5.33 TRIFORINE (116)

TOXICOLOGY

Triforine is the ISO-approved common name for *N*,*N*′-[piperazine-1,4-diylbis[(trichloromethyl)methylene)] diformamide (IUPAC), with CAS number 37273-84-0. Triforine is a systemic fungicide that acts by inhibition of sterol biosynthesis in the membranes of fungi (black spot, rust, powdery mildew).

Triforine was first evaluated by JMPR in 1977, when no ADI was established. When more toxicological data were made available to the Meeting for review in 1978, an ADI of 0–0.02 mg/kg bw was established. The Meeting reviewed triforine in 1997 within the periodic review programme of CCPR and reaffirmed the ADI of 0–0.02 mg/kg bw. No ARfD was established, because the establishment of ARfDs by JMPR was not common practice in 1997. Triforine was reviewed by the present Meeting as part of the periodic review programme of CCPR.

Some of the critical studies do not comply with GLP, as the data were generated before the implementation of GLP regulations. Overall, however, the Meeting considered that the database was adequate for the risk assessment.

Biochemical aspects

In single-dose or repeated-dose studies using a dose of 10 mg/kg bw, more than 80% of administered radiolabelled triforine was rapidly absorbed by both male and female rats. In a study using a single dose of 1000 mg/kg bw, only about 10–20% was absorbed. The AUC was about 2 times greater in male rats than in female rats. Triforine was widely distributed in the body. After a single low dose (10 mg/kg bw), more than 78% was excreted in urine, 5–6% in expired air and 12–14% in faeces (9–13% in bile). After a single high dose (1000 mg/kg bw), more than 77% was excreted in faeces and 11–19% in urine. The terminal elimination half-lives at 10 mg/kg bw were 125 hours in males and 95.7 hours in females. After 168 hours, the highest residues were seen in liver, red blood cells and kidney.

Triforine was extensively metabolized by cleavage of one of the two side-chains, followed by oxidation and conjugation of the side-chain metabolites with glucuronic acid or glutathione.

Toxicological data

Triforine is of low acute toxicity, with oral LD50s greater than 5000 mg/kg bw in rats and mice. The dermal LD50 in rats was greater than 2000 mg/kg bw. The inhalation LC50 in rats was greater than 5.12 mg/L. Triforine was not irritating to the eye or skin of rabbits. It was not a dermal sensitizer in guinea-pigs (Maurer optimization test).

Administration of triforine to mice, rats and dogs in repeated-dose toxicity studies (4-week and 13-week studies in mice, 4-week, 13-week and 2-year studies in rats and 13-week and 2-year studies in dogs) resulted in haemolytic anaemia and associated effects.

In a 4-week study of toxicity in mice, triforine was administered in the diet at a concentration of 0, 200, 1000 or 5000 ppm (equal to 0, 39.0, 195.8 and 982.1 mg/kg bw per day for males and 0, 45.2, 237.0 and 1284.3 mg/kg bw per day for females, respectively). The NOAEL was 1000 ppm (equal to 195.8 mg/kg bw per day), on the basis of mild haemolytic anaemia in mice of both sexes, slightly reduced body weight gain in males and increased relative liver weight in females at 5000 ppm (equal to 982.1 mg/kg bw per day).

In a 13-week study of toxicity in mice designed solely to determine the high dose for use in longer-term studies, triforine was administered in the diet at a concentration of 0 or 7000 ppm (equal to 1354 mg/kg bw per day for males and 2239 mg/kg bw per day for females). Evidence of mild haemolytic anaemia and moderately increased spleen and liver weights were seen in treated animals.

In a 4-week study of toxicity in rats, triforine was administered in the diet at a concentration of 0, 500, 2500 or 12 500 ppm (equal to 0, 49.7, 238.2 and 1233.7 mg/kg bw per day for males and 0, 48.5, 233.2 and 1180.8 mg/kg bw per day for females, respectively). A NOAEL was not identified in this study, as the incidence and severity of haemosiderin deposition in the spleen were increased in females of all dose groups.

In a 13-week study of toxicity in rats, triforine was administered in the diet at a concentration of 0 or 20 000 ppm (equal to mean achieved doses of 1630 mg/kg bw per day for males and 1945 mg/kg bw per day for females). Treated animals showed mild haemolytic anaemia and increased spleen and liver weights.

In a 13-week study of toxicity in rats, triforine was administered in the diet at a concentration of 0, 2500, 7000 or 20 000 ppm (equal to 0, 162.7, 453.6 and 1315.3 mg/kg bw per day for males and 0, 174.1, 491.4 and 1451.4 mg/kg bw per day for females, respectively). A NOAEL was not identified, as the incidence of marked haemosiderin deposition in the spleen was increased in female rats at 2500 ppm (equal to 174.1 mg/kg bw per day), the lowest dose tested.

In a 3-month study of toxicity in rats, triforine was administered in the diet at concentrations providing doses of 0, 10, 100 and 1000 mg/kg bw per day. The NOAEL was 10 mg/kg bw per day for males and females, based on haemolytic anaemia and increased liver weight at 100 mg/kg bw per day and above.

In a 14-week study of toxicity in rats, triforine was administered in the diet at a concentration of 0, 100 or 500 ppm (equal to 0, 6.0 and 30.4 mg/kg bw per day for males and 0, 6.9 and 34.0 mg/kg bw per day for females, respectively). The NOAEL was 500 ppm (equal to 30.4 mg/kg bw per day), the highest dose tested.

In a 90-day study of toxicity and neurotoxicity in rats, triforine was administered in the diet at a concentration of 0, 200, 2000 or 20 000 ppm (equal to 0, 13, 133 and 1344 mg/kg bw per day for males and 0, 15, 150 and 1540 mg/kg bw per day for females, respectively). The NOAEL for neurotoxicity was 20 000 ppm (equal to 1334 mg/kg bw per day), the highest dose tested. The NOAEL for all other effects was 200 ppm (equal to 13 mg/kg bw per day), based on evidence of haemolytic anaemia and changes in kidney and liver at 2000 ppm (equal to 133 mg/kg bw per day) and above.

In a 13-week study of toxicity in dogs, triforine was administered in the diet at a concentration of 0, 3500, 10 000 or 30 000 ppm (equal to 0, 83, 230 and 690 mg/kg bw per day for males and 0, 85, 240 and 730 mg/kg bw per day for females, respectively). A NOAEL was not identified, as signs of haemolytic anaemia were observed at all doses.

In a further 13-week study of toxicity in dogs, triforine was administered in the diet at a concentration of 0, 100, 600 or 3500 ppm (equal to 0, 3.6, 22.6 and 121.0 mg/kg bw per day for males and 0, 3.4, 21.3 and 120.7 mg/kg bw per day for females, respectively). The NOAEL was 100 ppm (equal to 3.4 mg/kg bw per day), on the basis of increased haemosiderin deposits in the liver, spleen and bone marrow at 600 ppm (equal to 21.3 mg/kg bw per day) and above.

