5.7 DICHLOBENIL (274)

TOXICOLOGY

Dichlobenil is the ISO-approved common name for 2,6-dichlorobenzonitrile (IUPAC), with CAS number 1194-65-6. It belongs to the group of benzonitrile compounds, which are used as herbicides. It inhibits the germination of actively dividing meristems and acts primarily on growing points and root tips.

Dichlobenil has not previously been evaluated by JMPR and was reviewed by the present Meeting at the request 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

Following gavage dosing of rats at 6 mg/kg bw, dichlobenil was extensively absorbed; about 89% and 97% of the administered dose were absorbed in males and females, respectively, in 7 days, based on a comparison of urinary excretion following oral and intravenous administration. Oral absorption after repeated exposure was at least 60%, irrespective of dose and sex. Liver, kidney and kidney fat reached the highest concentrations of radioactivity; concentrations peaked 1 hour after administration in these organs and in plasma. The elimination of the radioactivity associated with [^{14}C]dichlobenil following oral administration was rapid, with most (about 80%) being eliminated within 24 hours at the low dose. About 60% was excreted in the urine and about 20% in the faeces, irrespective of sex or dose regimen, within 9–10 days after initiation of exposure. Approximately 79% of the administered dose was excreted in the bile within 24 hours in bile duct–cannulated rats, suggesting enterohepatic recirculation.

Dichlobenil was extensively metabolized, and no parent compound was detected in the urine. Most of the urinary metabolites were conjugates, such as sulfate, glutathione (and derivatives) and glucuronic acid conjugates. In faeces, three major metabolites (each representing at least 5% of the administered dose) were observed, as well as parent compound (dichlobenil). The proportion of conjugated metabolites decreased with increasing dose, whereas the amount of parent compound in faeces increased. Dichlobenil was metabolized in rats via two metabolic pathways: the first pathway involves hydroxylation at the 3 or 4 position, followed by glucuronidation or sulfation, and the second pathway involves substitution of one chlorine atom by glutathione.

Toxicological data

The acute oral and dermal LD_{50}s were greater than 2000 mg/kg bw in rats and rabbits, respectively. The acute inhalation LC_{50} in rats was greater than 3.2 mg/L. Dichlobenil was non-irritating to rabbit skin and eyes. It was not a skin sensitizer in guinea-pigs, as determined by the Magnusson and Kligman maximization test.

The liver was the primary target organ in mice, rats, hamsters and dogs in repeated-dose toxicity studies.

In a 90-day toxicity study in mice using dietary dichlobenil concentrations of 0, 25, 125, 625 and 3125 ppm (equal to 0, 3.8, 19, 91 and 447 mg/kg bw per day for males and 0, 4.8, 24, 114 and 512 mg/kg bw per day for females, respectively), the NOAEL was 625 ppm (equal to 91 mg/kg bw per day), based on transient clinical signs of toxicity, decreased body weight gains, decreased feed consumption and liver toxicity (increased liver weights, clinical chemistry, severity of centrilobular hypertrophy and glycogen storage) at 3125 ppm (equal to 447 mg/kg bw per day).

In a 90-day toxicity study in hamsters using dietary dichlobenil concentrations of 0, 41, 209, 1289 and 7500/4648 ppm (equivalent to 0, 3, 16, 79 and 395/263 mg/kg bw per day, respectively), the NOAEL was 41 ppm (equivalent to 3 mg/kg bw per day), based on decreased weight and mineralization of the prostate and decreased absolute seminal vesicle and testicular weights at 209 ppm (equivalent to 16 mg/kg bw per day).

In a 90-day toxicity study in rats, dichlobenil was administered in the diet at a concentration of 0, 100, 1000 or 3000 ppm (equivalent to 0, 10, 100 and 300 mg/kg bw per day, respectively); an additional group of males was fed 10 000 ppm (equivalent to 1000 mg/kg bw per day). The NOAEL was 100 ppm (equivalent to 10 mg/kg bw per day), based on hepatocellular necrosis and inflammation in males seen at 1000 ppm (equivalent to 100 mg/kg bw per day).

In a 90-day toxicity study in dogs using dietary dichlobenil concentrations of 0, 50, 150 and 450 ppm (equivalent to 0, 1.3, 3.8 and 11 mg/kg bw per day), the NOAEL was 150 ppm (equivalent to 3.8 mg/kg bw per day), based on increased alkaline phosphatase, alanine aminotransferase and liver and kidney weights at 450 ppm (equivalent to 11 mg/kg bw per day).

In a 52-week toxicity study in dogs administered dichlobenil by capsule at a dose of 0, 1, 6 or 36 mg/kg bw per day, the NOAEL was 1 mg/kg bw per day, based on increases in liver weights, serum cholesterol, triglycerides, phospholipids, alkaline phosphatase and gamma-glutamyltransferase and periportal hypertrophy of the hepatocytes in males at 6 mg/kg bw per day.

In a 2-year toxicity study in dogs using dietary dichlobenil concentrations of 0, 20, 50 and 350 ppm (equivalent to 0, 0.5, 1.3 and 8.8 mg/kg bw per day), the NOAEL was 50 ppm (equivalent to 1.3 mg/kg bw per day), based on increases in liver weight, serum alkaline phosphatase and serum alanine aminotransferase (females only) and liver histopathology (leukocytic infiltration around the central veins and necrosis in males) seen at 350 ppm (equivalent to 8.8 mg/kg bw per day).

The overall NOAEL for the 1- and 2-year dog studies was 50 ppm (equivalent to 1.3 mg/kg bw per day), and the overall LOAEL was 6 mg/kg bw per day.

In a first toxicity and carcinogenicity study in hamsters (91 weeks for males and 78 weeks for females) using dietary dichlobenil concentrations of 0, 675, 1500 and 3375 ppm (equal to 0, 51, 117 and 277 mg/kg bw per day for males and 0, 55, 121 and 277 mg/kg bw per day for females, respectively), decreased body weight gain in males and females and increased relative liver weight in females were observed at all doses. No NOAEL could be identified. The NOAEL for carcinogenicity in male hamsters was 1500 ppm (equal to 117 mg/kg bw per day), based on an increased incidence of hepatocellular adenoma and carcinoma at 3375 ppm (equal to 277 mg/kg bw per day). No treatment-related tumours were observed in female hamsters.

In the second toxicity and carcinogenicity study in hamsters, dichlobenil was administered at a dietary concentration of 0, 5, 26, 132 or 675 ppm (equal to 0, 0.34, 1.69, 9.39 and 45.6 mg/kg bw per day for males and 0, 0.35, 1.78, 9.20 and 48.9 mg/kg bw per day for females, respectively) for 88 weeks (males) or 80 weeks (females). The NOAEL was 26 ppm (equal to 1.69 mg/kg bw per day), based on reduced secretion of the prostate and seminal vesicles at 132 ppm (equal to 9.39 mg/kg bw per day).

In a 2-year toxicity and carcinogenicity study in rats using dietary dichlobenil concentrations of 0, 50, 400 and 3200 ppm (equal to 0, 3.2, 29 and 241 mg/kg bw per day for males and 0, 3.2, 26 and 248 mg/kg bw per day for females, respectively), the NOAEL was 50 ppm (equal to 3.2 mg/kg bw per day), based on changes in clinical chemistry (increased blood urea nitrogen, cholesterol), gross pathology (enlarged liver and kidney) and histopathology (nephrosis, parathyroid hyperplasia) in males and enlarged liver with increased liver weight and polyploidy with hepatocytic swelling in the liver in females at 400 ppm (equal to 26 mg/kg bw per day). There was an increased incidence of hepatocellular tumours at 3200 ppm in both sexes, reaching statistical significance only in females. The NOAEL for carcinogenicity was 400 ppm (equal to 26 mg/kg bw per day).

The Meeting concluded that dichlobenil is carcinogenic in rats and hamsters.

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

The Meeting concluded that dichlobenil is unlikely to be genotoxic.

In view of the lack of genotoxicity and on the basis of other available toxicological information, the Meeting concluded that the mode of action for the increased incidences of hepatocellular adenomas and carcinomas in male and female rats and male hamsters, although not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that dichlobenil is unlikely to pose a carcinogenic risk to humans from the diet.

In a two-generation reproductive toxicity study in rats given diets containing dichlobenil at a concentration of 0, 60, 350 or 2000 ppm (equivalent to 0, 4, 23 and 130 mg/kg bw per day, respectively), the NOAEL for parental toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on decreased body weight gains during premating (males and females) and gestation (females) in both generations, decreased feed consumption during premating in both generations (males and females) and a decreased number of implantations per dam in F_1 females at 2000 ppm (equivalent to 130 mg/kg bw per day). The NOAEL for offspring toxicity was 60 ppm (equivalent to 4 mg/kg bw per day), based on decreased body weight during weaning in both generations seen at 350 ppm (equivalent to 23 mg/kg bw per day). The NOAEL for reproductive toxicity was 350 ppm (equivalent to 23 mg/kg bw per day), based on a decreased number of implantations per dam in F_1 females seen at 2000 ppm (equivalent to 130 mg/kg bw per day).

