day for females, respectively) for 4 weeks. Clinical signs were monitored daily. Body weights and feed consumption were measured weekly. At termination, all animals were necropsied after urine analysis, haematology and blood chemistry examinations. The brain, pituitary, thyroid (including parathyroid), thymus, heart, liver, spleen, kidneys, adrenals, and testes or ovaries were weighed. Histopathological examination was conducted for the livers and kidneys from all animals.

All animals survived the study. There were no treatment-related clinical signs or changes in body weight or feed consumption. In haematological analysis, a very slight increase in mean corpuscular haemoglobin was not considered to be treatment related. In blood biochemistry, glucose level was increased at 5000 ppm and above in males, triglyceride level was decreased at 20 000 ppm in males, and chloride concentration was very slightly increased at 5000 ppm and above in both sexes. Total protein and globulin levels were slightly, but statistically significantly, increased at all treated doses in males. In addition, albumin level was slightly increased at the two highest doses in males. All changes were not considered to be toxicologically significant because they were slight increases (< 10%) compared with the control values. In addition, relative liver weight was slightly (approximately 10%) increased at 5000 ppm and above in males, and absolute liver weight was increased at 20 000 ppm in males. These increases were not considered to be toxicologically significant, as there were no histopathological findings indicating hepatotoxicity. In the kidney, relative weight was increased at 20 000 ppm in males. Microscopically, basophilic tubules in the kidney were increased at incidences of 4/6 at 5000 ppm and 6/6 at 20 000 ppm in males. The severity of the lesion at 5000 ppm was less than that at 20 000 ppm. In females, no histopathological changes were observed.

The NOAEL for the 28-day rat oral toxicity study was 500 ppm (equal to 38.5 mg/kg bw per day), based on histopathological changes in the kidney in male rats at 5000 ppm (equal to 370 mg/kg bw per day) (Nakashima, 1999a).

In a 90-day oral toxicity study, cyazofamid (lot no. 9506; purity 95.5%) was administered in the diet to 12 F344/DuCrj rats of each sex per group for 13 weeks at 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 at 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 rats were checked daily for clinical signs. Body weights and feed consumption were measured weekly. Ophthalmological examination was conducted for the males and females in the control and high-dose groups at week 13. All animals were subjected to urine analysis at week 13. After the 13-week treatment period, the animals were subjected to haematology and blood biochemistry examinations, organ weight analysis, necropsy and histopathology. At necropsy, the brain, liver, kidneys, adrenals and testes were weighed.

There were no deaths and no treatment-related clinical signs during the study. There were no treatment-related differences in body weight or feed consumption. There were no ocular abnormalities in either sex at the high dose. In urine analysis, significant increases in protein and urine volume were observed in males at 5000 ppm (Table 10). Urinary pH was also significantly elevated in this group. Other statistically significant differences in urine analysis parameters were not observed in the males

Table 10. Summary of urine analysis, blood biochemistry, organ weight and microscopic findings in a 90-day oral toxicity study in rats

	Males					Females				
	0 ppm	**10 ppm**	**50 ppm**	**500 ppm**	**5 000 ppm**	**0 ppm**	**50 ppm**	**500 ppm**	**500 ppm**	**20 000 ppm**
Urine analysis										
Protein grade −/+/++[a]	1/9/2	0/6/6	0/9/3	1/5/6	0/4/8*	1/10/1	3/9/0	2/9/1	5/7/0	4/8/0
Volume (mL)	6.4	6.3	6.2	6.4	7.6**	4.9	5.0	4.7	4.8	6.0
pH										
6.0	–	–	–	–	–	–	–	3	4	–
6.5	1	6	8	7	–	–	3	5	4	–
7.0	–	4	3	4	–	1	7	3	3	–
7.5	7	2*	1**	1	2	2	2	1*	1*	4
8.0	8	–	–	–	10*	7	–	–	–	7
8.5	–	–	–	–	–	2	–	–	–	–
Blood biochemistry										
Chlorine (meq/L)	104.5	105.1	105.0	105.1	106.0**	108.0	107.9	108.6	107.4	108.5
Total cholesterol (mg/dL)	50	49	52	49	44**	56	59	58	57	55
Triglyceride (mg/dL)	130	135	132	132	99**	54	59	56	53	51
Organ weights										
Relative liver weight[b]	2.28	2.31	2.32	2.28	2.36	2.25	2.18	2.16	2.27	2.40*
Relative kidney weight[b]	0.59	0.57	0.58	0.56*	0.60	0.63	0.63	0.62	0.66*	0.66*
Histopathological findings										
Basophilic tubule in the kidney	0	0	0	0	12	0	0	0	0	0

eq: equivalents; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$

[a] Grade of urine protein: −: negative; +: slight; ++: moderate.

[b] Relative weight: absolute weight (g) / body weight (g) × 100.

Source: Nakashima (1999b)

or in all the females. There were no significant haematology changes in any treated group of either sex. In blood biochemistry, treatment-related changes were increased chlorine and total cholesterol levels and decreased triglyceride levels in males at 5000 ppm. No other treatment-related differences were noted in the analysis (Table 10).

No treatment-related macroscopic findings were observed. There was no difference in organ weights in males. In females, relative kidney weights at 5000 ppm and above and relative liver weights at 20 000 ppm were significantly increased compared with controls. Other changes noted were not related to dose. The very slight increases in relative kidney weights, which were less than 5% and not accompanied by alterations in parameters related to renal toxicity, were not considered to be treatment related. Microscopically, an incidence of basophilic tubules was significantly increased

in the kidneys of all male rats at 5000 ppm. This change was exclusively noted at the portion of the pars recta of the proximal tubules. There were no other microscopic treatment-related changes in any treated groups of females or in males in the 500 ppm group and lower. The kidney is considered a major target organ for cyazofamid in males based on the changes in urine analysis parameters and histopathological analysis.

The NOAEL for the 90-day oral toxicity study in rats was 500 ppm (equal to 29.5 mg/kg bw per day), based on kidney effects in males at 5000 ppm (equal to 295 mg/kg bw per day) (Nakashima, 1999b).

Dogs

In a 28-day oral toxicity study, cyazofamid (lot no. 9506; purity 96.4%) was administered by capsule to two Beagle dogs of each sex per group at 10, 100 or 1000 mg/kg bw per day. A control group received capsules only. Haematology, blood chemistry and urine analysis were performed prior to initiation of test material administration and at termination of the treatment period. All animals were necropsied, and the adrenals, brain, heart, liver, kidneys, ovaries or testes, and thyroid with parathyroids were weighed. All tissues were fixed except ear, cervix, vagina and tongue from the control group animals. All dogs in the treatment groups were examined microscopically.

There were no deaths during the treatment period. No treatment-related changes were observed in clinical signs, body weights, body weight gain, feed consumption, haematology, blood biochemistry, gross necropsy findings, organ weights or histopathology in any treatment group. Both high-dose females exhibited diffuse, unilateral mottling of one lobe of the lung, which may be explained by the histopathological findings suggesting inhalation of regurgitated stomach contents. Therefore, these findings were not considered to be treatment related.

There were no treatment-related effects observed in this study up to 1000 mg/kg bw per day, the highest dose tested (Savides, Yoshida & Watson, 1998).