In a 2-year study of toxicity in dogs, triforine was administered in the diet at a concentration of 0, 10, 40, 100 or 1000 ppm (equal to 0, 0.23, 0.93, 2.39 and 22.50 mg/kg bw per day for males and 0, 0.25, 0.99, 2.56 and 23.60 mg/kg bw per day for females, respectively). The NOAEL was 100 ppm (equal to 2.39 mg/kg bw per day), based on evidence of haemolytic anaemia, increased erythropoiesis and haemosiderin deposition in the liver and bone marrow at 1000 ppm (equal to 22.50 mg/kg bw per day).

As the pattern of changes in the 13-week and 2-year dog studies was similar, the overall NOAEL was 100 ppm (equal to 3.4 mg/kg bw per day). The overall LOAEL was 600 ppm (equal to 21.3 mg/kg bw per day).

In a 105-week carcinogenicity study in mice, triforine was administered in the diet at a concentration of 0, 70, 700 or 7000 ppm (equal to 0, 11.4, 117 and 1204 mg/kg bw per day for males

and 0, 15.9, 161 and 1570 mg/kg bw per day for females, respectively). The NOAEL for systemic toxicity was 70 ppm (equal to 11.4 mg/kg bw per day), based on a slight decrease in body weight gain and changes in large intestine in males at 700 ppm (equal to 117 mg/kg bw per day) and above. In males, higher incidences of hepatocellular adenoma and carcinoma at 7000 ppm were within the historical control ranges and not associated with an increase in the incidence of preneoplastic changes. In females, incidences of alveolar/bronchiolar adenoma, carcinoma and adenoma plus carcinoma at 7000 ppm were statistically significantly increased; the incidence of adenoma was slightly higher than the historical control range, whereas the incidence of carcinoma was within the historical control range. The NOAEL for carcinogenicity was 700 ppm (equal to 161 mg/kg bw per day), based on an increased incidence of lung tumours (predominantly adenomas) in females at 7000 ppm (equal to 1570 mg/kg bw per day).

In a non-GLP-compliant 2-year study of carcinogenicity in rats, triforine was administered in the diet at a concentration of 0, 25, 125, 625 or 3125 ppm (equal to mean achieved doses of 0, 1.2, 6.2, 31.2 and 158.7 mg/kg bw per day for males and 0, 1.5, 7.8, 38.6 and 195.4 mg/kg bw per day for females, respectively). The NOAEL for toxicity was 625 ppm (equal to 31.2 mg/kg bw per day), based on evidence of haemolytic anaemia at 3125 ppm (equal to 158.7 mg/kg bw per day). Triforine was not carcinogenic in this study.

In a subsequent 2-year GLP-compliant study of toxicity and carcinogenicity in rats, triforine was administered in the diet at a concentration of 0, 200, 2000 or 20 000 ppm (equal to 0, 10.3, 101 and 1038 mg/kg bw per day for males and 0, 13.1, 136 and 1436 mg/kg bw per day for females, respectively). The NOAEL for toxicity was 200 ppm (equal to 10.3 mg/kg bw per day), based on evidence of haemolytic anaemia and related changes at 2000 ppm (equal to 101 mg/kg bw per day) and above. Triforine was not carcinogenic in this study.

The Meeting concluded that triforine is carcinogenic in female mice, but not in male mice or male or female rats.

Triforine was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. It did not induce gene mutation in bacterial and mammalian cell lines, and it did not induce unscheduled DNA synthesis or DNA repair in rat hepatocytes in vitro. Structural chromosomal aberrations were inducible in vitro, but not in vivo, in a mouse bone marrow micronucleus test.

The Meeting concluded that triforine is unlikely to be genotoxic in vivo.

In view of the lack of genotoxicity in vivo, the absence of carcinogenicity in male mice and male and female rats and the fact that an increased incidence of lung tumours (predominantly adenomas) was observed only in female mice at the highest dose tested, the Meeting concluded that triforine is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats, triforine was administered in the diet at a concentration of 0, 500, 3000 or 20 000 ppm (equal to 0, 39.4, 252.5 and 1768.3 mg/kg bw per day for males and 0, 54.6, 323.3 and 2209.2 mg/kg bw per day for females, respectively). The NOAEL for reproductive toxicity was 20 000 ppm (equal to 1768.3 mg/kg bw per day), the highest dose tested. The NOAEL for parental toxicity was 500 ppm (equal to 39.4 mg/kg bw per day), based on reduced weight gain, increased spleen weight and haemosiderin deposition in spleen at 3000 ppm (equal to 252.5 mg/kg bw per day) and above. The NOAEL for offspring toxicity was 500 ppm (equal to 39.4 mg/kg bw per day), based on reduced preweaning body weight gain at 3000 ppm (equal to 252.5 mg/kg bw per day) and above.

In a study of developmental toxicity in rats dosed at 0, 200, 500 or 1000 mg/kg bw per day, the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested. There was no evidence of teratogenicity.

In a developmental toxicity study in which Himalayan rabbits were dosed at 0, 5, 25 or 125 mg/kg bw per day, the NOAEL for maternal toxicity was 25 mg/kg bw per day, based on a reduction in feed consumption and body weight at 125 mg/kg bw per day during the first days of

treatment. The NOAEL for embryo and fetal toxicity was 125 mg/kg bw per day, the highest dose tested.

In a second developmental toxicity study in which New Zealand White rabbits were dosed at 0, 6, 30 or 150 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day, based on reductions in feed consumption and body weight gain at 150 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 150 mg/kg bw per day, the highest dose tested.

In a third developmental toxicity study in which New Zealand White rabbits were dosed at 0 or 1000 mg/kg bw per day, a NOAEL for maternal toxicity and embryo/fetal toxicity was not identified, based on reductions in body weight gain and feed consumption in dams, a slight reduction in fetal weights and a delay in ossification in fetuses at 1000 mg/kg bw per day, the only dose tested. There was no evidence of teratogenicity.

The Meeting concluded that triforine is not teratogenic.

The Meeting concluded that triforine is not neurotoxic, based on the 90-day study in rats described previously.

In a 28-day dietary study in mice and rats, no immunotoxic effects were seen up to the highest dose tested (1115 mg/kg bw per day in both species).

The Meeting concluded that triforine is not immunotoxic.

Following a 28-day administration of triforine to mice at a dietary concentration of 7000 ppm (equal to 1555.3 mg/kg bw per day) and to rats at a dietary concentration of 20 000 ppm (equal to 1956.9 mg/kg bw per day), it was concluded that triforine does not have any marked stimulatory or inhibitory effect on hepatic xenobiotic metabolism and does not produce hepatic peroxisome proliferation in either species.

Toxicological data on metabolites and/or degradates

No metabolites or degradates have been identified in plants.

Human data

In reports on manufacturing plant personnel, no adverse health effects were noted, and no information on accidental or intentional poisoning in humans is available.

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

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw, derived from an overall NOAEL of 3.4 mg/kg bw per day from studies of toxicity in dogs. A safety factor of 100 was applied. The margin of exposure between the upper bound of the ADI and the LOAEL for lung tumours in female mice is greater than 50 000.

