DICHLOBENIL (274)

see also FLUOPICOLIDE (235)

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EXPLANATION

Dichlobenil is a herbicide belonging to the benzonitrile group of chemicals. It is a systemic herbicide, which acts by inhibition of cell wall (cellulose) biosynthesis, thereby inhibiting actively dividing meristems, germination of seeds, and damaging rhizomes. It is used for control of annual and perennial weeds, principally in orchard crops.

At the Forty-fifth session of the CCPR (2013), dichlobenil was scheduled for evaluation as a new compound by the 2014 JMPR.

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

IDENTITY

Common name: Dichlobenil

Chemical names

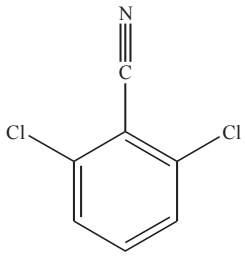
IUPAC: 2,6-Dichlorobenzonitrile

CAS: 2,6-Dichlorobenzonitrile

CAS number: 1194-65-6

Synonyms: Casoron

Structural formula:



Molecular formula: $C_7H_3Cl_2N$

Molecular weight: 172.0

SPECIFICATIONS

Specifications for dichlobenil have not been published by FAO.

PHYSICAL AND CHEMICAL PROPERTIES

Table 1a Physical and chemical properties of dichlobenil

Appearance	Off-white crystalline solid (technical material)
Melting point	143.8–144.3 °C (technical material)
Boiling point	270 °C (101.3 kPa)
Relative density	1.55
Water solubility	25 mg/L (25 °C)

Solubility in organic solvents	Acetone: 86 g/L (20 °C) Cyclohexane: 3.7 g/L (25 °C) Methanol: 17.2 g/L (20 °C) Dichloromethane: 151 g/L (20 °C) Ethyl acetate: 59.3 g/L (20 °C) Xylene: 53 g/L (25 °C)
Hydrolysis	Stable in pH 5, 7 and 9 unsterilized buffers at 22 ± 1 °C, with half-life >150 days (Buisman, 1988)
Aqueous photolysis (sterile pH 7 buffer in sealed ampoules at 25 ± 1ºC)	10 days under spring/summer sunlight at 40ºN latitude. Metabolites included 4-chloro-2(3*H*)benzoxazolone, 2,6-dichlorobenzoic acid, 2,6-dichlorobenzamide, 2-hydroxybenzonitrile, 2,6-dichloro-4-hydroxybenzonitrile, 2-chlorobenzonitrile and various small polar molecules (Boelhouwers *et al*., 1989)
Vapour pressure	0.14 Pa (25 °C)
Octanol/water partition coefficient ($log_{10}P_{OW}$)	2.70 (pH 3, 22 °C)
pH	6.7
Stability	Stable for 3 months at 54 °C
Explosive characteristics	Not explosive
Corrosive characteristics	Not corrosive

Table 1b Physico-chemical properties of 2,6-dichlorobenzamide

Appearance	Crystalline solid
Melting point	195–203 °C
Boiling point	335 °C (estimated)
Density	1.44 g/cm^3
Water solubility	2.7 g/L (20-25 °C)
Vapour pressure	0.35–4.35 × 10^{-3} Pa at 25 °C (various estimated values)
Octanol/water partition coefficient ($log_{10}P_{OW}$)	0.77 (Nakagawa *et al*, 1992)
Hydrolysis (5.19-5.26 mg/L ^{14}C-(U-phenyl) label in pH 5, 7, and 9 buffers at 25 ± 2 ºC in the dark)	No degradation over 33 days (Nag, 2000)
Aqueous photolysis (^{14}C-1-phenyl) label in sterile pH 5 buffer at 25 ºC irradiated with 40.3ºN summer sunlight in a 12 hour light/dark cycle)	Compound was not photolysed or hydrolysed over 31 days (Reynolds, 2000)

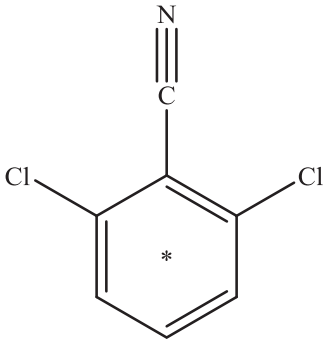
FORMULATIONS

GAPs have been reported for two formulations, a capsule suspension (CS) containing 168 g/L dichlobenil, and a granular formulation containing 40 g/kg dichlobenil.