In a prenatal developmental toxicity study in rats that used dichlobenil doses of 0, 20, 60 and 180 mg/kg bw per day, the NOAEL for maternal toxicity was 20 mg/kg bw per day, based on decreased body weight gain, feed consumption and feed efficiency during dosing seen at 60 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 180 mg/kg bw per day, the highest dose tested.

In a prenatal developmental toxicity study in rabbits that tested at dichlobenil doses of 0, 15, 45 and 135 mg/kg bw per day, the NOAEL for maternal toxicity was 45 mg/kg bw per day, based on decreased body weight gain and feed consumption during dosing at 135 mg/kg bw per day. The NOAEL for embryo and fetal toxicity was 45 mg/kg bw per day, based on an increased number of total resorptions per dam, increased post-implantation loss and increased incidences of external, visceral and skeletal malformations (e.g. open eyes, major fusion of sternebrae, cleft palate) seen at 135 mg/kg bw per day.

The Meeting concluded that dichlobenil is teratogenic in rabbits, but not in rats.

No evidence of immunotoxicity was observed in an immunotoxicity study in rats administered dichlobenil by gavage at a dose level of 0, 20, 60 or 180 mg/kg bw per day for 28 days.

The Meeting concluded that dichlobenil is not immunotoxic.

Dichlobenil produces harmful effects on nasal mucosa via dermal, inhalation and oral routes of exposure. These effects on nasal mucosa are reversible, and there is a threshold below which the effect was not observed.

Toxicological data on metabolites and/or degradates

The acute oral LD_{50}s for 2,6-dichlorobenzamide (BAM; a plant metabolite and soil degradate) were 1538 and 1144 mg/kg bw for male and female mice, respectively.

In a 13-week toxicity study in rats using dietary BAM concentrations of 0, 50, 180, 600 and 2300 ppm (equal to 0, 4, 14, 49 and 172 mg/kg bw per day, respectively), the NOAEL was 180 ppm (equal to 14 mg/kg bw per day), based on reduced skeletal muscle tone (males and females) and decreased body weight gain (females) seen at 600 ppm (equal to 49 mg/kg bw per day).

In a 2-year study of toxicity in dogs using dietary BAM concentrations of 0, 60, 100, 180 and 500 ppm (equivalent to 0, 1.5, 2.5, 4.5 and 12.5 mg/kg bw per day, respectively), the NOAEL was 180 ppm (equivalent to 4.5 mg/kg bw per day), based on decreased body weight and body weight gain seen at 500 ppm (equivalent to 12.5 mg/kg bw per day).

In a 2-year study of carcinogenicity in rats using dietary BAM concentrations of 0, 60, 100, 180 and 500 ppm (equal to 0, 2.2, 3.6, 6.5 and 19 mg/kg bw per day for males and 0, 2.8, 4.7, 8.5 and 25 mg/kg bw per day for females, respectively), the NOAEL was 100 ppm (equal to 4.7 mg/kg bw per day), based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci) seen in females at 180 ppm (equal to 8.5 mg/kg bw per day). The NOAEL for carcinogenicity was 180 ppm (equal to 8.5 mg/kg bw per day), based on a marginally significant increase in the incidence of hepatocellular adenomas seen in females at 500 ppm (equal to 25 mg/kg bw per day).

The Meeting concluded that BAM is carcinogenic in rats.

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

The Meeting concluded that BAM is unlikely to be genotoxic.

In view of the lack of genotoxicity and on the basis of other available toxicological information, the Meeting concluded that the mode of action for the increased incidence of hepatocellular adenomas in female rats, although not completely understood, is likely to involve a threshold. Therefore, the Meeting concluded that BAM is unlikely to pose a carcinogenic risk to humans from the diet.

In a three-generation reproductive toxicity study in rats given diets providing a BAM dose of 0, 60, 100 or 180 ppm (equivalent to 0, 4.0, 6.7 and 12 mg/kg bw per day, respectively), the NOAEL for parental toxicity, offspring toxicity and reproductive toxicity was 180 ppm (equivalent to 12 mg/kg bw per day), the highest dose tested.

In a prenatal developmental toxicity study in rabbits that administered BAM by gavage at a dose of 0, 10, 30 or 90 mg/kg bw per day, the NOAEL for maternal toxicity was 30 mg/kg bw per day, based on late abortion, thin appearance and severely decreased body weight gain and feed consumption during dosing at 90 mg/kg bw per day. The NOAEL for embryo/fetal toxicity was 30 mg/kg bw per day, based on increased incidences of late abortion and skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) malformations observed at 90 mg/kg bw per day.

The Meeting concluded that BAM is teratogenic in rabbits.

BAM produces harmful effects on nasal mucosa via intraperitoneal and oral routes of exposure. These effects on nasal mucosa are reversible, and there is a threshold below which the effect was not observed.

Human data

No information on employees working in dichlobenil manufacturing plants was provided.

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

Toxicological evaluation

Dichlobenil

The Meeting established an ADI of 0–0.01 mg/kg bw on the basis of an overall NOAEL of 1.3 mg/kg bw per day in 1- and 2-year dietary studies of toxicity in dogs, on the basis of liver toxicity (increased

liver weight, liver enzymes, cholesterol and triglycerides) at 6 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by an overall NOAEL of 1.69 mg/kg bw per day in a dietary carcinogenicity study in hamsters, based on reduced secretion of the prostate and seminal vesicles, decreased body weight gain, and hyperplasia of the adrenal cortex, small intestine and bone marrow observed at 9.39 mg/kg bw per day. The upper bound of the ADI provides a margin of exposure of at least 24 000 relative to the LOAEL for liver tumours in hamsters and rats.

An ARfD of 0.5 mg/kg bw was established on the basis of a NOAEL of 45 mg/kg bw per day in a study of developmental toxicity in rabbits, based on increased incidences of external, visceral and skeletal malformations (e.g. open eyes, major fusion of sternebrae, cleft palate) seen at 135 mg/kg bw per day. A safety factor of 100 was applied. This ARfD applies to women of childbearing age only. The Meeting concluded that it is not necessary to establish an ARfD for the remainder of the population in view of the low acute oral toxicity of dichlobenil and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

2,6-Dichlorobenzamide (BAM)

Based on a re-evaluation of the data, the Meeting withdrew the ADI and ARfD for 2,6-dichlorobenzamide (BAM) established by the 2009 JMPR as part of the evaluation of fluopicolide.

The Meeting established an ADI of 0–0.05 mg/kg bw for 2,6-dichlorobenzamide (BAM) on the basis of a NOAEL of 4.5 mg/kg bw per day in a 2-year dietary study of toxicity in dogs, based on decreased body weight and body weight gain at 12.5 mg/kg bw per day. A safety factor of 100 was applied. This ADI is supported by a NOAEL of 4.7 mg/kg bw per day in a 2-year dietary carcinogenicity study in rats, based on decreased body weight and body weight gain and an increased incidence of hepatocellular alteration (eosinophilic foci) in females observed at 8.5 mg/kg bw per day. The upper bound of the ADI provides a margin of exposure of at least 500 relative to the LOAEL for liver tumours in rats and also a 240-fold margin of exposure relative to the highest dose tested in a three-generation reproductive toxicity study in rats, at which no effects were observed.

The Meeting established an ARfD of 0.3 mg/kg bw for 2,6-dichlorobenzamide (BAM) on the basis of a NOAEL of 30 mg/kg bw per day in a developmental toxicity study in rabbits, based on increased incidences of skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) malformations seen at 90 mg/kg bw per day. A safety factor of 100 was applied. This ARfD applies to women of childbearing age only. The Meeting concluded that it is not necessary to establish an ARfD for the remainder of the population in view of the low acute oral toxicity of BAM and the absence of any other toxicological effects that would be likely to be elicited by a single dose.