The Meeting established an ARfD of 0.3 mg/kg bw based on the NOAEL of 25 mg/kg bw per day for reduced body weight gain and reduced feed intake in dams in the first days after dosing in a rabbit developmental toxicity study. The Meeting considered the early reduction in feed intake at 25 mg/kg bw per day in this study as not relevant because it was transient, not associated with reduced body weight gain and not observed at 30 or 150 mg/kg bw per day in a second rabbit study. A safety factor of 100 was applied.

Levels relevant to risk assessment of triforine

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity[a]	Toxicity	70 ppm, equal to 11.4 mg/kg bw per day	700 ppm, equal to 117 mg/kg bw per day
		Carcinogenicity	700 ppm, equal to 161 mg/kg bw per day	7 000 ppm, equal to 1 570 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity[a,b]	Toxicity	200 ppm, equal to 10.3 mg/kg bw per day	2 000 ppm, equal to 101 mg/kg bw per day
		Carcinogenicity	20 000 ppm, equal to 1 038 mg/kg bw per day[c]	–
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	20 000 ppm, equal to 1 768.3 mg/kg bw per day[c]	–
		Parental toxicity	500 ppm, equal to 39.4 mg/kg bw per day	3 000 ppm, equal to 252.5 mg/kg bw per day
		Offspring toxicity	500 ppm, equal to 39.4 mg/kg bw per day	3 000 ppm, equal to 252.5 mg/kg bw per day
	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[c]	–
Rabbit	Developmental toxicity study[d]	Maternal toxicity	25 mg/kg bw per day	125 mg/kg bw per day
		Embryo and fetal toxicity	125 mg/kg bw per day[c]	–
Dog	Thirteen-week and 2-year studies of toxicity[a,b]	Toxicity	100 ppm, equal to 3.4 mg/kg bw per day	600 ppm, equal to 21.3 mg/kg bw per day

[a] Dietary administration.

[b] Two or more studies combined.

[c] Highest dose tested.

[d] Gavage administration.

Estimate of acceptable daily intake (ADI)

0–0.03 mg/kg bw

Estimate of acute reference dose (ARfD)

0.3 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 triforine

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid; absorption is ~80% at low dose and 10–20% at high dose
Dermal absorption	No data
Distribution	Widely distributed
Potential for accumulation	None
Rate and extent of excretion	Rapid; at low dose, ~75% within 24 hours, mainly via urine; at high dose, 77–85% via faeces within 168 hours
Metabolism in animals	Extensively metabolized; cleavage of side-chain, followed by oxidation and conjugation with glucuronic acid or glutathione
Toxicologically significant compounds in animals and plants	Triforine
Acute toxicity	
Rat, LD_{50}, oral	> 5 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.12 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (Maurer optimization test)
Short-term studies of toxicity	
Target/critical effect	Haematopoietic system / haemolytic anaemia
Lowest relevant oral NOAEL	3.4 mg/kg bw (dog)
Lowest relevant dermal NOAEL	1 100 mg/kg bw, highest dose tested (rat)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Haematopoietic system / haemolytic anaemia
Lowest relevant NOAEL	10.3 mg/kg bw per day (rat)
Carcinogenicity	Lung tumours in female mice; unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Unlikely to be genotoxic in vivo
Reproductive toxicity	
Target/critical effect	No reproductive effect
Lowest relevant parental NOAEL	39.4 mg/kg bw per day
Lowest relevant offspring NOAEL	39.4 mg/kg bw per day
Lowest relevant reproductive NOAEL	1768.3 mg/kg bw per day, highest dose tested
Developmental toxicity	
Target/critical effect	None
Lowest relevant maternal NOAEL	25 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	125 mg/kg bw per day, highest dose tested (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	1334 mg/kg bw per day, highest dose tested

Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	1115 mg/kg bw per day, highest dose tested
Medical data	
	No adverse health effects reported in workers at triforine manufacturing plants

Summary

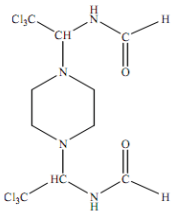
	Value	Study	Safety factor
ADI	0–0.03 mg/kg bw	Thirteen-week and 2-year studies of toxicity (dog)	100
ARfD	0.3 mg/kg bw	Developmental toxicity study (rabbit)	100

RESIDUE AND ANALYTICAL ASPECTS

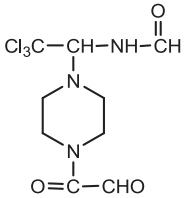
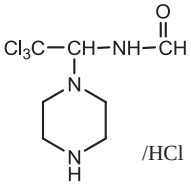
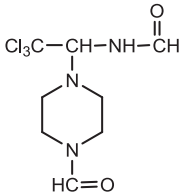
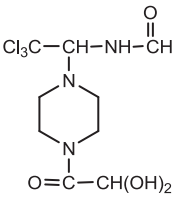
Triforine is a systemic fungicide for control of powdery mildews, rusts, scabs and rots. It was first evaluated by JMPR in 1977 (T), 1978 (T, R) and lastly in 1997 (T). The ADI for Triforine was 0–0.02 mg/kg bw and no ARfD was recommended by the previous JMPR. Triforine was scheduled at the Forty-fifth Session of the CCPR (2013) for the periodic re-evaluation of toxicity and residues by the 2014 JMPR.

The Meeting received information on identity, physical and chemical properties, animal and plant metabolism, environmental fate in soil, analytical methods, storage stability, use patterns, supervised trials, farm animal feeding studies and fates of residues in processing.

N,N'-{piperazine-1,4-diylbis[(trichloromethyl)methylene]}diformamide



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

WOS 2379	W 1084/W 1069	W 625	W 2379
			
N-{2,2,2-trichloro-1-[4-(2-oxoacetyl)piperazin-1-yl] ethyl}formamide	*N*-(2,2,2-trichloro-1-piperazin-1-yl-ethyl) formamide/ hydrochloride	*N*-[2,2,2-trichloro-1-(4-formyl piparazin-1-yl) ethyl]formamide	Hydrate of *N*-{2,2,2-trichloro-1-[4-(2-oxoacetyl)piperazin-1-yl] ethyl}formamide

Animal metabolism

The Meeting received animal metabolism studies with triforine in rats, lactating goat and laying hens. The metabolism and distribution of triforine in animals were investigated using the [^{14}C-piperazine] and [^{14}C-side chain]-labelled triforine.

Metabolism in rats was summarized and evaluated by the WHO panel of the JMPR in 2014.

Triforine is rapidly metabolized and excreted in rats. Highest residues were found in liver followed by kidney. Residues were lower in muscle and fat. Radioactive residues were identified in the excreta only. *N*-[2,2,2-trichloro-1-(piperazin-1-yl) ethyl]-formamide (W 1084), which is formed by the cleavage of a side chain, was the major component in urine of rats. It was excreted in urine as the glucuronide. The side-chain metabolites trichloroethanol including its glucuronide and *N*-acetylcysteine conjugate of 2,2,2-trichloroethylamine was detected in urine. W 1084 and triforine were detected in the faeces of rats.

Lactating goats were administered with [piperazine-^{14}C]-triforine as an oral dosage equivalent to a dietary level of 250 or 1000 ppm once daily for 3 consecutive days. The goats were sacrificed 4 hours or 6 days after the last treatment.