In a 90-day oral toxicity study, cyazofamid (lot no. 9506; purity 96.2%) was administered by capsule to four Beagle dogs of each sex per group at 40, 200 or 1000 mg/kg bw per day for 13 weeks. Control animals received empty capsules equal to the number of capsules required for the high-dose animals. Animals were observed twice daily for mortality and morbidity, cage-side observations were made for signs of toxic effects approximately 1 hour after test material administration, and a detailed physical examination of each animal was performed weekly. Feed consumption and body weights were measured weekly. An ophthalmological examination was performed on each animal prior to and after 13 weeks of treatment. Haematology, blood biochemistry and urine analysis were performed for all animals prior to treatment and after 45 days and 13 weeks of treatment. At blood sampling, the animals were fasted for 16–24 hours. After necropsy, the adrenal glands, brain, heart, liver, kidneys, ovaries or testes, and thyroid glands with parathyroid glands were weighed. Fixed tissues and organs were examined microscopically.

There were no deaths during the treatment period. No treatment-related clinical signs were observed in either sex. No treatment-related changes were noted in body weight, feed consumption or ophthalmology. There were no treatment-related effects on haematology or blood biochemistry, although no consistent or dose-related changes were observed. At necropsy, a small prostate gland was noted in one male at each of 40, 200 and 1000 mg/kg bw per day. The small prostate gland was observed to be immature microscopically and was considered to be within the normal range for the age of Beagles used in this study. Therefore, the change was not considered to be treatment related. For organ weights, the thyroid weight relative to brain weight was statistically significantly decreased in males at 1000 mg/kg bw per day. However, there was no decrease in thyroid weight relative to body weight, and there were no corresponding histopathological changes in the thyroid, indicating that the decrease was not biologically significant. No treatment-related histopathological changes were noted.

The NOAEL for the 90-day oral toxicity study in dogs was 1000 mg/kg bw per day, the highest dose tested (Savides, Lucas & Watson, 1998).

In a 1-year oral toxicity study, cyazofamid (lot no. 9506; purity 96.4%) was administered by capsule to six Beagle dogs of each sex per group at 40, 200 or 1000 mg/kg bw per day for 52 weeks. Control animals received empty capsules equal to the number of capsules required for the high-dose animals. Animals were observed twice daily for mortality and morbidity, cage-side observations were made for signs of toxic effects after administration, and a detailed physical examination of each animal was performed weekly. Feed consumption and body weights were measured weekly. An ophthalmological examination was performed prior to and after 52 weeks of treatment. Haematology, blood biochemistry and urine analysis were performed prior to treatment and after 14, 27, 40 and 52 weeks of treatment. The animals were fasted for 16–24 hours before blood sampling. At termination, all animals were necropsied, and the adrenal glands, brain, heart, liver, lungs, kidneys, ovaries or testes, spleen, and thyroid glands with parathyroid glands were weighed. After all tissues and organs were fixed, histopathological analysis was performed. A male (animal number: 300131) at 200 mg/kg bw per day was excluded from toxicological evaluation because it was medicated to improve soft stools/diarrhoea for 9 days.

There were no deaths throughout the treatment period. In all groups, sporadic clinical signs were observed, but no treatment-related changes were found. There were no treatment-related differences in body weight, body weight gain or feed consumption between the control and treated groups. There were no treatment-related ophthalmological effects. There were no treatment-related changes observed in haematology, blood biochemistry or urine analysis parameters. The increases in total bilirubin level at 40 and 1000 mg/kg bw per day in males at week 40 were not treatment related owing to a lack of dose dependency. At necropsy, no gross lesions were considered to be treatment related. Absolute spleen weights were dose-dependently lowered in all treated groups in males, and relative spleen weights were statistically significantly decreased at 1000 mg/kg bw per day in males; however, no corresponding histopathological findings were observed in the spleen of any dogs in this study (Table 11). The spleen weights in two males of the control group were approximately 50–100% higher than those of the remaining control group males. Although it was possible that incomplete exsanguination at necropsy resulted in artificial congestion in the spleen in these males, the absolute and relative spleen weights in males at 1000 mg/kg bw per day were still approximately 20% lower than those in control dogs after excluding these two males. The absolute spleen weights were also statistically significantly lower in females at 1000 mg/kg bw per day. Therefore, the decrease in spleen weights in both sexes in the 1000 mg/kg bw per day group was considered to be treatment related. Microscopically, atrophy of the thymus was increased in females at 1000 mg/kg bw per day, although the number of thymuses examined was not sufficient, owing to inadequate tissue sampling of the thymus. This change is not frequent in young adult dogs (Sato et al., 2008). Therefore, it could not be excluded that the thymus atrophy observed in females at 1000 mg/kg bw per day was treatment related.

The NOAEL for the 1-year oral toxicity study in dogs was 200 mg/kg bw per day, based on decreased spleen weight in both sexes and atrophy of the thymus in females at 1000 mg/kg bw per day (Savides & Watson, 1999).

(b) Dermal application

No data on toxicity following dermal application were provided by the company.

A 4-week dermal toxicity study of cyazofamid (lot number and purity were not provided) was conducted in Sprague-Dawley rats at concentrations of 0, 250, 500 and 1000 mg/kg bw per day. The NOAEL for systemic toxicity via dermal exposure was 1000 mg/kg bw per day, the highest dose tested (Food Safety Commission, 2014).

Table 11. Summary of blood biochemistry and pathological findings in 1-year dog toxicity study

	Males				Females			
	0 mg/kg bw per day	**40 mg/kg bw per day**	**200 mg/kg bw per day**	**1 000 mg/kg bw per day**	**0 mg/kg bw per day**	**40 mg/kg bw per day**	**200 mg/kg bw per day**	**1 000 mg/kg bw per day**
No. of dogs examined	6	6	5	6	6	6	6	6
Final body weight (kg)	13.61	12.06	11.51	11.93	10.23	10.17	9.85	10.37
Spleen weight, absolute (g)	86.75[a]	75	66	61*	78	56	66	66*
Spleen weight, relative ($g/g \times 10^3$)	6.478[a]	6.208	5.672	5.195	7.565	5.527	6.743	6.297
No. of thymuses examined	2	3	2	3	1	6	0	5
Atrophy, thymus	1	2	2	3	1	4	0	5
Severity of the atrophy (minimum/mild/moderate)	0/0/1	0/2/0	1/0/1	1/2/0	0/1/0	2/2/0	0/0/0	3/1/1

bw: body weight; *: $P < 0.05$

[a] Two males showing approximately 50–100% heavier spleens than those of the other control males were excluded.

Source: Savides & Watson (1999)

(c) Exposure by inhalation

Inhalation studies were not performed.

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Cyazofamid (lot no. 9506; purity 95.5 ± 0.7%) was administered in the diet to 60 CD-1 mice of each sex per group at 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) for 18 months. The high dose of 7000 ppm (equal to approximately 1000 mg/kg bw per day) was selected because it is a limit dose accepted by many regulatory agencies. The mice in the two control groups received basal diet. The animals were monitored twice daily for viability and clinical signs and checked for tumour formation by palpation. Body weights and feed consumption were periodically recorded. Blood smears were prepared for each surviving animal at 12 and 18 months. The mice were not fasted prior to the blood sample collection from the orbital sinus. Differential leukocytes from animals in the control and high-dose groups were counted at 12 and 18 months. All animals were subjected to necropsy. Adrenals, brain, liver with gallbladder, kidneys and testes were weighed. All organs and tissues were fixed and routinely processed for histopathological examination.