Levels relevant to risk assessment of dichlobenil

Species	Study	Effect	NOAEL	LOAEL
Hamster	Eighteen-month studies of toxicity and carcinogenicity[a,b]	Toxicity	26 ppm, equal to 1.69 mg/kg bw per day	132 ppm, equal to 9.39 mg/kg bw per day
		Carcinogenicity	1 500 ppm, equal to 117 mg/kg bw per day	3 375 ppm, equal to 277 mg/kg bw per day
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 3.2 mg/kg bw per day	400 ppm, equal to 26 mg/kg bw per day
		Carcinogenicity	400 ppm, equal to 26 mg/kg bw per day	3 200 ppm, equal to 241 mg/kg bw per day
	Two-generation study of reproductive toxicity[a]	Reproductive toxicity	350 ppm, equivalent to 23 mg/kg bw per day	2 000 ppm, equivalent to 130 mg/kg bw per day
		Parental toxicity	350 ppm, equivalent to 23 mg/kg bw per day	2 000 ppm, equivalent to 130 mg/kg bw per

Species	Study	Effect	NOAEL	LOAEL
				day
		Offspring toxicity	60 ppm, equivalent to 4 mg/kg bw per day	350 ppm, equivalent to 23 mg/kg bw per day
	Developmental toxicity study[c]	Maternal toxicity	20 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	180 mg/kg bw per day[d]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	45 mg/kg bw per day	135 mg/kg bw per day
		Embryo and fetal toxicity	45 mg/kg bw per day	135 mg/kg bw per day
Dog	One-[c] and 2-year studies of toxicity[a,b]	Toxicity	50 ppm, equivalent to 1.3 mg/kg bw per day	6 mg/kg bw per day

[a] Dietary administration.
[b] Two or more studies combined.
[c] Gavage administration, including capsules.
[d] Highest dose tested.

Levels relevant to risk assessment of BAM

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-year study of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 4.7 mg/kg bw per day	180 ppm, equal to 8.5 mg/kg bw per day
		Carcinogenicity	180 ppm, equal to 8.5 mg/kg bw per day	500 ppm, equal to 25 mg/kg bw per day
	Three-generation study of reproductive toxicity[a]	Reproductive toxicity	180 ppm, equivalent to 12 mg/kg bw per day[b]	–
		Parental toxicity	180 ppm, equivalent to 12 mg/kg bw per day[b]	–
		Offspring toxicity	180 ppm, equivalent to 12 mg/kg bw per day[b]	–
Rabbit	Developmental toxicity study[c]	Maternal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
		Embryo and fetal toxicity	30 mg/kg bw per day	90 mg/kg bw per day
Dog	Two-year study of toxicity[a]	Toxicity	180 ppm, equivalent to 4.5 mg/kg bw per day	500 ppm, equivalent to 12.5 mg/kg bw per day

[a] Dietary administration.
[b] Highest dose tested.
[c] Gavage administration.

Estimate of acceptable daily intake (ADI)

0–0.01 mg/kg bw

0–0.05 mg/kg bw for 2,6-dichlorobenzamide (BAM)

Estimate of acute reference dose (ARfD)

0.5 mg/kg bw (applies to women of childbearing age only)

0.3 mg/kg bw for 2,6-dichlorobenzamide (BAM) (applies to women of childbearing age only)

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 dichlobenil

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly and completely absorbed from gastrointestinal tract (at least 89% in 24 hours)
Dermal absorption	No data
Distribution	Rapidly distributed, highest concentrations in liver, kidney, kidney fat and brown fat; concentrations peaked in 1 hour
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid; about 80% excreted within first 24 hours following single low dose
Metabolism in animals	Extensive, 10 metabolites in urine (no parent) and four metabolites and parent compound in faeces
Toxicologically significant compounds in animals and plants	Dichlobenil and 2,6-dichlorobenzamide (BAM; plant metabolite)
Acute toxicity	
Rat, LD_{50}, oral	> 2 000 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 3.2 mg/L (4 hours)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Guinea-pig, dermal sensitization	Not sensitizing (maximization test)
Short-term studies of toxicity	
Target/critical effect	Liver
Lowest relevant oral NOAEL	1.3 mg/kg bw per day (dog)
Lowest relevant dermal NOAEL	≥ 1 000 mg/kg bw per day (rabbit)
Lowest relevant inhalation NOAEC	≥ 12 mg/m^3
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver and kidney (rat) and liver (hamster)
Lowest relevant oral NOAEL	1.69 mg/kg bw per day (hamster)
Carcinogenicity	Liver tumours (male hamster, male and female rats); unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	

Target/critical effect	Decreased body weights in adults and pups, decreased number of implantations per dam in F_1 females
Lowest relevant parental NOAEL	23 mg/kg bw per day
Lowest relevant offspring NOAEL	4 mg/kg bw per day
Lowest relevant reproductive NOAEL	23 mg/kg bw per day
Developmental toxicity	
Target/critical effect	Increased total resorptions, post-implantation loss and malformations (rabbit)
Lowest relevant maternal NOAEL	20 mg/kg bw per day (rat)
Lowest relevant embryo/fetal NOAEL	45 mg/kg bw per day (rabbit)
Neurotoxicity	
Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity NOAEL	180 mg/kg bw per day, highest dose tested
Medical data	
	No data provided

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

Acute toxicity	
Mouse, LD_{50}, oral	1140 mg/kg bw
Short-term studies of toxicity	
Target/critical effect	Body weight and body weight gain
Lowest relevant oral NOAEL	4.5 mg/kg bw per day (dog)
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver and body weight
Lowest relevant oral NOAEL	4.7 mg/kg bw per day (rat)
Carcinogenicity	Hepatocellular adenomas in female rats; unlikely to pose a carcinogenic risk to humans from the diet
Genotoxicity	
	Unlikely to be genotoxic
Reproductive toxicity	
Target/critical effect	None
Lowest relevant parental NOAEL	12 mg/kg bw per day, highest dose tested
Lowest relevant offspring NOAEL	12 mg/kg bw per day, highest dose tested
Lowest relevant reproductive NOAEL	12 mg/kg bw per day, highest dose tested
Developmental toxicity	
Target/critical effect	Increased incidences of skeletal (bipartite interparietal bone) and visceral (postcaval lung lobe agenesis) anomalies
Lowest relevant maternal NOAEL	30 mg/kg bw per day (rabbit)
Lowest relevant embryo/fetal NOAEL	30 mg/kg bw per day (rabbit)
Neurotoxicity	

Acute neurotoxicity NOAEL	No data
Subchronic neurotoxicity NOAEL	No data
Developmental neurotoxicity NOAEL	No data

Summary

	Value	Study	Safety factor
Dichlobenil			
ADI	0–0.01 mg/kg bw	One-year and 2-year studies of toxicity (dog)	100
ARfD[a]	0.5 mg/kg bw	Developmental toxicity study (rabbit)	100
2,6-Dichlorobenzamide (BAM)			
ADI	0–0.05 mg/kg bw	Two-year study of toxicity (dog)	100
ARfD[a]	0.3 mg/kg bw	Developmental toxicity study (rabbit)	100

[a] Applies to women of childbearing age only.

RESIDUE AND ANALYTICAL ASPECTS

See also FLUOPICOLIDE

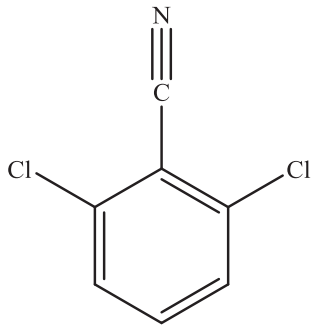
Dichlobenil is a benzonitrile herbicide for use by soil application in fruit crops, including stone fruit, grapes, and other berry crops. Dichlobenil was scheduled at the Forty-fifth Session of the CCPR in 2013 for evaluation as a new compound by the 2014 JMPR. Data was provided on physicochemical properties, metabolism in food producing animals and plants, environmental fates, methods of analysis, stability of residues in stored analytical samples, GAP information, supervised residue trials, processing studies, and animal feeding studies.

The Meeting received information on processing of oranges, strawberries, onions, lettuce head and peas.

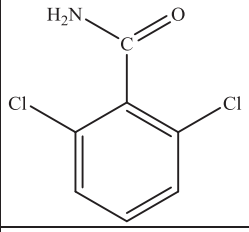
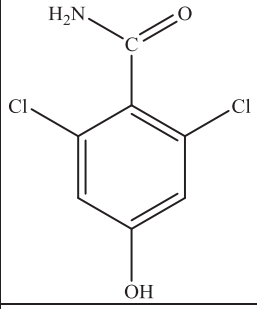
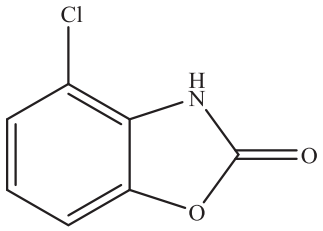
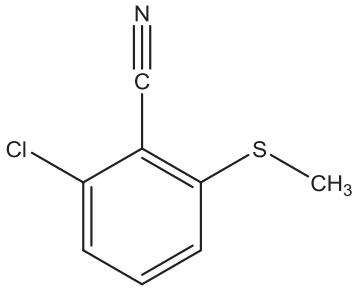
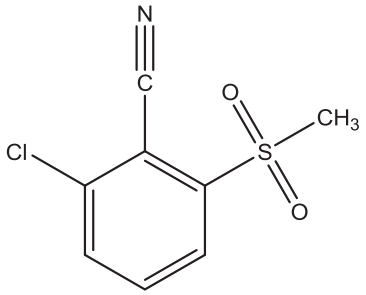
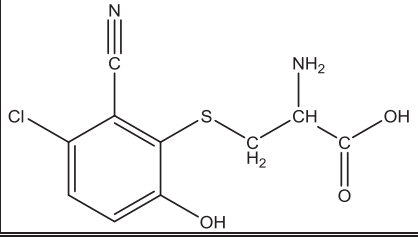
Processing factors calculated for the processed commodities for the above raw agricultural commodities, including previously estimated, are shown in the table below. STMP-Ps was calculated for processed commodities of strawberry, onion and peas for which maximum residue levels were estimated.