Radioactivity administered to the goats was rapidly eliminated in urine and faeces. A total of 47% and 72% of the applied radioactivity were eliminated in 24 hours, and a further 16 and 14% were excreted in the following 5 days by the 250 ppm and the 1000 ppm dose group goats, respectively.

Up to 68% of the residual radioactivity in the liver of goat sacrificed 4 hours after the last treatment was extracted. The extracted radioactivity consisted of at least five metabolite fractions. M1 and M2 represented unknown polar metabolite fractions whereas WOS 2379 and W 1084 were characterized. The fraction M1 represented 18% TRR. Triforine, WOS 2379 and W 1084 accounted for 15%, 15% and 13% TRR, respectively. Up to 14% of the residual radioactivity in the liver of goat sacrificed 6 days after the last treatment was extracted. This metabolite pattern was similar to that in the extracted radioactivity of the liver of goat sacrificed 4 hours after the last treatment.

Seventy-eight percent of the residual radioactivity in the kidneys of goats sacrificed 4 hours after the last treatment was extracted. The metabolite pattern of the extracted radioactivity was similar to that in the extracted radioactivity of the liver. The predominant fraction found in the extracted radioactivity of goat sacrificed 4 hours after the last treatment was metabolite fraction M1 (31% TRR). Triforine, WOS 2379 and W1084 represented 19%, 8.4% and 19% TRR, respectively. Analysis of the extracted radioactivity of the kidneys of a goat sacrificed 6 days after the last treatment showed a similar metabolite pattern in terms of their relative amounts in the extracts. The predominant fraction was M1 accounting for 11% TRR. Triforine represented 3.6% TRR.

Seventy-nine percent of the residual radioactivity in the muscle of goat sacrificed 4 hours after the last treatment was extracted. The predominant radioactive fraction was triforine at 41% TRR. The other metabolites accounted for 9.5% (W 1084), 13% (WOS 2379), 0.70% (M2) and 15% TRR (M1).

In one study, laying hens were orally administered with [piperazine-^{14}C]-triforine at the dietary dose equivalent to 500 or 2000 ppm in the feed once daily for 3 consecutive days. The hens were sacrificed 4 hours or 7 days after the last treatment.

The radioactivity administered to the hens was rapidly eliminated (54–84% in 56 hours after the first dose). In the following 7 days a further 10–15% of the administered radioactivity was excreted (76% excreted from 500 ppm dosed hens and 94% from 2000 ppm dosed hens).

The highest values in eggs were found about 4 to 5 days after the first treatment with [^{14}C] triforine.

In the other study, laying hens were administered with [side chain-^{14}C]-triforine for 10 consecutive days at a dose of 32 ppm in the feed. Radioactivity recovered in excreta during the 10 days accounted for about 85% of the total cumulative dose.

Total radioactivity in eggs increased steadily during the 10 days to a peak value of 1.6 mg equiv/kg (yolk) and 0.19 mg equiv/kg (white). The major components in egg white and egg yolk were the fraction A which accounted for 48% TRR (0.08 mg/kg) and the sulphate conjugate of trichloroethanol which accounted for 25% TRR (0.25 mg/kg), respectively. W 1069 was observed and accounted for 10% TRR (0.10 mg/kg) in egg yolk. Triforine accounted for 13% TRR(0.02 mg/kg) and 2.1% TRR(0.02 mg/kg) in egg white and egg yolk, respectively.

The fraction A in the protease-treated extract of liver was separated into five components each of which accounted for 0.07–0.40 mg equiv/kg (4–24% TRR). The sulphate conjugate of trichloroethanol was present in liver accounting for 9% TRR. There appeared to be a small amount of triforine in liver (2.9% TRR, 0.05 mg/kg).

The main components in muscle were the trichloroethanol sulphate conjugate and W 1069 each accounting for about 22% TRR (0.05 mg equiv/kg). Triforine was present at 8.4% TRR (0.02 mg/kg).

Triforine accounted for 18% TRR (0.02 mg/kg) and the sulphate conjugate of trichloroethanol accounted for 36% TRR (0.03 mg equiv/kg) in fat.

Triforine was also found in skin at low levels (0.01 mg/kg). The retention time of the main fraction corresponded to that of trichloroethanol sulphate (56% TRR, 0.13 mg/kg).

In animal metabolism studies, triforine, W 1084/W 1069, WOS 2379 and trichloroethanol sulphate were predominantly found in tissues of lactating goats and laying hens. The major component in milk and egg white was the polar fraction consisting of several components but they were not identified. Triforine was identified in egg white and egg yolk. Excretion, distribution and triforine and its metabolites found in excreta of lactating goats and laying hens were similar to those in rats.

Plant metabolism

The Meeting received plant metabolism studies performed on apples, tomatoes and cucumber with triforine ^{14}C-labelled in two carbons at the side chain, and on barley with triforine ^{3}H-labelled at piperazine ring ([side chain-^{14}C] and [piperazine-^{3}H]).

In an outdoor apple metabolism study, a number of fruits or leaves of apple were treated at random on the surface with [side chain-^{14}C]-triforine at a rate of 1.2 g ai/L in a series of small droplets. Treated apple fruits were harvested 2 weeks after the last of five successive applications with 8-day intervals. After five successive applications of [^{14}C] triforine, 32% (fruit) and 22% (leaf) of the applied radioactivity were recovered. On an average, 1.36 mg equiv/kg was recovered in the treated fruits.

The major component in the surface washes and extracts of fruits was identified as triforine accounted for 73–79% TRR (0.88–1.2 mg/kg) two weeks after the last application. Several minor components were observed in the extracts and each of them accounted for 1–2% TRR.

In an indoor tomato metabolism study, a number of fruits or leaves of tomato were treated at random on the surface with [side chain-^{14}C]-triforine at a rate of 1.2 g ai/L in a series of small droplets. The treated tomatoes were harvested at 2 hours and 3 days after the last of four successive applications with 8–10 days intervals. The initial surface washes of treated tomatoes at harvest contained, on an average, 92% (2 hours after the last application) and 91% (3 days after the last application) of TRR. Acetonitrile extracts of washed and homogenised tomatoes accounted for, on an average, 5.8% TRR (2 hours after the last application) and 6.2% TRR (3 days after the last

application). The TRR from the treated tomatoes accounted for, on an average, 16 (2 hours after the last application) and 9.7 (3 day after the last application) mg equiv/kg.

Triforine in the surface washes and extracts accounted for 91–93% TRR (7.6–19 mg/kg) in tomatoes taken at 2 hours and 3 days after the final application of [^{14}C] triforine. The extracts also contained several minor components each accounting for, on an average, 0.05–1.1% TRR.

In an indoor cucumber metabolism study, a number of fruits or leaves of cucumber were treated at random on the surface with [side chain-^{14}C]-triforine at a rate of 1.2 g ai/L in a series of small droplets. The treated cucumbers were harvested 3 days after the last of four successive applications at 7-day intervals. The surface washes of treated cucumbers at harvest contained, on an average, 85% of TRR. Extracts of washed and homogenised cucumber peel and flesh accounted for, on an average, 7.5% TRR (peel) and 1.4% TRR (flesh). The TRR from the treated cucumbers accounted for, on an average, 2.2 mg equiv/kg.