Mortality in the treated groups was comparable with that in the control groups. There were no treatment-related clinical signs or changes in body weight, feed consumption, haematology or blood biochemistry. Cyazofamid did not affect organ weights or cause any macroscopic changes. Microscopically, non-neoplastic and neoplastic lesions were comparable between the treated and control groups.

The NOAEL for the 18-month oral toxicity and carcinogenicity study in mice was 7000 ppm (equal to 985 mg/kg bw per day), the highest dose tested (O'Meara & Watson, 1999).

Rats

Cyazofamid (lot no. 9506; purity 95.5%) was administered in the diet to 85 F344/DuCrj rats of each sex per group for 104 weeks 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 a concentration of 0, 50, 500, 5000 and 20 000 ppm (equal to 0, 2.01, 20.2, 208 and 856 mg/kg bw per day, respectively) for females. Fifty rats of each sex per group were allocated to the carcinogenicity study group at the end of the 104 weeks, and 35 rats of each sex per group were used for the interim kill. All rats were observed daily for clinical signs. Body weights of all rats were periodically recorded, and mean body weights per dose group were obtained from the data on the carcinogenicity study group. Feed consumption was periodically recorded for the carcinogenicity study group only. At 25, 52 and 78 weeks after the treatment, 10 rats of each sex per group in the interim kill group had blood withdrawn for haematology and blood biochemistry and were necropsied. Before the necropsy, these rats were subjected to urine analysis. The remaining rats in the interim kill group were not used for these assays. The surviving rats in the control and high-dose groups were examined after 104 weeks of treatment. Before termination, 10 rats of each sex per group were examined for urine analysis, and these animals were then subjected to haematology, blood biochemistry and organ weight measurement after 104 weeks of treatment. All animals in the main group were subjected to necropsy. Histopathological examination was performed on the animals killed after 52 and 104 weeks of treatment and the animals found dead or killed in extremis during the treatment period.

Cyazofamid did not affect mortality in any treated groups. In clinical observations, the incidences of bradypnoea were increased in males at 500 ppm and above. However, there were no treatment-related abnormalities in the respiratory tracts at these doses, suggesting that the increases were not treatment related. Body weight changes in males and feed consumption in both sexes of the treated groups were comparable with those in control group animals. Body weights in females at 20 000 ppm were slightly (approximately 5%), but continuously, depressed during weeks 3–100, indicating that the slight depression was treatment related. At termination, no treatment-related changes at ophthalmological examination were found in either sex in the highest-dose group. In urine analysis, slight but statistically significant increases in urinary volume were observed at week 77 in males and at weeks 51 and 77 in females at the highest dose (Table 12). Other parameters related to urinary function, such as increased kidney weights, were found in the same dose group. In addition, the kidney was the target site of this compound at the same dose in the short-term rat study. Therefore, the increase in urinary volume were considered to be a treatment-related adverse effect. Increases in blood sodium and chloride levels at week 25 in males were not considered to be treatment related because the changes in electrolytes were very slight (approximately 1%) and transitory. The slight increase in blood urea nitrogen (approximately 15%) observed in males at 5000 ppm at week 51 was considered to be a treatment-related change. Relative kidney weights in males at week 26 at 5000 ppm and in females at weeks 26 and 78 at 5000 ppm and higher were statistically significantly increased (Table 12). The increases in relative kidney weight and blood urea nitrogen in males at 5000 ppm were slight but considered to be treatment related, because the kidney was the target of cyazofamid. Although relative kidney weights were increased in females at the highest dose at weeks 26 and 52, the increases were not considered to be treatment related owing to the lack of corresponding changes indicating renal toxicity at this dose. Increased liver weights occurred at the highest dose tested. As no change indicating hepatotoxicity was observed, the increases in liver weight were not considered to be toxicologically significant. In histopathological examination, the incidences of neoplastic changes in all treated groups were also comparable with those in control group animals of both sexes.

Cyazofamid was not carcinogenic in rats at dietary concentrations up to 5000 ppm (equal to 171 mg/kg bw per day) for males and 20 000 ppm (equal to 856 mg/kg bw per day) for females. The slight but primary toxicity target of cyazofamid was the kidney in rats.

The NOAEL for 2-year oral toxicity in male rats was 500 ppm (equal to 17.1 mg/kg bw per day), based on kidney effects at 5000 ppm (equal to 171 mg/kg bw per day). The NOAEL for carcinogenicity in male rats was 5000 ppm (equal to 171 mg/kg bw per day), the highest dose tested (Nakashima, 1999c). The NOAELs for both of these end-points in females were higher than the NOAELs in males.

Table 12. Summary of treatment-related changes observed in the 2-year oral toxicity study in rats

	Males					Females				
	0 ppm	**10 ppm**	**50 ppm**	**500 ppm**	**5 000 ppm**	**0 ppm**	**50 ppm**	**500 ppm**	**5 000 ppm**	**20 000 ppm**
Urinary volume (mL)										
Week 25	6.8	6.7	6.5	6.6	7.3	4.2	3.7	4.8	4.0	5.0
Week 51	6.3	6.7	6.8	6.1	6.9	3.5	3.7	3.7	4.2	5.6**
Week 77	6.9	6.9	6.5	7.1	8.4*	5.3	5.5	5.1	6.8	7.8*
Week 103	7.1	7.1	8.2	7.6	7.9	5.4	5.5	6.2	5.8	6.7
BUN (mg/dL)										
Week 52	13.8	13.4	13.7	14.8	15.6*	16.6	17.8	17.1	17.8	16.5
Kidney weight, absolute (mg)										
Week 26	1 930	1 938	1 910	1 939	2 058*	1 210	1 189	1 142	1 232	1 194
Week 52	2 231	2 177	2 283	2 142	2 158	1 391	1 316	1 389	1 436	1 395
Week 78	2 317	2 260	2 277	2 325	2 382	1 475	1 518	1 526	1 553	1 508
Week 104	2 343	2 376	2 367	2 551	2 536	1 623	1 601	1 652	1 634	1 686
Kidney weight, relative (g/g bw × 100)										
Week 26	0.53	0.52	0.52	0.54	0.55*	0.60	0.60	0.60	0.63**	0.64**
Week 52	0.51	0.50	0.52	0.51	0.53	0.60	0.59	0.62	0.65*	0.64
Week 78	0.53	0.51	0.51	0.54	0.56	0.56	0.62*	0.60	0.62*	0.63**
Week 104	0.54	0.53	0.57	0.58	0.59	0.59	0.57	0.58	0.60	0.62
Liver weight										
Absolute (g), week 26	7.42	7.55	7.53	7.33	8.04*	4.33	4.20	4.07*	4.43	4.37
Relative (g/g bw × 100)	2.02	2.03	2.06	2.05	2.15*	2.14	2.11	2.13	2.26	2.35**

BUN: blood urea nitrogen; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: Nakashima (1999c)

2.4 Genotoxicity

The genotoxicity of cyazofamid was investigated in a comprehensive array of studies. One in vivo (mouse micronucleus test in bone marrow) and four in vitro genotoxicity tests (a bacterial assay for reverse mutations, a DNA repair test [rec-assay], a clastogenicity test in human lymphocytes and a gene mutation assay in mouse lymphoma cells) were conducted (Table 13).