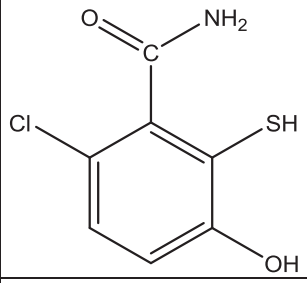
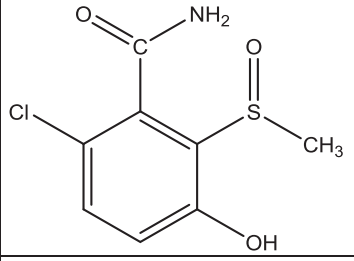
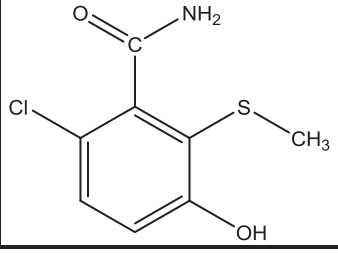
Chemical name and structure

The IUPAC name for dichlobenil is 2,6-dichlorobenzonitrile.



The following metabolites are discussed below:

2,6-Dichlorobenzamide (BAM)	
2,6-Dichloro-4-hydroxybenzamide	
4-Chloro-2(3*H*)benzoxazolone	
6-Chloro-2-methylthiobenzonitrile	
6-Chloro-2-methylsulfonylbenzonitrile	
S-(3-Chloro-2-cyano-6-hydroxyphenyl)-cysteine	

6-Chloro-3-hydroxy-2-thiobenzamide	
6-Chloro-3-hydroxy-2-methylsulfinylbenzamide	
6-Chloro-3-hydroxy-2-methylthiobenzamide	

Animal metabolism

The Meeting received information on the metabolism of ^{14}C-phenyl labelled dichlobenil in rats, lactating goats, and laying hens, and for the metabolism of ^{14}C-phenyl labelled 2,6-dichlorobenzamide (a significant plant metabolite) in lactating goats and laying hens.

Animal metabolism: dichlobenil

In rats, dichlobenil is metabolized via two metabolic pathways: hydroxylation at the 3 or 4 position followed by glucuronidation or sulphation and the second pathway includes substitution of one chlorine atom by glutathione, followed by cleavage and oxidation of the glutathione moiety to cysteine and ultimately thiol and sulfonyl derivatives.

Lactating goats were dosed orally, twice daily, with ^{14}C-phenyl dichlobenil at 10 mg/kg bw/day for three days. Milk was collected twice daily, with blood samples being collected just prior to and at various intervals after the first and fifth doses. The goats were sacrificed 15 hours after the final dose.

The majority of the administered dose was excreted (39–45% in urine, 24–31% in faeces, 2.9–4.0% in cage wash, and 0.06 to 0.33% in milk). Carcass tissue accounted for 3.2 to 3.4 % of the administered radioactivity, while 1.1–3.3% remained in GI tract tissue, and 0.4–3.3% GI tract contents.

Levels in milk peaked after about 2 days of dosing, at 1.30 mg eq./kg. At sacrifice, total residues of 27 mg eq./kg were found in liver, 4.1 mg eq./kg in kidney, 2.4 mg eq./kg in fat, and 0.42 mg eq./kg in muscle. The proportions of residue extracted were variable, at 65–70% of TRR in milk, 65% in liver, 26% in fat, 13% in muscle, and 28% in kidney.

In muscle, a metabolite tentatively identified as 2-chloro-6-methylthiobenzonitrile predominated, at 11% TRR, with smaller amounts of S-(3-chloro-2-cyano-6-hydroxyphenyl)-cysteine (0.9% TRR) and an unidentified compound (1.3% TRR).

In fat, parent comprised 60% of the identified residue (16% of TRR), with the other 40% 2-chloro-6-methylsulfonylbenzonitrile (10% TRR).

In milk, S-(3-chloro-2-cyano-6-hydroxyphenyl)-cysteine comprised 11–21% TRR, 2-chloro-6-methylsulfonylbenzonitrile 14-38% of TRR, with 3.9–12% of an unidentified component; parent was only present at ≤ 1%.

In liver, the majority of residue was present as S-(3-chloro-2-cyano-6-hydroxyphenyl)-cysteine (95% of that identified, or 62% of TRR), with a small amount of 2-chloro-6-methylsulfonylbenzonitrile (3% of TRR).

In kidney, the largest component was 2-chloro-6-methylthiobenzonitrile (23% of TRR), with smaller amounts (1.1–2.5% of TRR) of S-(3-chloro-2-cyano-6-hydroxyphenyl)-cysteine, 2-chloro-6-methylsulfonylbenzonitrile, and an unidentified component.

Laying hens were dosed orally with ^{14}C-phenyl dichlobenil at 9.8–10.2 mg/kg bw/day for three days. The dose was administered twice daily, eggs were collected twice daily, with blood samples being collected just prior to and at various intervals after the first and fifth doses. The hens were sacrificed 15 hours after the final dose.

The majority of the administered dose was excreted, with a mean of 67% recovered in excreta, 4.2% in cage washings, and 6.1% in the GI tract at sacrifice. Eggs accounted for 0.44% of the dose, with levels not reaching a plateau during the study.

The proportions of residue extracted were variable, at 60% of TRR in eggs, 90% in liver, 15% in fat, 8% in muscle, and 39% in kidney.

In fat and eggs, 100% of the extracted residue was unchanged parent compound (15% and 60% of the TRR in fat and eggs, respectively). In muscle, the largest component was the tentatively identified 2-chloro-6-methylthiobenzonitrile (6.4% of TRR), with parent compound comprising 1.6% of TRR. Similar ratios were observed for liver (2-chloro-6-methylthiobenzonitrile; 85% of TRR and parent; 5.4% of TRR). Kidney contained a number of metabolites, the cysteine conjugate of hydroxylated dichlobenil (17% of TRR), parent (9.8% of TRR), 2-chloro-6-methylsulfonylbenzonitrile (5.1% of TRR), and 2-chloro-6-methylthiobenzonitrile (4.3% of TRR), with small amounts (1.2-1.6% of TRR) of two unidentified components.

Metabolism of dichlobenil in goats and hens was similar, although the degree of metabolism appeared to be higher in goats. The chief metabolic pathway was conjugation with cysteine, replacing one of the chlorine atoms, with or without hydroxylation at an adjacent position on the ring. This was followed by cleavage and oxidation of the cysteine moiety to yield methylthio and methylsulfonyl derivatives.

There are strong similarities to the metabolism of dichlobenil in rats, with hydroxylation and glutathione conjugation followed by cleavage of the glutathione moiety taking place in rats, goats and hens.

The only residue of significance in fruit is 2,6-dichlorobenzamide, with no parent being present. Only uses in fruit crops are under consideration at this stage, therefore the Meeting considers the metabolism of dichlobenil in animals to be of secondary importance to the animal metabolism of 2,6-dichlorobenzamide.

Animal metabolism: 2,6-dichlorobenzamide

A lactating goat was dosed orally with ^{14}C-phenyl 2,6-dichlorobenzamide at 13 ppm in feed daily for five days. Milk was sampled twice daily, and the animals were sacrificed 23 hours after the final dose.

The majority of the dose was excreted. In the low dose animal, 62% of the TAR was recovered in urine, 17% in faeces, 3.4% in cage washings, and 0.26% in milk. TRRs in milk reached a plateau of 0.048 mg eq./kg on day 3 of dosing. Total residues in muscle and fat at sacrifice were 0.25 and 0.024 mg eq./kg respectively, with higher residues in the metabolic organs, 9.4 and 1.2 mg eq./kg in liver and kidney respectively.

High proportions of the residue were extracted from the matrices using column extraction methods: > 90% in all cases.

Except for liver, unchanged 2,6-dichlorobenzamide was the most significant residue component, at 0.75 mg eq./kg in kidney (63% of TRR), 0.19 mg eq./kg in muscle (77% of TRR), 0.009 mg eq./kg in fat (40% of TRR), and 0.038 mg eq./kg (80% of TRR) in 72 hour milk.

In kidney, the cysteine conjugate derivatives 6-chloro-3-hydroxy-2-thiobenzamide and 6-chloro-3-hydroxy-2-methylthiobenzamide were also found at 0.25 mg eq./kg (21% of TRR) and 0.23 mg eq./kg (19% of TRR) respectively.