The major radioactive component was identified as triforine in the surface washes and extracts accounted for 87–88% TRR (1.9 mg/kg) in cucumber taken 3 days after the final application of [^{14}C] triforine. The extracts also contained several minor components each accounting for means of 0.3–2% TRR.

In the first indoor barley metabolism study, [piperazine-^{3}H]-triforine was applied to barley plants grown in plastic pots as soil drenching. The leaves were harvested at 15 and 30 days after treatment.

Triforine was identified as a major component in the barley leaves, amounting to 58% TRR at 15 days after the treatment and 43% TRR at 30 days after the treatment. W 1084 was also observed in the 0.1M HCl extract (8.4–13% TRR).

In the second indoor barley metabolism study, the leaves of barley plants root-treated with [piperazine-^{3}H]-triforine were collected 30 days after treatment. The major component was identified as triforine, accounted for 45% TRR, and W 1084 and piperazine were also observed.

In the third outdoor barley metabolism study, the plants (during the stem extension stage when the second node of the stem was formed and the next-to-last leaf was just visible) were sprayed with an aqueous emulsion of a mixture of the commercial formulation of triforine and [piperazine-^{3}H]-triforine at a rate of 0.25 kg ai/ha. Barley was harvested when ripe, and straw and grain were analysed separately.

The methanol soluble radioactive residue contained triforine and its metabolites which were free in barley straw and grain. Triforine accounted for 0.034 mg/kg (18% TRR) in straw and 0.0018 mg/kg in grain (13% TRR). W 1084 was identified as a minor component. Two other radiolabelled components were identified in straw: glycine at 0.043 mg/kg (33% TRR) and iminodiacetic acid at 0.021 mg/kg (17% TRR), respectively.

In the plant metabolism studies, triforine was the major component of the residues found in all plants studied.

Environmental fate

The Meeting received information on aerobic degradation in soil, photolysis on soil surface and hydrolytic degradation study.

In soil under the aerobic conditions, the DT_{50} ranged from 1–70 days at 20 °C. Many minor degradation products were detected in the extracts during the study. Most of the radioactivity was recovered from natural components. Mineralization was up to 45%. Minor degradates were identified as W 625, WOS 2379, piperazine and W 1069, but all of them were less than 3% TAR.

In soil photolysis study, the degradation was biphasic. The photodegradation half-life of triforine was 11 hours of artificial sunlight or 0.5 natural sunlight days for phase 1 (hours 0 to 8). For

phase 2 (hours 8 to 48), the half-life was 71 hours of artificial sunlight equivalent to 3.2 natural sunlight days.

In summary, triforine was rapidly and completely degraded in soil and is unlikely to be taken up by crops from the soil after soil treatment.

Methods of analysis

The Meeting received description of validation data on analytical methods for residues of triforine in plant and animal commodities.

In most of the methods for the determination of triforine in plant, homogenized samples were extracted with acetone, and the extract was partitioned into organic solvent and the active substance was degraded by heating with dilute sulphuric acid. Chloral hydrate thus formed was determined by GC-ECD. The methods of analysis with GC-ECD for a range of matrices were validated with acceptable recoveries with the LOQs of 0.01 mg/kg for triforine.

New methods using LC-MS or LC-MS/MS were developed for analysing triforine directly. In the methods, homogenized samples were extracted with acetone, and the extract was purified with SPE cartridge clean-ups. The methods of analysis with LC-MS or LC-MS/MS for a range of matrices were validated with acceptable recoveries with the LOQs of 0.01 mg/kg except for tomato paste for which the LOQ was 0.05 mg/kg.

In the methods for animal commodities, homogenized samples were extracted with acetone, or were diluted with water. Triforine and possible metabolites containing the Cl_3C-CH group in the acetone extract or diluted homogenate were degraded by heating with dilute sulphuric acid. Chloral hydrate thus formed was determined by GC-ECD. These methods were validated with acceptable recoveries with the LOQ of 0.001–0.003 mg/kg for milk and animal tissues, and 0.01 mg/kg for egg.

The Meeting was aware that the QuEChRS-multi residue method was validated for most plant matrices with LOQs of 0.01–0.02 mg/kg for triforine.

Stability of residues in stored analytical samples

The Meeting received information on the freezer storage stability of triforine in plant (apples, cherries, plums, peaches, blueberries and hops) and their processed (beer) commodities. Analysis was done by the common moiety method.

Using the common moiety method, storage stability results indicated that residues with common moiety including triforine were stable for at least 1 month in processed hops (beer), at least 6 months in plums and hops (dried cones), at least 12 months in apples, cherries, peaches and blueberries. However, according to the result of plant metabolism study on tomato, triforine seems stable for at least 5 months.

Definition of the residue

In the lactating goat metabolism studies, TRRs in liver and kidney were higher than those in milk, muscle and fat. Triforine, WOS 2379 and W 1084 accounted for 15%, 15% and 13% TRR in liver, and 19%, 8.4% and 19% TRR in kidney. The polar unknown fraction M1 represented 18% TRR in liver and 31% TRR in kidney. In the laying hen metabolism studies, TRR in liver was also higher than those in other tissues. In the study using [side chain-^{14}C]-triforine, the trichloroethanol sulphate conjugate and W 1069 were the main components in muscle (22% TRR) and egg yolk (10–25% TRR).

The analytical methods for animal commodities provided determine only the residues of triforine and metabolites containing the Cl_3C-CH group converted to chloral hydrate which is formed by heating in acidic condition. No method of analysis was available for quantification of triforine alone.

The Meeting decided that triforine and its metabolites containing the Cl_3C-CH group were suitable analytes for enforcement purposes and dietary risk assessment for animal commodities.

The octanol/water coefficient (log P_{ow}) of triforine was 2.2 at 20 °C. In the lactating goat and the laying hen metabolism studies, triforine and its metabolites found in muscle were 2–100 times higher than those in fat. Fractionation of whole milk showed that 32% of the radioactivity was found in cream and 76% was found in skim milk. In the lactating goat feeding study, triforine and its metabolites were detected at 0.003–0.007 mg/kg in liver, kidney and muscle but less than LOQ (< 0.003 mg/kg) in fat. The Meeting considered the residue of triforine is not fat soluble.

In plant metabolism studies, parent triforine was the major component (43–93% TRR) in apple, tomato, cucumber and barley. Several metabolites identified accounted for < 10% TRR.

In most of the analytical methods for triforine in plant, since chloral hydrate formed by heating with dilute sulphuric acid was quantified with GC-ECD, triforine and its metabolites containing piperazine ring were simultaneously measured. As the predominant residue was the parent compound in the plant metabolism study, using the common moiety method would result in only slight over-estimation of residues if PHI was short. Recently LC-MS and LC-MS/MS methods were available for determining triforine only.

The Meeting decided that parent triforine was a suitable analyte for enforcement purposes and dietary risk assessment in plant commodities.