Cyazofamid demonstrated no mutagenic properties in *Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and a tryptophan-dependent mutant of *Escherichia coli* (strain CM891), with or without metabolic activation (S9). Cyazofamid did not damage DNA repair in *Bacillus subtilis* strains H17 and M45. Cyazofamid did not induce clastogenicity in the mammalian chromosomal aberration test using human lymphocytes. In mutation assays in mammalian cells, cyazofamid did not induce mutation in L5178Y mouse lymphoma cells. In in vivo genotoxicity testing, cyazofamid did not induce chromosomal aberrations in mouse bone marrow cells.

Table 13. Summary of genotoxicity studies with cyazofamid

Test	Test object	Concentration	Lot no./purity	Result	Reference
In vitro					
Bacterial reverse mutation	*Salmonella typhimurium* (strains TA98, TA100, TA1535 and TA1537) and a tryptophan-dependent mutant of *Escherichia coli* (strain CM891)	50, 150, 500, 1 500 and 5 000 µg/plate	9506/95.5%	Negative ±S9	Kitching (1998)
DNA repair test (rec-assay)	*Bacillus subtilis* recombination-wild (rec+) strain H17 and recombination-deficient (rec−) strain M45	250, 500, 1 000, 2 000, 4 000 and 8 000 µg/disc	LAP-0298/ 90.4%	Negative	Akanuma (1998)
Clastogenicity in mammalian cells	Mammalian chromosomal aberration test in human lymphocytes	1.563, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/mL	9506/95.5%	Negative	Akhurst (1998)
Mutation assay in mammalian cells	L5178Y mouse lymphoma cells	0.5, 1, 5, 10, 25, 50, 75 and 100 µg/mL	9506/95.5%	Negative ±S9	Kirkpatrick (1998)
In vivo					
Micronucleus test	Mouse bone marrow polychromatic erythrocytes	500, 1 000 and 2 000 mg/kg bw by gavage to ICR mice of both sexes	9506/95.5%	Negative	Proudlock & Dawe (1998)

bw: body weight; S9: 9000 × *g* supernatant fraction from rat liver homogenate

In conclusion, cyazofamid was tested for genotoxicity in an adequate range of in vitro and in vivo assays. No evidence of genotoxicity was found.

2.5 Reproductive and developmental toxicity

(a) Reproductive toxicity

Two reproductive toxicity studies were conducted using rats. In a single-generation reproductive toxicity study, cyazofamid (lot no. 9506; purity 95.5 ± 0.7%) was administered via the diet to 10 Sprague-Dawley rats of each sex per group at 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) during the 8-week growth phase. The parental animals were treated continuously throughout the entire study, and the offspring were exposed to the test diets throughout the lactation period. Body weights and feed consumption were recorded weekly during the 8-week growth period. Each female was randomly assigned for mating with a male in the same group for a 2-week period. After a check for mating, females were weighed on days 0, 7, 14 and 20 of gestation. Following delivery, dams were weighed on days 0, 7 and 14. Litters were culled to eight pups, if possible, on day 4 of lactation. The resulting offspring were held for a 14-day lactation period.

Necropsies were not conducted on any animals except to verify pregnancy of any females dying during the study.

There were no treatment-related changes in clinical signs, body weight or feed consumption. Two females in the 20 000 ppm group were euthanized when they showed poor condition at parturition and before terminal kill. Fetuses were observed in utero in these rats. As similar deaths were observed at all dose levels, including controls in a subsequent two-generation study, these deaths were considered not to be due to treatment.

Reproductive parameters, including mating, fertility and gestation indices, in the treated groups were comparable with those in the control group. No external malformations in offspring were observed. No effects on the number of stillborn pups, sex ratio, pup survival or physical condition of the pups during lactation were observed. Mean body weights of pups were comparable between the control and treated groups during the 14-day lactation period.

The NOAELs for maternal, reproductive and offspring toxicity in the single-generation reproductive toxicity study in rats were 20 000 ppm (equal to 1327 mg/kg bw per day), the highest dose tested (O'Meara, Serrone & Douglass, 1996).

In a two-generation reproductive toxicity study, cyazofamid (lot no. 9506; purity 95.5%) was administered in the diet to 30 Sprague-Dawley rats of each sex per group at a concentration of 0, 200, 2000 or 20 000 ppm. The parental animals of each generation were treated continuously throughout the growth, mating, gestation, lactation and resting phases of the study, and the offspring were exposed to the test diets throughout the lactation period. The growth period for the F_0 parental animals was 10 weeks. The animals were then mated for 2 weeks to produce the F_{1a} litters. After the F_{1a} litters were weaned, the parents were given a rest period of at least 1 week prior to mating for the F_{1b} litters. Selection for the F_2 generation was made from the weaned F_{1a} litters. The growth period for the F_{1a} generation was 12 weeks. The F_{1a} parental animals then went through a reproduction sequence similar to that for the F_0 animals to produce the F_{2a} and F_{2b} litters. On day 4 of lactation, each litter size was adjusted to eight pups. All animals, including pups, were monitored at least twice daily for mortality and clinical signs of toxicity. For parental animals, physical examinations, body weights and feed consumption were recorded at regular intervals. F_0 and F_1 parental males were necropsied following mating for the F_{1b} or F_{2b} litters, respectively. Females were necropsied on day 21 of the F_{1b} or F_{2b} lactation period. Representative organs and tissues fixed from 10 rats of each sex per group were examined microscopically from the control and high-dose animals.

During the reproductive phase, evidence of mating, date of delivery, number, weight and sex of pups, and any abnormalities observed in the offspring were recorded. Number, weight and sex of pups were recorded at regular intervals during lactation. All animals on study were necropsied. A complete set of tissues (above) was collected and preserved from 10 F_{2a} males and 10 F_{2a} females from each group at weaning. No histopathology was conducted on these tissues.

Average chemical intakes in the F_0 and F_1 generations are shown in Table 14.

Table 14. Chemical intake in two-generation reproductive toxicity study in rats

	Dose (mg/kg bw per day)		
Generation	**200 ppm**	**2000 ppm**	**20 000 ppm**
F_0 males	9.5	94.2	958
F_0 females	13.4	134	1 340
F_1 males	8.9	89.2	936
F_1 females	13.7	138	1 400

bw: body weight; F_0: first parental generation; F_1: second parental generation; ppm: parts per million
Source: O'Meara & Watson (1998)

In parental animals, there were no treatment-related effects on mortality, clinical observations, feed consumption or gross necropsy observations for either the F_0 or F_1 animals. Microscopically, there were no findings in tissues examined that were considered to be related to treatment. During the growth phase, body weights of treated F_0 and F_1 males were comparable with those of the control group animals.