In liver, the most significant residue component was 6-chloro-3-hydroxy-2-thiobenzamide at 6.6 mg eq./kg (70% of TRR), followed by 6-chloro-3-hydroxy-3-methylthiobenzamide at 1.6 mg eq./kg (17% of TRR). Much smaller amounts of 2,6-dichlorobenzamide and 6-chloro-3-hydroxy-2-methylsulfinylbenzamide (0.09–0.16 mg eq./kg, or 0.98–1.7% of TRR) were also found in liver.

Small amounts of a component postulated as dichlobenil only on the basis of retention time comparison with a standard were found in muscle and fat, at 0.01 mg eq./kg (4.4% of TRR) and 0.005 mg eq./kg (24% of TRR) respectively.

The only component found in milk apart from 2,6-dichlorobenzamide was 6-chloro-3-hydroxy-2-thiobenzamide, at 0.006 mg eq./kg (13% of TRR).

Laying hens were dosed orally with ^{14}C-phenyl 2,6-dichlorobenzamide at a mean level of 12 ppm in feed daily for five days. Eggs were sampled twice daily, and the birds were sacrificed 23 hours after the final dose.

Around half the administered dose for the low dose group was excreted, with 49% recovered from excreta and 1.5% from cage washings. In the low dose hens 3.7% of the dose was recovered in eggs, with levels not having peaked over the five day dosing period, reaching 2.4 mg eq./kg in the final day eggs. Total residues of 8.6 mg eq./kg were found in liver and 5.0 mg eq./kg in kidney. Residues were lower in fat, skin and muscle, at 1.3, 1.9 and 1.9 mg eq./kg.

Extraction was essentially quantitative, with > 95% of the TRR extracted from all matrices.

With the exception of muscle and egg, where a single additional unidentified metabolite was found at < 6% of the TRR, the extracted residue in hen matrices comprised only unchanged 2,6-dichlorobenzamide.

While 2,6-dichlorobenzamide is largely unmetabolized by hens, the metabolism of 2,6-dichlorobenzamide in goats is somewhat similar to that of dichlobenil, with the main pathway being cysteine conjugation replacing one of the chlorine atoms, followed by cleavage and oxidation of the cysteine side chain to yield metabolites such as 6-chloro-3-hydroxy-2-thiobenzamide, 6-chloro-3-hydroxy-2-methylthiobenzamide, and 6-chloro-3-hydroxy-2-methylsulfinylbenzamide.

Plant metabolism

The Meeting received information on the metabolism of ^{14}C-phenyl labelled dichlobenil in apples and grapes.

Single outdoor grown apple trees were treated in mid-February at late dormancy with a soil application of a liquid formulation of ^{14}C-phenyl dichlobenil at 6.7 kg ai/ha, the GAP for most fruit crops. Total radioactive residues were determined in soil samples collected at various intervals, as well as in immature apples collected at 77 and 107 days after soil application, and mature fruit collected at harvest (137 days after application).

Total residues in apples were low, at 0.012, 0.028, and 0.042 mg eq/kg at 77, 107, and 137 (mature harvest) days after application, respectively. The majority of the residue in mature apples, 81% of the TRR, or 0.034 mg eq./kg, could be extracted with methanol. The only residue identified in apples was 2,6-dichlorobenzamide, at 0.024 mg eq./kg, or 57% of the TRR. Unidentified components were all < 0.01 mg eq/kg.

Small plots of outdoor grown grapevines were treated in mid-February at late dormancy with a soil application of a liquid formulation of ^{14}C-phenyl dichlobenil at 6.7 kg ai/ha. Total radioactive residues were determined in soil samples collected at various intervals, as well as in immature fruit collected at 159 and 190 days after soil application, and mature grapes collected at harvest (222 days after application).

The total radioactive residues in grapes were similar at the three sampling intervals, 0.32, 0.29, and 0.39 mg eq./kg at 159, 190 and 222 days respectively. Nearly all the residue in mature grapes collected at harvest could be extracted with methanol (99% of TRR, or 0.39 mg eq./kg). The most significant residue component in grapes was by far 2,6-dichlorobenzamide, at 0.34 mg eq./kg, or 87% of the TRR. A second metabolite, 2,6-dichloro-4-hydroxybenzamide was found at < 0.01 mg eq./kg, or 2% of the TRR, while unidentified components totalled 0.06 mg eq./kg, or 15% of the TRR.

The only significant metabolic pathway for dichlobenil in fruits is hydrolysis to give 2,6-dichlorobenzamide.

Environmental fate

As all residue trials considered by the Meeting involve soil application, in accordance with the FAO Manual, data for aerobic soil metabolism, soil surface photolysis, and hydrolysis were evaluated.

Dichlobenil

In an aerobic metabolism study conducted over 50 weeks at 24 ºC in sandy loam soil, dichlobenil was principally lost through volatilization of the unchanged parent compound (57% of TAR after 50 weeks), and also by metabolism to 2,6-dichlorobenzamide (13% TAR), formation of bound residue (10% TAR), and mineralization to $^{14}CO_2$ (< 3%). The DT_{50} for dichlobenil was 13 weeks.

The Meeting noted that dichlobenil has low solubility in water, 14.6 mg/L at 20 °C. Hydrolysis of dichlobenil was insignificant, with no significant degradation in unsterilized aqueous buffer solutions over 150 days.

2,6-Dichlorobenzamide

The DT_{50} values for 2,6-dichlorobenzamide were up to 261 days (37 weeks) in soil. The Meeting noted that 2,6-dichlorobenzamide has a significantly higher water solubility than dichlobenil (2.7 g/L), and is very stable to hydrolysis.

The Meeting concluded that dichlobenil is principally lost from soil through volatilization of the parent compound, with secondary degradation pathways including metabolism to 2,6-

dichlorobenzamide, binding to soil, and mineralization to CO_2 Dichlobenil is relatively persistent, with a DT_{50} in soil of 13 weeks. Hydrolysis is not a significant pathway for degradation.

Residues in succeeding crops

No information on residues of dichlobenil in following crops was received by the Meeting, however such data is not needed given that only uses in permanent crops are under consideration.

Based on the available soil degradation data for 2,6-dichlorobenzamide in soil, the Meeting considers that there is potential for accumulation of soil residues of 2,6-dichlorobenzamide from application in multiple years. Modelling of multiple year soil accumulation using the DT_{50} value of 261 days for 2,6-dichlorobenzamide as a worst case indicates that residues of 2,6-dichlorobenzamide will reach a steady state level of 1.6× the level resulting from a single application.

Methods of analysis

The Meeting received details of analytical methods for dichlobenil and 2,6-dichlorobenzamide residues in plant and animal matrices.

Analyses of dichlobenil in plant commodities involved extraction with ethyl acetate/hexane in the presence of anhydrous sodium sulphate, with clean-up by solid phase extraction using deactivated neutral alumina as the solid phase. Residues were determined by GC-ECD or GC-MS/MS. The method LOQ is 0.01 or 0.02 mg/kg.

Analyses of 2,6-dichlorobenzamide in plant commodities involved extraction with ethyl acetate in the presence of anhydrous sodium sulphate and filter pulp or diatomaceous earth. The extracts were cleaned up by solid phase extraction (neutral alumina), followed by analysis using GC-ECD or GC-MS/MS. The method LOQ is 0.003–0.03 mg/kg.

Mean recoveries in plant matrices were generally within the acceptable range of 70–120%, with the exception of 2,6-dichlorobenzamide fortified in grape juice at 0.003 mg/kg. However, acceptable recoveries were achieved at fortifications of 0.03 and 0.30 mg/kg.

Residues of dichlobenil and 2,6-dichlorobenzamide in animal commodities were determined using a GC-MS method. Fat samples were homogenized with hexane then partitioned into acetonitrile. Kidney, muscle and liver samples were homogenized with water and extracted with ethyl acetate. Residues were transferred into hexane and partitioned into acetonitrile. Milk samples were extracted with ethyl acetate, and the residues transferred into hexane, which was cleaned up by solid phase extraction (Florisil). An LOQ of 0.01 mg/kg was achieved for both analytes in all matrices.

Mean recoveries in animal matrices were within the acceptable range of 70–120%.

Suitable methods are therefore available for determination of both dichlobenil and 2,6-dichlorobenzamide residues in plant and animal commodities with an LOQ of 0.01 mg/kg for each analyte.

Multiresidue methods were not provided to the Meeting. However, the Meeting noted that the USA FDA multiresidue method PAM I has been successfully validated for dichlobenil and 2,6-dichlorobenzamide. The Meeting also noted successful validations of the QuEChERS method for determination of both dichlobenil and 2,6-dichlorobenzamide residues.

Stability of residues in stored analytical samples

Storage stability data for residues of dichlobenil and 2,6-dichlorobenzamide in plant and animal commodities were generated as part of the residue trial and animal feeding studies.