The Meeting recommended the following residue definition:

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities: *Triforine*

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: *Triforine and its metabolites determined as chloral hydrate expressed as triforine*

The residue is not fat soluble

Residue of supervised residue trials on crops

The Meeting received supervised trial data for the foliar application of triforine on apple, pear, cherry, plum, apricot, nectarine, peach, raspberry, blueberry, black currant, grape, cranberry, strawberry, cucumber, squash, melon, peppers, eggplant, tomato, common bean, barley and wheat. Residue trials were conducted in Australia, Brazil, Canada, Denmark, France, Germany, Greece, Hungary, Italy, Japan, Mexico, New Zealand, South Africa, South Korea, the United Kingdom (UK) and the USA. Most of the supervised residue trials employed the common moiety method, while the results of plant metabolism studies showed that triforine was the main component of residues in crops.

Labels were available from a number of countries in North and South America, Africa, Asia and Oceania describing the registered uses of triforine.

Pome fruits

Apple

Data were available from supervised trials conducted on apples in the USA, Canada, Australia, Germany, France and Brazil.

The GAP on apples in Canada was five foliar applications at a maximum rate of 0.475 kg ai/ha between tight cluster and petal fall stage. Trials in the USA and Canada on apples were conducted with foliar applications of EC formulation. Triforine residues in apple from the trials in Canada matching GAP of Canada were (n=1): 0.041 mg/kg.

Trials in Australia on apples were conducted with one to ten foliar applications of EC formulation (GAP: four foliar applications at a maximum spray concentration of 0.023 kg ai/hL with a PHI of 1 day). Triforine residue trials on apples in the Australia did not match the GAP of Australia.

Trials in Germany and France on apples were conducted with foliar applications of EC formulation. No GAP from European countries was available for apple.

The GAP on apples from Brazil was three foliar applications at a maximum concentration of 0.024 kg ai/hL with a PHI of 5 days. Triforine residues in apple from the trials in Brazil matching Brazilian GAP were (n=2): < 0.02 mg/kg (2).

As the data were insufficient for estimating a maximum residue level, the Meeting agreed to withdraw its previous recommendation for apple.

Pear

Data were available from a supervised trial on pears in Australia. No GAP from Australia was available for pear.

The Meeting agreed that estimation of maximum residue level was not possible for pear.

Stone fruits

Cherry

Data were available from supervised trials on cherries conducted in the USA, Canada and Germany.

The GAP on cherries in Canada was for three foliar applications at a maximum spray concentration of 0.014 kg/hL or a rate of 0.475 kg ai/ha between early and full bloom stages. Triforine residue in cherries from the trials in Canada and the USA matching GAP of Canada was (n=1): 0.007 mg/kg.

Trials in Germany on cherries were conducted with foliar applications of an EC formulation. No GAP from European countries was available for cherries.

The Meeting agreed to withdraw its previous recommendation for cherries.

Plum

Data were available from supervised trials on plums conducted in the USA, Canada, Germany, France and South Africa.

The GAP on plums in Canada is for three foliar applications at a maximum spray concentration of 0.014 kg/hL or a rate of 0.475 kg ai/ha between early and full bloom stages. No trials on plums in Canada and the USA matched the GAP of Canada.

Trials in Germany and France were conducted with foliar applications of an EC formulation. No GAP from European countries was available for plums.

The GAP on plums in South Africa was two foliar applications at a rate of 0.87 kg ai/ha with a PHI of 3 days. No trials for plums in South Africa matched the GAP of South Africa.

The Meeting agreed to withdraw its previous recommendation for plums.

Apricot

Data were available from supervised trials on apricots from the USA, France, Greece and Italy.

Trials in the USA on apricots were conducted with one to three foliar applications of an EC formulation at a spray concentration of 0.018 kg ai/hL. No GAP of the USA was available.

Trials in France, Greece and Italy on apricots were conducted with three foliar applications of DC formulation at a spray concentration of 0.038 kg ai/hL. No GAP from European countries was available for apricots.

The Meeting agreed that estimation of maximum residue level was not possible for apricots.

Nectarine

Data were available from supervised trials on nectarines from the USA.

Trials in the USA on nectarines were conducted with one to four foliar applications of an EC formulation at a spray concentration of 0.015–0.018 kg ai/hL. No GAP from the USA was available.

The Meeting agreed that the estimation of maximum residue level was not possible for nectarines.

Peach

Data were available from supervised trials on peaches from the USA, Canada, France, Greece, Japan, Brazil and South Africa.

The GAP on peaches in Canada is three foliar applications at a maximum spray concentration of 0.014 kg/hL or a rate of 0.475 kg ai/ha between early and full bloom stages. Triforine residue in peaches from trials in Canada and the USA matching GAP of Canada was (n=1): 0.02 mg/kg.

The GAP on peaches in Brazil is for three foliar applications at a maximum spray concentration of 0.024 kg ai/hL with a PHI of 3 days. Triforine residues in peaches from trials in Brazil matching GAP were (n=2): < 0.01 and 9.4 mg/kg.

Trials in France and Greece on peaches were conducted with one to eight foliar applications of a DC formulation at a rate of 0.36–0.38 kg ai/ha. No GAP from European countries was available for peaches.

The GAP on peaches in South Africa is two foliar applications at a maximum rate of 0.67 kg ai/ha with a PHI of 3 days. No trials from South Africa on peaches matched the GAP of South Africa.

The GAP on peaches in Japan is five foliar applications at a maximum spray concentration of 0.018 kg ai/hL with a PHI of 1 day. Triforine residues in peaches from the trials in Japan matching GAP of Japan were (n=2): 0.77 and 1.4 mg/kg.

The Meeting considered the data insufficient for the estimation of a maximum residue level, and agreed to withdraw its previous recommendation for peaches.

Berries and other small fruits

Raspberries, Red, black

Data were available from supervised trials on raspberries in France. No GAP from European countries was available for raspberry.

The Meeting agreed that estimation of maximum residue level was not possible for raspberry.

Blueberries

Data were available from supervised trials on blueberries in Canada.

The GAP on blueberries in Canada (except for Eastern Canada) is four foliar applications at a maximum rate of 0.570 kg ai/ha from bud break to 10–14 days after early bloom with a PHI of 60 days. Triforine residues in blueberries from the trials conducted in Canada (except for Eastern Canada) matching this GAP were (n=2): < 0.01 and 0.015 mg/kg.

The GAP for Eastern Canada (only) is three foliar applications at a maximum rate of 0.570 kg ai/ha from leaf-bud break to pink-bud stage with a PHI of 60 days. Triforine residues in blueberries from the trials conducted in Eastern Canada matching this GAP were (n=3): < 0.01(3) mg/kg.

Based on the trials on blueberries in Canada, the Meeting estimated a maximum residue level of 0.03 mg/kg to replace its previous recommendation (1 mg/kg) for blueberry. The Meeting also estimated an STMR and an HR for triforine in blueberry of 0.01 and 0.018 mg/kg, respectively. The highest residue concentration in an individual sample was selected for HR.

Currant, Black

Data were available from supervised trials on black currants in Germany and UK. No GAP from European countries was available for black currants.

The Meeting agreed to withdraw its previous recommendation for black currants.

Grapes

Data were available from supervised trials on grapes from Germany, Mexico and New Zealand.

Trials in Germany on grapes were conducted with one to three foliar applications of an EC formulation at a rate equivalent to 0.28–0.57 kg ai/ha. No GAP from European countries was available for grapes.