Body weights for the F_0 females and the low- and mid-dose F_1 females were comparable with those of the control group animals. The mean body weights for the F_1 females at 20 000 ppm were slightly, but statistically significantly, lower after weaning up to mating compared with the mean weights in the control group (Table 15). The change in body weight observed just after weaning in the F_1 generation was considered to be a slight effect on offspring. During the gestation and lactation periods, body weights in F_0 and F_1 females at 20 000 ppm were slightly, but statistically significantly, depressed at many time points (Table 16). Findings similar to those seen in the F_0 females were noted in the F_1 females – that is, the mean body weights for the F_1 females at 20 000 ppm were slightly, but statistically significantly, lower during pregnancy/lactation compared with the control mean weights (Table 16). These continuous body weight decreases in F_0 and F_1 females during the gestation and lactation periods were considered to be a treatment-related effect on the parent. During the growth phase, the body weights of treated F_0 and F_1 males were similar to those of the control group animals. Body weights of the F_0 females and the low- and mid-dose F_1 females were comparable with the mean body weight of the control group.

In reproductive parameters, mating and fertility indices and gestation length were not affected by treatment with cyazofamid. No malformations were increased by the treatment in either generation. No effects on the pup sex ratio, pup survival or physical condition of the pups during lactation were observed. The body weights of pups on day 21 in all generations at 20 000 ppm were slightly (approximately 10%), but statistically significantly, lower than those in the control group (Table 17). This common change in all generations was considered to be a treatment-related adverse effect. The number of stillborn pups showed a slightly increased tendency at 20 000 ppm through the F_{1a}, F_{1b} and F_{2a} generations. This difference from control was not apparent for the F_{2b} generation. The survivability of pups in the treated groups from days 1 to 21 was comparable with that of the control group for all litters. Therefore, these changes were not considered to be a biologically significant effect. Although the numbers of pups and litters with dilated pelvis were increased in the F_{1a} generation at 20 000 ppm, the number in other generations was varied, indicating that the increase was not toxicologically significant. No findings that were considered to be treatment related were observed at the necropsy of the pups.

In this two-generation reproductive toxicity study in rats, 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) (O'Meara & Watson, 1998).

(b) Developmental toxicity

Rats

Cyazofamid (lot no. 9506; purity 95.5%) was administered by gavage to six pregnant female Sprague-Dawley rats per group at 0, 20, 100, 500 or 1000 mg/kg bw per day during gestation days (GDs) 0–19. Animals were checked twice daily for mortality and for obvious signs of toxicity. They were also subjected to a detailed physical examination on days 0–20 of gestation. Body weights and feed consumption were measured on days 0, 3, 6, 9, 12, 15, 18 and 20 of gestation. All females were necropsied on GD 20, and gravid uterine weights, number of corpora lutea, number of implantation sites, number of live fetuses and number of resorptions were recorded. Each fetus was weighed, sexed and individually identified. A gross external examination for defects, including observations of the palate, and for external sex determination was performed on each fetus. All fetuses were sacrificed and discarded.

Table 15. F_1 female body weights during non-reproduction periods

Dietary concentration (ppm)	Body weight (g)														
	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 21	Week 22
0	72.4	107.7	144.4	167.9	192.7	213.9	228.8	241.8	257.6	264.6	267.2	273.4	279.9	313.0	313.1
200	70.8	106.4	142.2	167.0	190.4	211.5	228.7	240.0	255.0	265.0	267.8	275.6	281.7	312.7	312.6
2 000	71.5	108.0	145.9	172.7	197.2	219.3	233.9	245.1	258.7	269.1	272.6	280.9	286.1	313.6	315.2
20 000	66.2*	100.1*	135.6**	160.6	183.2*	203.5*	220.8	231.7	244.4*	252.9	257.5	263.5	269.0	294.8*	296.0

ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: O'Meara & Watson (1998)

Table 16. Female body weights during gestation and lactation in two-generation reproductive toxicity study in rats

Dietary concentration (ppm)	Body weight (g)							
	Gestation				Lactation			
	Day 0	Day 7	Day 14	Day 20	Day 0	Day 7	Day 14	Day 21
F_0 females for F_{1a} litters								
0	259.0	289.2	319.7	386.1	302.4	310.0	323.0	309.1
200	261.6	294.5	323.7	383.1	301.0	313.8	323.0	304.1
2 000	251.8	281.5	313.6	370.7	296.1	305.6	317.1	306.7
20 000	251.9	280.9	308.3	371.6	286.8	298.8	309.4	296.9
F_0 females for F_{1b} litters								
0	300.4	330.8	362.1	425.5	336.2	344.9	353.6	334.2
200	302.6	333.9	359.9	425.6	338.4	344.2	349.2	328.1
2 000	296.5	323.4	349.5	418.0	334.2	342.9	346.0	330.5
20 000	287.8	310.0*	338.4*	407.2	317.7	330.6	336.7*	325.7

Dietary concentration (ppm)	Body weight (g)							
	Gestation				Lactation			
	Day 0	Day 7	Day 14	Day 20	Day 0	Day 7	Day 14	Day 21
F_{1a} females for F_{2a} litters								
0	275.3	306.3	332.1	390.3	309.8	323.1	333.0	315.6
200	274.6	304.1	332.7	393.9	316.0	330.1	334.5	318.6
2 000	279.9	306.1	332.9	395.4	312.2	324.8	337.0	320.0
20 000	260.5*	286.7*	313.6*	375.0	291.5*	305.0**	312.1**	302.6
F_{1a} females for F_{2b} litters								
0	310.2	336.7	372.0	439.6	351.6	357.7	361.8	337.2
200	307.1	334.9	369.1	439.4	349.7	355.5	356.0	335.0
2 000	307.4	330.5	362.3	429.3	347.4	348.5	356.8	339.1
20 000	294.7*	318.5*	351.5	416.7	333.4	335.6**	342.3*	327.1

F_1: first filial generation; F_2: second filial generation; ppm: parts per million; *: $P < 0.05$; **: $P < 0.01$
Source: O'Meara & Watson (1998)

Table 17. Body weight of pups in the two-generation reproductive toxicity study in rats

Day	Body weight (g)															
	F_{1a}				F_{1b}				F_{2a}				F_{2b}			
	0 ppm	200 ppm	2 000 ppm	20 000 ppm	0 ppm	200 ppm	2 000 ppm	20 000 ppm	0 ppm	200 ppm	2 000 ppm	20 000 ppm	0 ppm	200 ppm	2 000 ppm	20 000 ppm
0	6.2	6.5	6.6*	6.3	6.3	6.3	6.4	6.4	6.1	6.5*	6.3	6.2	6.2	6.4	6.1	6.3
7	16.2	17.3	17.3	15.8	16.8	17.2	17.0	16.2	16.2	17.1	16.3	15.8	16.7	17.5	16.5	16.5
21	52.1	53.5	54.1	48.2*	54.1	52.5	54.5	49.4*	52.9	55.1	52.5	49.3*	55.1	56.5	54.3	31.5*

F_1: first filial generation; F_2: second filial generation; ppm: parts per million; *: $P < 0.05$

Source: O'Meara & Watson (1998)

No treatment-related clinical signs were observed. One dam at 100 mg/kg bw per day showing lower body weight and feed consumption was noted to be emaciated and had laboured breathing from GDs 15 to 20. Macroscopic findings of the dam, such as red discoloration of the lungs and a fluid-filled thoracic cavity with adhesions of thoracic organs, suggested an administration error. Similar abnormalities were not observed in other dams at the same dose. Therefore, the abnormalities found in the dam at 100 mg/kg bw per day were not considered to be related to the chemical treatment. The mean number of corpora lutea, uterine implantation sites, live fetuses and resorptions per pregnant female were not affected by the treatment at any dose.