The data showed that residues of dichlobenil and 2,6-dichlorobenzamide were stable in plant samples for at least the period for which the residue trial and animal feeding study samples were stored between collection and analysis (up to 11 months in the case of grapes and grape juice).

Storage stability of 2,6-dichlorobenzamide residues in animal matrices was acceptable over the storage period in the feeding study (up to 71 days), while dichlobenil residues were stable in muscle, fat and milk. Poor stability was observed in liver and kidney, however based on the available metabolism and feeding data, detectable residues of dichlobenil parent compound are not expected in any animal commodities.

Definition of the residue

Plant metabolism studies were available for dichlobenil in fruit crops. In metabolism studies in apples and grapes where radiolabelled compound was applied to the soil, dichlobenil was metabolised almost exclusively to 2,6-dichlorobenzamide, which comprised 57% of the TRR in mature apples, and 86% in mature grapes. No dichlobenil parent compound was found in either apples or grapes, and only one other component, 2,6-dichloro-4-hydroxybenzamide, was found, albeit at a very low level, < 0.01 mg eq./kg, or 2.1% of the TRR, in grapes only. The JMPR considered that this was not of greater toxicological concern than 2,6-dichlorobenzamide and was covered by the toxicological evaluation of 2,6-dichlorobenzamide and dichlobenil.

It is further noted that in residue trials in stone fruit, grapes, and bush berry crops, where both dichlobenil and 2,6-dichlorobenzamide were both analysed, only 2,6-dichlorobenzamide was found at quantifiable levels.

Given that dichlobenil is almost exclusively metabolised to 2,6-dichlorobenzamide in the edible portions of fruit crops, the Meeting proposes that only 2,6-dichlorobenzamide be included in the residue definition for dichlobenil in plant commodities for both compliance with MRLs and dietary risk assessment.

In considering a residue definition for animal commodities, the Meeting noted that livestock are only likely to be exposed to 2,6-dichlorobenzamide residues through feeding, as parent compound is unlikely to be found in feeds, while 2,6-dichlorobenzamide may be present in finite amounts. Therefore, only the livestock metabolism studies for 2,6-dichlorobenzamide are of significance.

In hens, 2,6-dichlorobenzamide was largely unchanged, being the only residue detected in fat, liver and kidney, and the major residue in muscle and eggs. One unidentified component was detected in muscle and eggs but at low levels (< 6% of the TRR). In goats, unchanged 2,6-dichlorobenzamide was the largest residue component in kidney, muscle, fat, and milk. In liver, 6-chloro-3-hydroxy-2-thiobenzamide was the largest component, with 2,6-dichlorobenzamide only being present at low levels. The JMPR considered that this metabolite and others found in lactating goats were not of greater toxicological concern than 2,6-dichlorobenzamide and were covered by the toxicological evaluation of 2,6-dichlorobenzamide and dichlobenil. Given that 2,6-dichlorobenzamide is not significantly metabolised in poultry, and is the most significant residue in the milk and all tissues except liver of goats dosed with the compound, the Meeting proposes that only 2,6-dichlorobenzamide will be included in the residue definition for dichlobenil in animal commodities for both compliance with MRLs and for dietary risk assessment.

The $\log_{10}P_{ow}$ of 2,6-dichlorobenzamide (0.77) indicates low fat solubility. In the goat and hen metabolism studies, residues of 2,6-dichlorobenzamide were higher in muscle than in fat. Similar observations were made in the cattle feeding study. The Meeting concluded that the residues are not fat soluble.

It is noted that 2,6-dichlorobenzamide is not uniquely a metabolite of dichlobenil, as it is also a metabolite of the fungicide fluopicolide, evaluated by JMPR in 2009. A residue definition of *fluopicolide* was established for compliance with the MRLs for plant and animal commodities, and *fluopicolide* and *2,6-dichlorobenzamide*, measured separately, for estimation of dietary intake for plant and animal commodities. Fluopicolide parent compound is the major residue resulting from use of fluopicolide. When 2,6-dichlorobenzamide is present in the absence of fluopicolide, it is most likely to have resulted from use of dichlobenil.

The Meeting considered residues of 2,6-dichlorobenzamide resulting from use of both dichlobenil and fluopicolide for establishment of MRLs and for dietary risk assessment.

The Meeting proposed the following definition of the residue (for compliance with the MRL and for dietary risk assessment, for plant and animal commodities): *2,6-dichlorobenzamide*.

The residue is not fat soluble.

Residues of supervised residue trials on crops

The Meeting received supervised trial data for soil application of dichlobenil to stone fruit (peaches, cherries and plums) and berry fruit (grapes, raspberries, blackberries, cranberries and blueberries).

The Meeting noted that residues of 2,6-dichlorobenzamide may arise from use of fluopicolide as well as from use of dichlobenil. Therefore, the Meeting has estimated maximum residue levels and STMR and HR values for 2,6-dichlorobenzamide in crops treated with fluopicolide using the data evaluated by the 2009 Meeting.

Stone fruits

Cherries

The critical GAP for dichlobenil in cherries is in the USA, where soil application at 6.7 kg ai/ha can be made in late autumn (15 November to 15 February) or early spring (up to 1 May). A harvest withholding period is not specified.

In trials on cherries conducted in the USA, 1 × 7.4–7.6 kg ai/ha soil application was made to cherry trees bearing immature fruit, with mature fruit being harvested 28 days later. Residues of 2,6-dichlorobenzamide were < 0.003 (12), and 0.004 (2) mg/kg.

The Meeting noted that most of the trials were conducted with application significantly later than specified on the label (in June or July rather than prior to 1 May), after fruit set. Insufficient trials matching GAP are available. Therefore, the Meeting did not estimate a maximum residue level for cherries.

Peaches

The GAP for dichlobenil in peaches in Canada is soil application at 7 kg ai/ha in late autumn or early spring, with no more than 7 kg ai/ha per season. A harvest withholding period is not specified.

In trials on peaches conducted in the USA, 1 × 6.8–7.7 kg ai/ha soil application was made, with fruit being harvested 20-68 days later. Residues of 2,6-dichlorobenzamide in peaches were < 0.01, 0.01, 0.02, and 0.04 mg/kg.

The Meeting determined that there were insufficient data for establishment of a maximum residue level in peaches.

Plums

The GAP for dichlobenil in plums in Canada is soil application at 7 kg ai/ha in late autumn or early spring, with no more than 7 kg ai/ha per season. A harvest withholding period is not specified.

In trials on plums conducted in the USA, 1 × 6.7–7.9 kg ai/ha soil application was made, with fruit being harvested 139-154 days later. Residues of 2,6-dichlorobenzamide in plums were < 0.01 and 0.45 mg/kg.

The Meeting determined that there were insufficient data for establishment of a maximum residue level in plums.

Berries and other small fruits

Blueberries

The GAP in the USA for blueberries involves soil application at 6.7 kg ai/ha, with application in late autumn (15 November to 15 February) or early spring (up to 1 May). No withholding period is specified.

In trials conducted on blueberries, a single soil application of dichlobenil was made in spring at 4.3 kg ai/ha. Residues of 2,6-dichlorobenzamide in blueberries at normal harvest (at a PHI of 51–58 days) were < 0.01 (3), and 0.015 mg/kg.

The Meeting determined that there were insufficient data for establishment of a maximum residue level in blueberries. Further, the Meeting noted that the trials were not conducted at the label rate, and the results were not amenable to the use of proportional scaling given that application was made at below GAP and the majority of the results were <LOQ.

Caneberries

The critical GAP for blackberries and raspberries in the USA is a soil application at 4.5 kg ai/ha made in late autumn (15 November to 15 February) or early spring (up to May 1). No withholding period is specified.

In trials conducted on blackberries and raspberries in the USA, where a single soil application was made at 4.3–4.9 kg ai/ha during spring (between mid-April and mid-May) or early summer (one trial with a mid-June application), residues of 2,6-dichlorobenzamide in mature blackberries and raspberries at normal harvest (44-89 DAT) were < 0.01 (2), 0.01, 0.02, 0.021, 0.031, 0.035, 0.056, and 0.067 mg/kg.

The Meeting noted that although a number of trials were conducted with application after 1 May, there did not appear to be a correlation between the application timing/interval and the residue level, and considered that all the trials were sufficiently robust to provide a realistic estimate of the residues.

The Meeting noted the potential for accumulation of residues of 2,6-dichlorobenzamide from application in successive years and agreed to scale the above values to account for multiple year applications by the factor of 1.6× determined from modelling of the multiple year soil accumulation. The resultant scaled data set was: < 0.016 (2), 0.016, 0.032, 0.034, 0.050, 0.056, 0.090, and 0.11 mg/kg.

Based on the above scaled data set, the Meeting estimated a maximum residue level of 0.2 mg/kg for the subgroup caneberries, along with an STMR of 0.034 mg/kg, and an HR of 0.13 mg/kg (the scaled highest residue from an individual sample).