METABOLISM AND ENVIRONMENTAL FATE

The metabolism of dichlobenil has been investigated in grapes, rats, laying hens and lactating goats. Metabolism of the major plant metabolite 2,6-dichlorobenzamide has also been investigated in lactating goats and laying hens.

Metabolism studies for dichlobenil and 2,6-dichlorobenzamide were conducted with compound uniformly labelled with carbon-^{14} in the benzyl ring.



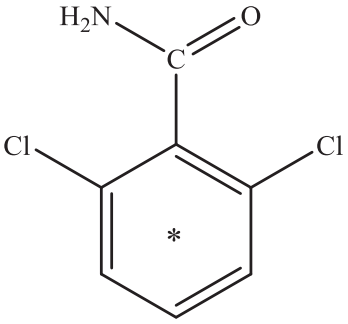
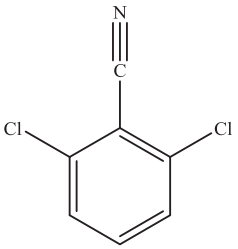
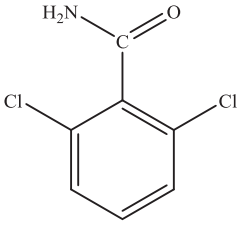
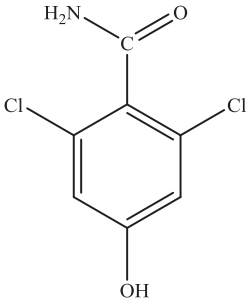
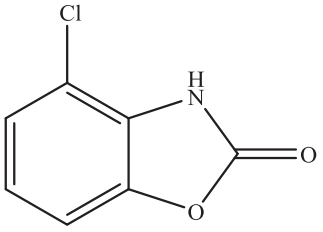
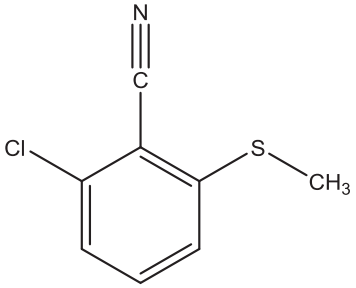
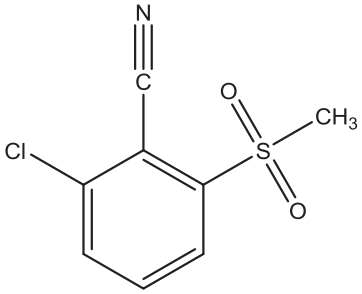
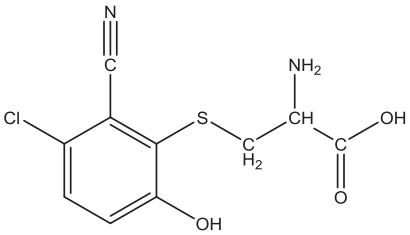
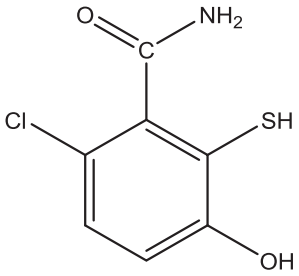
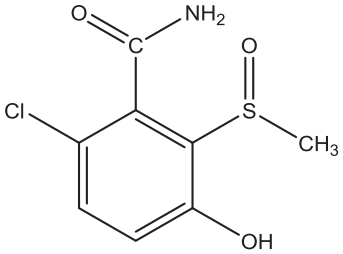
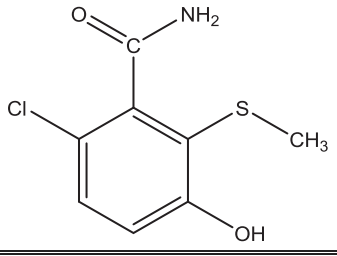


Figure 1: [^{14}C-U-phenyl]-dichlobenil and [^{14}C-U-phenyl]-dichlorobenzamide using in the metabolism studies

Table 2 Metabolites of dichlobenil and 2,6-dichlorobenzamide

Code name, common name	IUPAC name	Structure	Where found
Dichlobenil (parent compound)	2,6-Dichlorobenzonitrile		
BAM	2,6-Dichlorobenzamide		Grapes, apples, soil
BAM-4-OH	2,6-Dichloro-4-hydroxybenzamide		Grapes
	4-Chloro-2(3*H*)benzoxazolone		Aqueous photolysis