Cyazofamid had no effect on fetal weights except for the decreased weights of fetuses whose dam was affected by an administration error. The decrease was a secondary effect on the dam and was not treatment related. The sex distribution of the fetuses was comparable between the control and treatment groups. No malformations or variations were noted during the external examinations. No external malformations or variations were seen in the control group fetuses.

The NOAELs for maternal and embryo/fetal toxicity in rats were 1000 mg/kg bw per day, the highest dose tested (Rodwell, 1999a).

Rabbits

Cyazofamid (lot no. 9506; purity 95.5%) was administered to six mature, time-mated female New Zealand White rabbits per group by gavage at 0, 20, 100, 500 or 1000 mg/kg bw per day during GDs 4–28. Does were checked twice daily for mortality and for obvious signs of toxicity. They were also subjected to a detailed physical examination daily from receipt through GD 29. Body weights and feed consumption were measured on GDs 0, 4, 6, 9, 12, 15, 18, 21, 24, 27 and 29. All does were necropsied on GD 29, and gravid uterine weights were recorded. The number of corpora lutea was counted, and implantation parameters were examined. A gross external examination for defects, including observations of the palate, was performed on each fetus.

No treatment-related mortality occurred in the course of the study. One female at 1000 mg/kg bw per day died on GD 9 due to an intubation error, but all other animals survived to termination. No treatment-related abnormalities or changes in body weight or feed consumption were noted. At necropsy, there were no treatment-related changes in maternal macroscopic postmortem findings. White fluid in the trachea and white discoloured lungs were observed in the animal that died following dosing on GD 9. No treatment-related effects on uterine implantation parameters, including the numbers of corpora lutea, implantation sites, preimplantation and postimplantation losses, live and dead fetuses, and early and late resorptions, were noted in any treated group. In the findings on fetuses, no effects of treatment with cyazofamid on fetal weight, malformations or variations were observed.

The NOAELs for maternal and embryo/fetal toxicity in rabbits were 1000 mg/kg bw per day, the highest dose tested (Rodwell, 1999b).

2.6 Special studies

(a) Acute neurotoxicity

Cyazofamid (lot no. 9507; purity 95.7%) was administered by single dose gavage to 10 young Sprague-Dawley rats of each sex per group at 0, 80, 400 or 2000 mg/kg bw. After the treatment, mortality and morbidity were checked once a day. The neurobehavioural assessment, such as a functional observational battery and a motor activity test, was conducted before initiation of treatment and approximately 30–60 minutes, 7 days and 14 days after dosing. Fourteen days after dosing, the surviving animals were necropsied. After necropsy, six rats of each sex per group were fixed by perfusion for neuropathological examination.

No treatment-related changes in mortality, body weight, feed consumption, cage-side observations, parameters of neurobehavioural assessment, or macroscopic and microscopic neuropathological analysis were observed at any time point examined.

The NOAEL for acute neurotoxicity in rats was 2000 mg/kg bw, the highest dose tested (Ridder, 2000).

(b) Subchronic neurotoxicity

Cyazofamid (lot no. 9801-2; purity 94.9%) was administered to 12 Sprague-Dawley rats of each sex per group for 13 weeks at a dietary 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). All animals were observed twice daily for mortality and moribundity. Detailed physical examinations, body weights and feed consumption were recorded weekly. Functional observational battery and locomotor activity data were recorded for all animals during pretreatment and at weeks 3, 7 and 12. The functional observational battery, including handling, open-field, sensory, neuromuscular and physiological observations, was conducted on all animals. At the termination of the study, all animals were necropsied and fixed with buffered 4.0% paraformaldehyde solution. Fixed brain weight and brain dimensions (length [excluding olfactory bulbs] and width) were recorded. Neuropathological examination was performed using six selected rats of each sex in the control and 20 000 ppm groups.

There were no treatment-related changes in mortality or in handling, open-field, sensory, neuromuscular and physiological observations in the functional observational battery or macroscopic and microscopic findings. Males in the 20 000 ppm group showed slightly, but statistically significantly, lower (approximately 15% lower than that in the control group) body weight gain in the first week of treatment. The mean body weight gain in males at 20 000 ppm before treatment was significantly higher (3.4%) than that in the control group. Feed consumption was not affected during the first week of treatment. Therefore, the decrease was considered to be a treatment-related adverse effect, but it was not a neurotoxicity indicator. At 10 weeks, the body weight gain in females at 20 000 ppm was slightly (9 g at 0 ppm and 2 g at 20 000 ppm) lower, without a change in feed consumption. The transient change was not considered to be treatment related, but was an incidental effect. The only statistically significant difference in locomotor activity between the control and test substance–exposed groups during the exposure period when the six subintervals were evaluated was a lower mean ambulatory count in females at 20 000 ppm at week 12. The mean overall ambulatory count was not affected. No other changes indicating neurotoxicity were observed. Therefore, this transient difference was considered to be incidental.

The NOAEL for general toxicity was 2000 ppm (equal to 156 mg/kg bw per day), based on slightly lower body weight gain during the early phase of treatment at 20 000 ppm (equal to 1356 mg/kg bw per day). The NOAEL for subchronic neurotoxicity in rats was 20 000 ppm (equal to 1356 mg/kg bw per day), the highest dose tested (Toot, 2012).

(c) Immunotoxicity

Seven-week-old female CD-1 mice (10 mice per group) were administered cyazofamid (lot no. 9506; purity 95.0%) in the diet at 0, 600, 3000 or 6000 ppm (equal to 0, 136, 599 and 1381 mg/kg bw per day, respectively) for 28 days. All animals were immunized with an intravenous injection of sheep red blood cells on day 24. The animals in the positive control group were administered an intraperitoneal injection of cyclophosphamide monohydrate (50 mg/kg bw per day) once daily for 4 consecutive days (study days 24–27). All animals were checked for mortality, moribundity and clinical signs. Detailed physical examinations were performed approximately weekly. Body weights and feed consumption were recorded approximately twice weekly. Blood samples for possible immunoglobulin M (IgM) antibody analysis were withdrawn from all animals at termination (on day 28). After necropsy, thymus and spleen weights were measured. The thymus was fixed for possible future microscopic examination, and the spleen was subjected to immunotoxicological evaluation, the IgM antibody-forming cell (AFC) assay.

All animals survived to termination. There were no treatment-related clinical signs or findings. Although occasional slightly, but statistically significantly, lower mean body weight gains were noted

in the 3000 and 6000 ppm groups, these differences were not considered to be treatment related owing to the lack of a dose–response relationship. No abnormalities in macroscopic findings or changes in thymus weights or spleen weights were observed in the cyazofamid-treated groups.

In the AFC assay, cyazofamid treatment showed no significant suppression of the humoral immune response. Statistically significantly lower spleen cell numbers, mean specific activity and mean total spleen activity values were noted in the positive control group.

The NOAEL for immunotoxicity in mice was 6000 ppm (equal to 1381 mg/kg bw per day), the highest dose tested (Wasil, 2012).