Cranberries

The GAP for cranberries in the USA is soil application at 4.5 kg ai/ha made in late autumn or early spring (no more than 4.5 kg ai/ha in a 12-month period).

In trials conducted on cranberries in the USA, where a single soil application was made during spring at 4.8-5.7 kg ai/ha, residues of 2,6-dichlorobenzamide in cranberries at normal harvest were 0.02 (2) mg/kg.

The Meeting determined that there were insufficient data for establishment of a maximum residue level in cranberries.

Grapes

GAP for grapes in the USA involves soil application at 6.7 kg ai/ha made in early spring, with a harvest withholding period not specified.

In trials conducted in the USA on grapes, where a single soil application was made between the end of dormancy, and the beginning of fruit set, at a rate of 6.7-8.3 kg ai/ha, residues of 2,6-dichlorobenzamide in grapes at normal harvest were 0.004, 0.029, 0.033, 0.042 (2), and 0.058 mg/kg.

The Meeting determined that six trials was insufficient for estimation of a maximum residue level for grapes.

Residues of 2,6-dichlorobenzamide in grapes resulting from use of fluopicolide (2009 evaluation) were: < 0.01 (28), 0.01 (2), 0.013, 0.014, 0.015, 0.02 (2), 0.026, 0.03, 0.037, and 0.04 mg/kg.

Based on the 2,6-dichlorobenzamide residues arising from use of fluopicolide, the Meeting estimated a maximum residue level of 0.05 mg/kg for grapes, together with an STMR of 0.01 mg/kg and an HR of 0.04 mg/kg.

Crops with a fluopicolide use but no dichlobenil use

Bulb vegetables

Residues of 2,6-dichlorobenzamide in bulb onions arising from use of fluopicolide were < 0.01 mg/kg (7). The Meeting estimated a maximum residue level of 0.01* mg/kg for 2,6-dichlorobenzamide in bulb onions, together with STMR and HR values of 0.01 mg/kg.

Residues of 2,6-dichlorobenzamide in Welsh onions arising from use of fluopicolide were < 0.01 (2), and 0.01 mg/kg. The Meeting estimated a maximum residue level of 0.02 mg/kg for 2,6-dichlorobenzamide in Welsh onions, together with STMR and HR value of 0.01 mg/kg.

Brassica vegetables

In the data set for head cabbage (with wrapper leaves) used by the 2009 Meeting for fluopicolide MRL estimation and dietary risk assessment, residues of 2,6-dichlorobenzamide were < 0.01 (6), and 0.02 mg/kg.

Residues of 2,6-dichlorobenzamide in Brussels sprouts arising from use of fluopicolide were < 0.01 (8) mg/kg.

In the USA data set for broccoli used by the 2009 Meeting for MRL estimation and dietary risk assessment for Flowerhead brassicas were < 0.01 (6) mg/kg.

The Meeting noted that 2,6-dichlorobenzamide residues of < 0.01 (6), 0.02, and 0.04 mg/kg were found in head cabbage grown as a rotational crop.

The Meeting agreed to combine the head cabbage dataset for 2,6-dichlorobenzamide residues resulting from in-crop use of fluopicolide with the dataset for residues of 2,6-dichlorobenzamide in head cabbage resulting from use of fluopicolide in a preceding crop:

< 0.01 (12), 0.02 (2), and 0.04 mg/kg

Recognizing that residues of 2,6-dichlorobenzamide could occur in brassica vegetables other than cabbage grown in rotation with a crop treated with fluopicolide, the Meeting decided to estimate a group maximum residue level of 0.05 mg/kg, together with an STMR of 0.01 mg/kg and an HR of 0.04 mg/kg, based on the combined head cabbage dataset.

Fruiting vegetables, Cucurbits

In the dataset for melons used by the 2009 Meeting for estimation of a group MRL for fruiting vegetables, Cucurbits, residues of 2,6-dichlorobenzamide were not detected: < 0.01 (9) mg/kg. It is noted that residues of 2,6-dichlorobenzamide were not detected in other cucurbit crops. The Meeting estimated a group maximum residue level of 0.01* mg/kg for fruiting vegetables, Cucurbits, together an STMR and an HR of 0.01 mg/kg.

Fruiting vegetables, other than Cucurbits

In the data set in peppers, sweet and peppers, Chilli used by the 2009 Meeting to estimate a group maximum residue level for fruiting vegetables, other than Cucurbits, residues of 2,6-dichlorobenzamide were: < 0.01 (10) mg/kg. It is noted that residues of 2,6-dichlorobenzamide were not detected in other non-cucurbit fruiting vegetable crops. The Meeting estimated a group maximum residue level of 0.01* mg/kg for 2,6-dichlorobenzamide in fruiting vegetables, other than Cucurbits, together with an STMR and an HR of 0.01 mg/kg.

As residues of 2,6-dichlorobenzamide were not detected in chilli peppers treated with fluopicolide in the trials reported by the 2009 JMPR, the Meeting estimated an MRL of 0.01* mg/kg for 2,6-dichlorobenzamide in peppers, Chili, dried, together with an STMR and an HR of 0.01 mg/kg.

Leafy vegetables

The spinach data set was used by the 2009 Meeting to estimate a group MRL for leafy vegetables. Residues of 2,6-dichlorobenzamide in spinach arising from use of fluopicolide were 0.02, 0.03, 0.06, 0.07 (2), 0.09, and 0.19 mg/kg. The Meeting estimated a maximum residue level of 0.3 mg/kg for 2,6-dichlorobenzamide in leafy vegetables, together with an STMR of 0.07 mg/kg and an HR of 0.19 mg/kg.

Celery

In the dataset considered by the 2009 Meeting, residues of 2,6-dichlorobenzamide resulting from use of fluopicolide in celery were < 0.01 (4), 0.01, 0.03 and 0.04 mg/kg. The Meeting estimated a maximum residue level of 0.07 mg/kg for 2,6-dichlorobenzamide in celery, together with an STMR of 0.01 mg/kg and an HR of 0.04 mg/kg.

Rotational crops

Residues arising in rotational brassica crops are covered in the appropriate section above.

Low levels of 2,6-dichlorobenzamide residues were found in rotational pulse and cereal forages and fodders after application of fluopicolide to a preceding crop.

Residues of 2,6-dichlorobenzamide in rotational faba beans were < 0.01 (8) mg/kg.

The Meeting estimated a maximum residue level of 0.01* mg/kg for faba bean (dry), together with an STMR and an HR of 0.01 mg/kg. The Meeting agreed to extrapolate these values to establish a group maximum residue level for pulses.

Residues of 2,6-dichlorobenzamide in faba bean forage were < 0.01 (3), 0.01, 0.03, 0.06 (2), and 0.10 mg/kg (as received).

The Meeting estimated a median residue of 0.02 mg/kg, and a highest residue of 0.10 mg/kg for bean forage on an as received basis. The Meeting agreed to extrapolate these values to legume animal feeds.

Residues of 2,6-dichlorobenzamide in rotational wheat were < 0.01 (9) mg/kg.

The Meeting estimated a maximum residue level of 0.01* mg/kg for wheat, together with STMR and HR values of 0.01 mg/kg. The Meeting agreed to extrapolate these values to estimate a group maximum residue level and STMR/HR values for cereal grains.

Residues of 2,6-dichlorobenzamide in rotational wheat forage were < 0.01 (6), 0.01 (2), and 0.02 mg/kg (as received basis).

The Meeting estimated a median residue of 0.01 mg/kg and a highest residue of 0.02 mg/kg for wheat forage on an as received basis. The Meeting agreed to extrapolate these figures to cereal forage.

Residues of 2,6-dichlorobenzamide in rotational wheat hay (stalks and/or ears) were < 0.01 (6), 0.01, 0.03, and 0.06 mg/kg (as received).

As it was not clear that the samples had been dried, these values were converted to a dry weight basis assuming the 25% dry matter content for wheat forage: < 0.04 (6), 0.04, 0.12, and 0.24 mg/kg.

Residues of 2,6-dichlorobenzamide in rotational wheat straw were < 0.01 (7), 0.01, and 0.03 mg/kg on an as received basis.

Based on the hay data, the Meeting estimated a maximum residue level of 0.4 mg/kg for wheat straw and fodder, dry. The Meeting agreed to extrapolate this to straw and fodder (dry) of cereal grains.

The Meeting estimated a median residue of 0.04 mg/kg and a highest residue of 0.24 mg/kg for cereal hays based on the wheat hay data set and a median residue of 0.01 mg/kg and a highest residue of 0.03 mg/kg for cereal straws based on the wheat straw data set.

Fate of residues during processing

Plums

A processing study was provided for plums but as there was insufficient data to estimate a maximum residue level in plums, it will not be considered further.

Grapes

A processing study in grapes was provided. Grapes from a plot treated with dichlobenil at a target rate of 8.74 kg ai/ha were processed into juice and raisins, and processing factors of 1.4 and 2.8 respectively were determined for 2,6-dichlorobenzamide.