Trials in Mexico on grapes were conducted with one foliar application of EC formulation at a rate equivalent to 0.28 kg ai/ha. No GAP from Mexico was available for grapes.

The GAP on grapes in New Zealand is four foliar applications at a rate of at least 0.38 kg ai/ha with a PHI 14 days. No trials in New Zealand on grapes matched the GAP of New Zealand.

The Meeting agreed that estimation of maximum residue level was not possible for grapes.

Cranberry

Data were available from supervised trials on cranberries from the USA.

The GAP on cranberries in Canada was three foliar applications at a maximum rate of 0.570 kg ai/ha with a PHI of 60 days. No trials for cranberries from the USA matching the GAP of Canada were available.

The Meeting agreed that estimation of maximum residue level was not possible for cranberry.

Strawberry

Data were available from supervised trials on strawberries from Mexico, Brazil and Japan.

The GAP on strawberries in Mexico was four foliar applications at a maximum rate of 0.19 kg ai/ha with a PHI of 14 days. Triforine residue in strawberries from the trials in Mexico matching GAP of Mexico was (n=1): 0.57 mg/kg.

The GAP on strawberries in Brazil was four foliar applications at a maximum rate of 0.23 kg ai/ha with a PHI of 2 days. No trials in Brazil on strawberries matched the GAP of Brazil.

The GAP on strawberries in Japan is five foliar applications at a spray concentration equivalent to 0.009 kg ai/hL with a PHI of 1 day. Triforine residues in strawberries from the trials in Japan matching GAP of Japan were (n=4): 0.20, 0.39, 0.48 and 0.78 mg/kg.

The Meeting considered that the data was insufficient for estimating a maximum residue level, the Meeting agreed to withdraw its previous recommendation for strawberry.

Gooseberry

No supervised trials on gooseberry were available.

The Meeting agreed to withdraw its previous recommendation for gooseberry.

Brassica vegetables

Brussels sprouts

No supervised trials on Brussels sprouts were available.

The Meeting agreed to withdraw its previous recommendation for Brussels sprouts.

Fruiting vegetables, Cucurbits

Cucumber

Data were available from supervised trials on cucumbers from the USA, Canada, Mexico, Hungary, France and Germany.

Trials from the USA on cucumbers were conducted with four or five foliar applications of an EC formulation at a rate equivalent to 0.29–0.57 kg ai/ha. No GAP from the USA was available for cucumber.

One trial in Canada on cucumbers was conducted with one foliar application of an EC formulation at a rate of 0.29 kg ai/ha. No GAP from Canada was available for cucumber.

The GAP on cucumbers in Mexico is three foliar applications at a maximum rate of 0.29 kg ai/ha with a PHI of 7 days. Triforine residue in cucumber from a trial in Mexico matching the GAP of Mexico was (n=1): 0.066 mg/kg.

Trials in Hungary, France and Germany on cucumbers were conducted but no GAP from European countries were available.

The Meeting considered that the data was insufficient for estimating a maximum residue level for cucumbers.

Squash

Data were available from supervised trials on summer squash and winter squash from the USA, France and Germany. No GAP from the USA or European countries was available for squash.

The Meeting agreed that estimation of maximum residue level was not possible for squash.

Melon

Data were available from supervised trials on melon from the USA, Mexico, France, Italy and Japan.

Trials in the USA on melons were conducted with one or five foliar applications of an EC formulation at a rate of 0.23–0.46 kg ai/ha. No GAP from the USA was available for melons.

The GAP on melons in Mexico is three foliar applications at a maximum rate of 0.29 kg ai/ha with a PHI of 7 days. Triforine residue in melon from one trial in Mexico matching GAP of Mexico was (n=1): 0.039 mg/kg.

Trials from Italy and France on melon were conducted with one or four foliar applications of an EC formulation at a rate of 0.20–0.29 kg ai/ha. No GAP from European countries was available for melons.

The GAP on melon in Japan is six foliar applications at a spray concentration of 0.009 mg/hL with a PHI of 1 day. Triforine residues in melon from the trials in Japan were (n=2): < 0.005 and 0.006 mg/kg.

The Meeting considered the data was insufficient for estimating a maximum residue level for melons.

The Meeting agreed to withdraw its previous recommendation for fruiting vegetables, cucurbits.

Fruiting vegetables, other than Cucurbits

Peppers

Data were available from supervised trials on peppers from Mexico, Japan and South Korea.

The GAP on peppers in Mexico is three foliar applications at a maximum rate of 0.29 kg ai/ha with a PHI of 14 days. Triforine residue in peppers from the trials in Mexico matching GAP of Mexico was (n=1): 0.12 mg/kg.

The GAP on peppers in Japan is three foliar applications at a spray concentration of 0.018 kg ai/hL with a PHI of 14 days. Triforine residues in peppers from trials in Japan matching GAP of Japan were (n=2): 0.06 and 0.22 mg/kg.

The GAP on chili peppers in South Korea is two foliar applications at a spray concentration equivalent to 0.019 kg ai/ha with a PHI of 7 days. Tis residue in peppers from the trial in South Korea matching GAP of South Korea was (n=1): 0.35 mg/kg.

The Meeting considered that the data was insufficient for estimating a maximum residue level for peppers.

Egg plant

Data were available from supervised trials on egg plants in Mexico and Japan.

The GAP on egg plants in Mexico is three foliar applications at a maximum rate of 0.29 kg ai/ha with a PHI of 15 days. Triforine residue in egg plants from a trial in Mexico matching GAP of Mexico was (n=1): 0.066 mg/kg.

The GAP on eggplants in Japan is five foliar applications at a spray concentration of 0.018 kg ai/hL with a PHI of 1 day. Triforine residues in egg plants from the trials in Japan matching GAP of Japan were (n=5): 0.25, 0.28, 0.29, 0.38 and 0.39 mg/kg.

Based on the trials on egg plants in Japan, the Meeting estimated a maximum residue level, an STMR and an HR for triforine in egg plants of 1, 0.29 and 0.39 mg/kg, respectively.

Tomato

Data were available from supervised trials on tomatoes in the USA, Mexico, Denmark and Japan.

One trial from Denmark on tomatoes was conducted with one foliar application of EC formulation at a rate equivalent to 0.95 kg ai/ha. No GAP of European countries were available for tomatoes.

The GAP on tomatoes in Japan was three foliar applications at a spray concentration of 0.018 kg ai/hL with a PHI of 1 day. Triforine residues in tomatoes from trials in Japan matching GAP of Japan were (n=5): 0.14, 0.17, 0.26, 0.28 and 0.56 mg/kg.

The GAP on tomatoes in Mexico is four foliar applications at a maximum rate of 0.38 kg ai/ha with a PHI of 3 days. Triforine residues in tomatoes from the trials in Mexico matching GAP of Mexico were (n=5): 0.083, 0.096, 0.13, 0.27 and 0.40 mg/kg.

Trials from the USA on tomatoes were conducted with four or five foliar applications of an EC formulation at a rate of 0.18–0.41 kg ai/ha. Triforine residues in tomatoes from the trials in the USA matching GAP of Mexico were (n=3): 0.072, 0.17 and 0.28 mg/kg.