(d) Studies on metabolites

Data on the acute toxicity in rats and the in vitro genotoxicity of CCIM (a first intermediate metabolite in rats, metabolite in goat muscle and fat and plants), CCIM-AM (large animals, plants), CCBA (a major urinary metabolite in rats) and CTCA (plants and soil) were provided.

CCIM

Acute toxicity. CCIM (lot no. 9809; purity 99.0%), a major intermediate metabolite of cyazofamid in animals, was administered to Sprague-Dawley rats (five rats of each sex per group) at 100, 160, 256, 410 or 656 mg/kg bw by gavage. All rats were monitored for toxic signs 30 minutes and 1, 3 and 6 hours after dosing and daily for 14 days. Body weights were recorded weekly. Each animal was subjected to necropsy either immediately after being found dead or 14 days after treatment. LD_{50} values were calculated from the mortality rates.

Deaths occurred 3 hours after test material administration in males and from 3 hours to 1 day in females. A summary of their mortality is shown in Table 18. No animals died at 100 or 160 mg/kg bw. Clinical signs included a decrease in spontaneous motor activity, prone position and sedation, tremors, ptosis and salivation. The prone position was observed at all dose levels, and the incidences of clinical signs were increased in a dose-dependent manner (Table 19). For animals found dead during the study, the most predominant necropsy observations included red-coloured lungs and digestive tracts and oedema in the lungs and small intestines. All surviving animals gained weight throughout the study, and the body weights in the treated groups were comparable with those in the control group. There were no abnormalities noted in animals surviving to study termination.

All surviving animals gained weight throughout the study. The LD_{50}s for CCIM in rats were 324 mg/kg bw in males and 443 mg/kg bw in females (Shutoh, 1999a).

Genotoxicity. An in vitro genotoxicity study of CCIM (lot no. 9809; purity 99.0%) was conducted. Reverse mutation tests were performed in *Escherichia coli* WP2 uvrA and four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537). The treatment doses were 20, 39, 78, 156, 313, 625 and 1250 µg/plate, with and without metabolic activation (S9 mix). After incubation in the presence or absence of S9 mix, the number of revertant colonies was counted.

The mean number of revertant colonies in plates treated with CCIM was similar to that of the corresponding solvent control in all strains, with or without S9 mix. Toxicity to the tester strains was observed at 625 µg/plate and higher in the TA strains and at 1250 µg/plate in the WP2 strain. The mean number of revertant colonies in positive controls was at least 3-fold higher than the solvent control numbers, indicating that CCIM was non-mutagenic to bacteria under the conditions of this study.

CCIM was concluded to be non-mutagenic in vitro (Matsumoto, 1999a).

Table 18. Mortality in rats treated with a single dose of CCIM

	Mortality													
	Males							Females						
Dose (mg/kg bw)	30 min	1 h	3 h	6 h	Day 1	Days 2–14	Final mortality	30 min	1 h	3 h	6 h	Day 1	Days 2–14	Final mortality
100	0	0	0	0	0	0	0/5	0	0	0	0	0	0	0/5
160	0	0	0	0	0	0	0/5	0	0	0	0	0	0	0/5
256	0	0	3	0	0	0	3/5	0	0	1	0	0	0	1/5
410	0	0	2	0	0	0	2/5	0	0	2	0	0	0	2/5
656	0	0	5	0	0	0	5/5	0	0	3	0	1	0	4/5

bw: body weight
Source: Shutoh (1999a)

Table 19. Summary of clinical signs in rats treated with a single dose of CCIM

	100 mg/kg bw		160 mg/kg bw		256 mg/kg bw		410 mg/kg bw		656 mg/kg bw	
Clinical signs	Fd	Tk	Fd	Tk	Fd	Tk	Fd	Tk	Fd	Tk
Males										
Number of rats	0	5	0	5	3	2	2	3	5	0
No abnormalities detected	–	3	–	1	0	1	1	0	0	–
Prone position	–	2	–	4	3	0	1	3	2	–
Decreased spontaneous motor activity	–	0	–	0	0	1	0	2	0	–
Crawling	–	0	–	1	3	0	0	1	2	–
Deep respiration	–	0	–	0	1	0	0	1	0	–
Sedation	–	0	–	4	2	1	0	3	5	–
Tremor	–	0	–	0	0	0	0	1	0	–
Ptosis	–	0	–	0	0	1	0	3	0	–

	100 mg/kg bw		160 mg/kg bw		256 mg/kg bw		410 mg/kg bw		656 mg/kg bw	
Clinical signs	**Fd**	**Tk**	**Fd**	**Tk**	**Fd**	**Tk**	**Fd**	**Tk**	**Fd**	**Tk**
Salivation	–	0	–	0	1	0	0	0	0	–
Soiled fur in the nasorostal region	–	0	–	0	0	0	0	1	0	–
Females										
Number of rats	0	5	0	5	1	4	2	3	4	1
No abnormalities detected	–	4	–	1	0	1	0	0	0	0
Prone position	–	1	–	4	1	3	2	2	3	0
Decreased spontaneous motor activity	–	0	–	1	0	2	1	3	1	0
Crawling	–	0	–	1	1	1	1	1	0	0
Deep respiration	–	0	–	0	1	0	1	2	0	0
Sedation	–	0	–	3	1	2	1	3	4	0
Tremor	–	0	–	0	1	0	0	0	0	1
Ptosis	–	0	–	0	1	0	1	2	1	0
Salivation	–	0	–	0	1	0	1	0	0	0
Soiled fur in the nasorostal region	–	0	–	0	0	0	0	1	0	0
Hunched position	–	0	–	0	0	0	0	0	1	1

bw: body weight; Fd: found dead; Tk: terminal kill
Source: Shutoh (1999a)

CCIM-AM

Acute toxicity. CCIM-AM (lot no. 9809; purity 99.6%) was administered to Sprague-Dawley rats (five of each sex per group) at 3000 mg/kg bw by gavage. All rats were monitored for toxic signs 30 minutes and 1, 3 and 6 hours after dosing and daily for 14 days. Body weights were recorded weekly. Each animal was subjected to necropsy either immediately after being found dead or 14 days after treatment. LD_{50} values were calculated from the mortality rates.

No deaths or clinical signs were observed. The treatment did not affect body weight or macroscopic examination. The LD_{50}s for CCIM-AM in both sexes were greater than 3000 mg/kg bw (Shutoh, 1999b).

Genotoxicity. An in vitro genotoxicity study of CCIM-AM (lot no. 9809; purity 99.6%) was conducted. Reverse mutation tests were performed in *Escherichia coli* WP2 uvrA and four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537). The treatment doses were 78, 156, 313, 625 and 1250 μg/plate, with and without metabolic activation (S9 mix). After incubation in the presence or absence of S9 mix, the number of revertant colonies was counted.

The mean number of revertant colonies in plates treated with CCIM-AM was similar to that of the corresponding solvent control in all strains, with or without S9 mix. Toxicity to the tester strains was not observed. A 2-fold or greater increase in the mean number of revertant colonies compared with the solvent control was not observed in any strain at any dose of CCIM-AM in the reverse mutation tests, with and without metabolic activation, indicating that CCIM-AM was non-mutagenic to bacteria under the conditions of this study.