Based on the processing factor of 2.8 and the grape MRL of 0.05 mg/kg, the Meeting estimated a maximum residue level of 0.15 mg/kg for dried grapes. Based on the STMR and HR of 0.01 and 0.04 mg/kg respectively for grapes, the Meeting estimated an STMR-P of 0.028 mg/kg, and an HR-P of 0.11 mg/kg for dried grapes.

Based on the processing factor of 1.4, and the grape MRL of 0.05 mg/kg, the Meeting estimated a maximum residue level of 0.07 mg/kg for grape juice. Based on the STMR and HR values for grapes, the Meeting estimated an STMR-P of 0.014 mg/kg and an HR-P of 0.056 mg/kg.

Grape pomace is used as a feed for cattle. However, the processing study did not include results for pomace. Given that pomace consists of the dry matter remaining after manufacture of juice or wine, the Meeting estimated an STMR-P value of 0.028 mg/kg for grape pomace, dry, based on the processing data for raisins.

Tomatoes

As residues of 2,6-dichlorobenzamide were not detected in tomatoes, the Meeting confirmed the median residues and STMRs of 0.01 mg/kg estimated by the 2009 Meeting for for tomato pomace, juice, paste and ketchup.

Residues in animal commodities

Feeding studies

A feeding study for 2,6-dichlorobenzamide in lactating dairy cattle was provided to the Meeting.

Lactating Holstein dairy cow were dosed daily by capsule with 2,6-dichlorobenzamide at the equivalent of 0.6, 1.6 and 5.3 ppm in the dry weight diet for 28 consecutive days. Milk was collected throughout, and the cattle were slaughtered within 6 hours of the final dose for tissue sampling. 2,6-Dichlorobenzamide residues were found at 0.011 mg/kg in milk from the 1.6 ppm group, and at 0.031 mg/kg in milk from the 5.3 ppm group. Residues of 2,6-dichlorobenzamide were found in most tissues at all feeding levels, except for fat. The relationships between dose and residues of 2,6-dichlorobenzamide in muscle, kidney and liver were linear.

Farm animal dietary burden

Dietary burden calculations incorporating all commodities considered by the current and 2003 Meetings for beef cattle, dairy cattle, broilers and laying poultry are presented in Annex 6. The calculations are made according to the livestock diets of the USA/Canada, the European Union, Australia and Japan as laid out in the OECD table.

	US/CAN		EU		AU		Japan	
	Max.	Mean	Max.	Mean	Max.	Mean	Max.	Mean
Beef cattle	0.064	0.010	0.31	0.063	0.38	0.077	0,019	0.002
Dairy cattle	0.25	0.054	0.091	0.031	0.39	0.075	0.031	0.013
Poultry – broiler	0.009	0	0.01	0	0.012	0	0.022	0.029
Poultry – layer	0.009	0	0.096	0.017	0.012	0	0.006	0

Animal commodity maximum residue levels

The highest dietary burden for cattle is for Australia (maximum of 0.39 ppm for both beef and dairy cattle and mean values of 0.077 mg/kg for beef cattle and 0.075 mg/kg for dairy cattle).

Scaling the residues observed in milk at a feeding level of 1.6 ppm for the expected Maximum Feeding Level of 0.39 ppm shows that residues are not expected to be found above the LOQ in milk. The Meeting estimated a maximum residue level of 0.01* mg/kg for milk, together with an STMR of 0.01 mg/kg.

The calculated maximum residues for muscle, liver and kidney are tabulated below.

Tissue	Regression equation (forced through origin)	Calculated residue for 0.39 ppm residues of 2,6-dichlorobenzamide in feed (mg/kg)
Liver	y = 0.0804×	0.031
Kidney	y = 0.01×	0.004
Muscle	y = 0.0128×	0.005
Fat	Scaled from residue at LOQ (0.01 mg/kg) at 1.6 ppm feeding level	0.002

The calculated mean residues for muscle, liver and kidney are tabulated below.

Tissue	Regression equation (forced through origin)	Calculated residue for 0.075 ppm residues of 2,6-dichlorobenzamide in feed (mg/kg)
Liver	y = 0.0584×	0.004
Kidney	y = 0.0078×	0.0006
Muscle	y = 0.0114×	0.0009
Fat	Scaled from residue at LOQ (0.01 mg/kg) at 1.6 ppm feeding level	0.0004

Based on the calculated residues, the Meeting estimated a maximum residue level of 0.04 mg/kg for edible offal, mammalian, together with an STMR of 0.01 mg/kg and an HR of 0.031 mg/kg. The Meeting estimated maximum residue levels of 0.01* mg/kg for meat (mammalian, except marine mammals) and mammalian fats, together with STMR and HR values of 0.01 mg/kg.

Poultry

The highest dietary burden for poultry is for European laying hens (mean of 0.017 ppm and maximum of 0.096 ppm).

A poultry feeding study was not provided to the Meeting. In a metabolism study for 2,6-dichlorobenzamide in laying hens, after dosing at 10 ppm daily for five days, residues of 2,6-dichlorobenzamide were 8.5 mg/kg in liver, 4.8 mg/kg in kidney, 1.8 mg/kg in skin, 1.9 mg/kg in muscle, 1.3 mg/kg in fat, and 2.0 mg/kg in eggs.

Scaling the residues in hen matrices for the expected maximum and mean feeding levels in poultry, the following median and highest residues were calculated.

Tissue	Maximum residue	Median residue
Liver	0.081	0.014
Kidney	0.046	0.008
Muscle	0.018	0.003
Fat	0.012	0.002
Eggs	0.019	0.003

Based on the calculations for eggs, the Meeting estimated a maximum residue level of 0.03 mg/kg for eggs, together with an STMR of 0.01 mg/kg and an HR of 0.019 mg/kg.

Based on the calculations for liver, the Meeting estimated a maximum residue level of 0.1 mg/kg for poultry edible offal, together with an STMR of 0.014 mg/kg and an HR of 0.081 mg/kg.

Based on the calculations for muscle, the Meeting estimated an MRL of 0.03 mg/kg for poultry meat, together with an STMR of 0.01 mg/kg and an HR of 0.018 mg/kg.

Based on the calculations for fat, the Meeting estimated an MRL of 0.02 mg/kg for poultry fats, together with an STMR of 0.01 mg/kg and an HR value of 0.012 mg/kg.

RECOMMENDATIONS

Definition of the residue (for compliance with the MRL and for estimation of dietary intake for plant and animal commodities): *2,6-Dichlorobenzamide*.

The residue is not fat soluble.

DIETARY RISK ASSESSMENT

Long-term intake

The ADI for dichlobenil is 0–0.01 mg/kg bw. Finite residues of dichlobenil parent compound are not expected to be found in edible commodities, and parent compound has not been recommended for inclusion in the residue definition for dietary risk assessment. The Meeting concluded that the long-term intake of residues of dichlobenil when used in ways that have been considered by the JMPR is unlikely to present a public health concern.

The ADI for 2,6-dichlorobenzamide is 0–0.05 mg/kg bw. The International Estimated Dietary Intakes (IEDIs) for 2,6-dichlorobenzamide arising from both dichlobenil and fluopicolide were calculated for the 17 GEMS/food cluster diets using STMRs/STMR-Ps estimated by the current Meeting (see Annex 3 of the 2014 JMPR Report). The calculated IEDIs were 0–1% of the maximum ADI (0.05 mg/kg bw). The Meeting concluded that the long-term intakes of residues of 2,6-dichlorobenzamide, resulting from the uses of dichlobenil considered by the current Meeting and from the uses of fluopicolide considered by the 2009 Meeting are unlikely to present a public health concern.

Short-term intake

The ARfD for dichlobenil is 0.5 mg/kg bw (for women of childbearing age only). Finite residues of dichlobenil parent compound are not expected to be found in edible commodities, and parent compound has not been recommended for inclusion in the residue definition for dietary risk assessment. The Meeting concluded that the short-term intake of residues of dichlobenil when used in ways that have been considered by the JMPR is unlikely to present a public health concern.

The ARfD for 2,6-dichlorobenzamide is 0.3 mg/kg bw (for women of childbearing age only). The International Estimated Short-Term Intakes (IESTIs) for 2,6-dichlorobenzamide arising from dichlobenil and fluopicolide were calculated for food commodities and their processed commodities using HRs/HR-Ps or STMRs/STMR-Ps estimated by the current Meeting and by the 2009 Meeting as part of the evaluation of fluopicolide (see Annex 4 of the 2014 JMPR Report). The calculated IESTIs were 0–2% of the ARfD for all commodities. The Meeting concluded that the short-term intake of residues of 2,6-dichlorobenzamide, when dichlobenil and fluopicolide are used in ways that have been considered by the JMPR, is unlikely to present a public health concern.