The Meeting decided to use the triforine residue data from the trials in Mexico and the USA. Triforine residues in tomatoes from the trials in Mexico and the USA matching GAP of Mexico were (n=8): 0.072, 0.083, 0.096, 0.13, 0.17, 0.27, 0.28, 0.40 mg/kg.

Based on the data, the Meeting estimated a maximum residue level of 0.7 mg/kg to replace its previous recommendation (0.5 mg/kg). The Meeting also estimated an STMR and an HR for triforine in tomato of 0.15 and 0.40 mg/kg, respectively.

Legume vegetables

Common bean

Data were available from supervised trials on common beans from Brazil and South Africa.

The GAP on beans in Brazil is three foliar applications at a maximum rate of 0.29 kg ai/ha with a PHI of 10 days. Triforine residue in bean seeds from the trials in Brazil matching GAP of Brazil was (n=1): < 0.01 mg/kg.

The GAP on beans in South Africa is for foliar application(s) with spray at a rate of 0.29 kg ai/ha with a PHI of 3 days (the maximum numbers of applications not specified). Triforine residue in beans from one trial in South Africa matching GAP of South Africa was (n=1): 0.44 mg/kg.

As the available data was insufficient for estimating a maximum residue level, the Meeting agreed to withdraw its previous recommendation for common bean (pods and immature seeds).

Cereal grains

Data were available from supervised trials on barley in France. No GAP from European countries was available for barley.

Data were available from supervised trials on wheat in Austria, France, UK and Brazil. No GAP from European countries and Brazil was available for wheat.

No other information was available for any other cereal grains.

The Meeting agreed that the estimation of maximum residue level was not possible for cereal grains.

The Meeting agreed to withdraw its previous recommendation for cereal grains.

Animal feedstuffs

Barley straw and forage

Data were available from supervised trials on barley straw and forage in France. No GAP from European countries was available for barley straw and forage.

The Meeting agreed that the estimation of a maximum residue level was not possible for barley straw and forage.

Wheat straw and forage

Data were available from supervised trials on wheat straw and forage in France and UK. No GAP from European countries was available for wheat straw and forage.

The Meeting agreed that the estimation of a maximum residue level was not possible for wheat straw and forage.

Fate of residues during processing

Residues in processed commodities

The fate of triforine residues following the processing of plums, grapes and tomatoes was made available to the Meeting. Estimated processing factors and the derived STMR-Ps are summarized in the Table below.

Processing factors, STMR-P for food

Raw agricultural commodity (RAC)	Processed commodity	Calculated processing factors [a]	PF (Mean or best estimate)	RAC STMR (mg/kg)	STMR-P (mg/kg)
Tomato	Juice	0.74 [b], 0.76 [b]	0.75	0.15	0.11
	Paste	< 0.008	< 0.008		< 0.001
	Puree	0.14, 2.3 [b], 2.6 [b]	2.3		0.35
	Wet pomace	< 0.12	< 0.12		< 0.018
	Dry pomace	1.6	1.6		0.24

[a] Each value represents a separate study.
[b] RAC was dipped in EC formulation solution.

Residues in animal commodities

Estimated maximum and mean dietary burdens of farm animals

The maximum and mean dietary burdens were calculated using the median residue of triforine in dry tomato pomace estimated at the current Meeting on a basis of the OECD Animal Feeding Table.

Summary of livestock dietary burdens (ppm of dry matter diet)

Livestock dietary burden, triforine, ppm of dry matter diet								
	US-Canada		EU		Australia		Japan	
	Max	Mean	Max	Mean	Max	Mean	Max	Mean
Beef cattle	0	0	0	0	0.027 [a]	0.027 [b]	0	0
Dairy cattle	0	0	0	0	0.027	0.027 [c]	0	0
Broilers	0	0	0	0	0	0	0	0
Layers	0	0	0	0	0	0	0	0

[a] Highest maximum beef cattle dietary burden suitable for MRL estimates for mammalian meat, fat, edible offal and milk
[b] Highest mean beef cattle dietary burden suitable for STMR estimates for mammalian meat, fat and edible offal

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

Farm animal feeding studies

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

Lactating dairy goats

Lactating dairy goats were dosed with triforine for 30 days at doses equivalent to 5, 15 and 50 ppm in the diet. Residues of triforine were at or less than the LOQ (0.001 mg/L) in whole milk at the 5 ppm of feeding level except on sampling day 29 (< 0.001–0.003 mg/L). In the highest dose group (50 ppm feed), triforine residues in milk reached a plateau at the level of 0.002–0.010 mg/L after 3 days. In tissues, no measurable residues were found in fat, liver, kidney and muscle of the 5 ppm feed group. In the 15 ppm feed group, triforine concentration slightly exceeded the LOQ in several samples. In the 50 ppm feed group, residues were detected in all of the analysed tissues with exception of muscle in one animal and fat of all animals. The maximum values in kidney and liver were 0.009 and 0.012 ppm, respectively.

Animal commodities maximum residue levels

For MRL estimation, the residue in the animal commodities is triforine.

The maximum dietary burden for beef and dairy cattle was 0.027 ppm. The maximum dietary burden for beef and dairy cattle was 0.54% of the lowest dose of 5 ppm in feed of the lactating goat feeding study. In the lactating goat feeding study at 5 ppm, triforine was at < 0.01 mg/kg in milk and < 0.01 mg/kg in liver.

The Meeting estimated a maximum residue level of 0.01* mg/kg and an STMR of 0 mg/kg in milk.

The Meeting estimated a maximum residue level of 0.01* mg/kg, an STMR of 0 mg/kg and an HR of 0 mg/kg in mammalian meat and fat.

The Meeting estimated a maximum residue level of 0.01* mg/kg, an STMR of 0 mg/kg and an HR of 0 mg/kg in mammalian edible offal.

RECOMMENDATIONS

On the basis of the data from supervised trials, the Meeting concluded that the residue levels listed below are suitable for estimating maximum residue limits and for IEDI and IESTI assessment.

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for plant commodities: *Triforine.*

Definition of the residue (for compliance with the MRL and for estimation of dietary intake) for animal commodities: *Triforine and its metabolites determined as chloral hydrate expressed as triforine.*

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The International Estimated Daily Intakes (IEDIs) of triforine were calculated for the 17 GEMS/Food cluster diets using STMRs/STMR-Ps estimated by the current Meeting (Annex 3 of the 2014 Report). The ADI is 0–0.03 mg/kg bw and the calculated IEDIs were 0–2% of the maximum ADI. The Meeting concluded that the long-term intakes of residues of triforine, resulting from the uses considered by current JMPR, are unlikely to present a public health concern.

Short-term intake

The ARfD for triforine is 0.3 mg/kg bw. The International Estimate of Short Term Intakes (IESTIs) for triforine were calculated for the food commodities for which STMRs or HRs were estimated by the present Meeting and for which consumption data were available. The results are shown in Annex 4 of the 2014 JMPR Report. The IESTIs were 0–5% of the ARfD for children and the general population. The Meeting concluded that the short-term intake of residues of triforine from other uses that have been considered by the present Meeting is unlikely to present a public health concern.