CCIM-AM was concluded to be non-mutagenic in vitro (Matsumoto, 1999b).

CTCA

Acute toxicity. CTCA (lot no. 9809; purity 99.7%) was administered to Crj:CD rats (five of each sex per group) at 763, 1221, 1953, 3125 or 5000 mg/kg bw by gavage. All rats were monitored for toxic signs 30 minutes and 1, 3 and 6 hours after dosing and daily for 14 days. Body weights were recorded weekly. Each animal was subjected to necropsy either immediately after being found dead or 14 days after treatment. LD_{50} values were calculated from the mortality rates.

Mortalities in the 763, 1221, 1953, 3125 and 5000 mg/kg bw groups were 0/5, 0/5, 0/5, 4/5 and 4/5 in males and 0/5, 1/5, 2/5, 5/5 and 5/5 in females, respectively. The deaths occurred from day 3 to day 8 after administration in males and from day 1 to day 7 in females. Various clinical signs, such as emaciation, hunchback position, decreases in or loss of spontaneous motor activity, bradypnoea, sedation, stupor, hypothermy, ptosis, soiled fur in the naso-orbital region, and soiled fur in the perianal region, were observed in many animals at dose levels of 1953 mg/kg bw and above in males and 1221 mg/kg bw and above in females. These signs began to appear from 3 hours after administration and disappeared by day 11. The body weights of survivors were reduced in one male and two females on day 7. At necropsy of surviving animals, smaller sizes of the thymus and testes were noted.

Based on the mortalities observed, the LD_{50}s for CTCA were considered to be 2947 and 1963 mg/kg bw for males and females, respectively (Shutoh, 1999c).

Genotoxicity. An in vitro genotoxicity study of CTCA (lot no. 9809; purity 99.6%) was conducted. Reverse mutation tests were performed in *Escherichia coli* WP2 uvrA and four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537). The treatment doses were 78, 156, 313, 625, 1250, 2500 and 5000 μg/plate, with and without metabolic activation (S9 mix). After incubation in the presence or absence of S9 mix, the number of revertant colonies was counted.

The mean number of revertant colonies in plates treated with CTCA was similar to that of the corresponding solvent control in all strains, with or without S9 mix. Toxicity to the tester strains was not observed. A 2-fold or greater increase in the mean number of revertant colonies compared with

the solvent control was not observed in any strain at any dose of CTCA in the reverse mutation tests, with and without metabolic activation, indicating that CTCA was non-mutagenic to bacteria under the conditions of this study.

CTCA was concluded to be non-mutagenic in vitro (Matsumoto, 1999c).

Dimethylsulfamic acid

Acute toxicity. Dimethylsulfamic acid (a predictive metabolite produced during the pathway from cyazofamid to the first metabolite CCIM in animals, plants, soil and water; lot no. 9902; purity 99.0%) was administered at 1000, 1600, 2560, 4096 or 6554 mg/kg bw by gavage to Crj:CD rats (five of each sex per group). All rats were monitored for toxic signs 30 minutes and 1, 3 and 6 hours after dosing and daily for 14 days. Body weights were recorded weekly. Each animal was subjected to necropsy either immediately after being found dead or 14 days after treatment. LD_{50} values were calculated from the mortality rates.

Mortalities in the 1000, 1600, 2560, 4096 and 6554 mg/kg bw groups were 0/5, 0/5, 0/5, 5/5 and 5/5 for males and 0/5, 0/5, 1/5, 5/5 and 5/5 for females, respectively. The animals died 1 hour after administration in males and from 1 hour after administration to day 2 in females. Many animals of both sexes showed clinical signs suggesting general poor condition, such as prone position, decrease in or loss of spontaneous motor activity and sedation. Bradypnoea, stupor, hypothermy, lacrimation and ptosis were also noted in the rats killed when moribund. All survivors gained weight on days 7 and 14, showing recovery. No macroscopic abnormalities were noted in surviving animals at terminal kill.

Based on the mortalities observed, the LD_{50}s for dimethylsulfamic acid were considered to be 3238 and 2948 mg/kg bw for males and females, respectively (Shutoh, 1999d).

Genotoxicity. An in vitro genotoxicity study of dimethylsulfamic acid (lot no. 9809; purity 99.6%) was conducted. Reverse mutation tests were performed in *Escherichia coli* WP2 uvrA and four tester strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537). The treatment doses were 313, 625, 1250, 2500 and 5000 µg/plate, with and without metabolic activation (S9 mix). After incubation in the presence or absence of S9 mix, the number of revertant colonies was counted.

The mean number of revertant colonies in plates treated with dimethylsulfamic acid was similar to that of the corresponding solvent control in all strains, with or without S9 mix. Toxicity to the tester strains was not observed. A 2-fold or greater increase in the mean number of revertant colonies compared with the solvent control was not observed in any strain at any dose of dimethylsulfamic acid in the reverse mutation tests with and without metabolic activation, indicating that dimethylsulfamic acid was non-mutagenic to bacteria under the conditions of this study.

Dimethylsulfamic acid was concluded to be non-mutagenic in vitro (Matsumoto, 1999d).

3. Observations in humans

Based on information from manufacturing plants, as well as a review of published literature, no reported incidents of cyazofamid poisoning in humans have been found. No epidemiological studies involving cyazofamid were found in a review of published literature.

Comments

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 dimethylsulfamic 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 (Huhtanen & Savides, 1998a,b,c; McFadden, Yoshida & Huhtanen, 1999; Murray & Savides, 1999).

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 2000 mg/kg bw in rats (Yoshida, Brock & Watson, 1998a,b; Yoshida & Watson, 1999). The inhalation LC_{50} was greater than 5.5 mg/L in rats (Ulrich, 1998). Cyazofamid was slightly irritating to the skin and eyes of rabbits. Cyazofamid was not sensitizing in the guinea-pig maximization test (Yoshida, Brock & Watson, 1998c,d,e).

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 (O'Meara, Serrone & Lorence, 1996).

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) (Nakashima, 1999b).

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 (Savides, Lucas & Watson, 1998).

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 and atrophy of the thymus in females at 1000 mg/kg bw (Savides & Watson, 1999).

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 (O'Meara & Watson, 1999).

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 (Nakashima, 1999c).

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 (Akanuma, 1998; Akhurst, 1998; Kirkpatrick, 1998; Kitching, 1998; Proudlock & Dawe, 1998).

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 (O'Meara, Serrone & Douglass, 1996).

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) (O'Meara & Watson, 1998).

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 (Rodwell, 1999a).

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 (Rodwell, 1999b).

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 (Ridder, 2000).

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 20 000 ppm (equal to 1356 mg/kg bw per day), the highest dose tested (Toot, 2012).

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, respectively), the NOAEL for immunotoxicity was 6000 ppm (equal to 1381 mg/kg bw per day), the highest dose tested (Wasil, 2012).

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 (Murray & Savides, 1999).

Acute toxicity studies on 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 (Shutoh, 1999a). CCIM did not show evidence of genotoxicity in vitro (Matsumoto, 1999a).

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.

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]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	20 000 ppm, equal to 936 mg/kg bw per day[b]	–

Species	Study	Effect	NOAEL	LOAEL
		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	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	> 2 000 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 (highest dose tested; 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

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