Code name, common name	IUPAC name	Structure	Where found
Methylthio-dichlobenil	6-Chloro-2-methylthiobenzonitrile		Goat, hen (tentative)
Methylsulfonyl-dichlobenil	6-Chloro-2-methylsulfonylbenzonitrile		Goat, hen
Dichlobenil-OH-cysteine	S-(3-Chloro-2-cyano-6-hydroxyphenyl)-cysteine		Goat, hen
BAM-OH-SH	6-Chloro-3-hydroxy-2-thiobenzamide		Goat metabolite of BAM
Methylsulfinyl-BAM-OH	6-Chloro-3-hydroxy-2-methylsulfinylbenzamide		Goat metabolite of BAM
Methylthio-BAM-OH	6-Chloro-3-hydroxy-2-methylthiobenzamide		Goat metabolite of BAM

Animal metabolism

Rats

The metabolism of dichlobenil in rats was investigated and was evaluated by the WHO Panel of the 2014 JMPR.

Rats: dichlobenil

Following oral gavage dosing of rats, dichlobenil was rapidly absorbed: about 89 and 97% of the administered dose in male and female in 7 days, respectively, based on comparison of urinary excretion following oral and intravenous routes of exposure. Oral absorption after repeated exposure is at least 60%, irrespective of dose and sex, based on radiolabel recovered from urine, faeces and cage wash. Liver, kidney and kidney-fat retained the highest concentrations of radiolabel, peaking 1 hour after administration. The elimination of the radioactivity associated with [^{14}C]dichlobenil following oral administration was rapid, with the majority (about 80%) of the radioactivity being eliminated within 24 hours at the low dose level. Around 60% is excreted in the urine and around 20% in the faeces, irrespective of sex or dose regime, 9 to 10 days after initiation of exposure. Approximately 79% of the administered dose was excreted in the bile in 24 hours in bile duct cannulated rats. Ten metabolites were identified in urine, although no parent compound was identified in urine. Most metabolites were conjugates such as sulphate, glutathione (and derivatives) and glucuronic acid conjugates. In faeces, three major metabolites (each representing 5% of the administered dose or more) were observed as well as parent compound (dichlobenil). The proportion of conjugated metabolites decreases with increasing and multiple doses, while the amount of parent compound in faeces increases. No chemical names were provided for the identified metabolites. Dichlobenil is metabolized in rats via two metabolic pathways: hydroxylation at the 3 or 4 position followed by glucuronidation or sulphation and the second pathway include substitution of one chlorine atom by glutathione.

Lactating goats: dichlobenil

Metabolism of [^{14}C-U-phenyl]-dichlobenil was investigated in lactating goats (Cameron and Dunsire, 1988 and Ruijten *et al*, 1988). Three animals were dosed orally twice daily by capsule for three consecutive days at the same dose of 10 mg/kg bw/day (one animal, goat 10P, as a pilot study and the other two, goats 7M and 8M, comprising the main study). Two further goats were dosed with unlabelled material at the same level.

The goats were kept in metabolism cages during the feeding phase of the study. Fresh drinking water and hay were available *ad libitum*, while commercially available diet was provided twice daily at milking. The dose was administered orally twice daily at approximately 0900 and 1700 using gelatin capsules. Actual dose levels were 9.9–10.2 mg/kg bw/day (mean = 10.0 mg/kg bw/day). Feed consumption was not recorded. Based on an estimated dry matter consumption figure of 4% of the body weight per day, these doses would equate to a mean of 250 ppm in feed.

The goats were milked twice daily and milk was sampled. Blood samples were taken prior to first and fifth doses, and at intervals of 30 minutes to 8 hours after each of these doses. A final blood sample was taken just prior to sacrifice. The goats were sacrificed 15 hours after the final dose, and samples of liver, kidney, muscle, fat, gastrointestinal (GI) tract and contents, and the remaining carcass were collected.

Total radioactive residues in liquid samples (urine, cage wash and plasma) were mixed with scintillation cocktail and analysed by liquid scintillation counting (LSC). Prior to combustion and LSC of the captured $^{14}CO_2$, faeces and residual carcass samples were homogenised with water, tissue samples were finely chopped, while the GI tract samples were separated into tissue and contents, with the contents being homogenised in water, and the tissue being minced then homogenised in water with carboxymethylcellulose.

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.

Table 3 Total radioactive residues in milk after dosing of dichlobenil at 250 ppm

Sampling interval (hours)	Concentration (mg eq./kg)			
	Goat 10P	Goat 7M	Goat 8M	Mean
Predose	0	0	0	0
0-8	0.96	0.95	0.95	0.95
8-24	0.73	0.87	0.76	0.79
24-32	0.85	1.46	1.58	1.30
32-48	0.77	0.90	0.90	0.86
48-56	1.12	1.28	1.48	1.29
56-71	0.91	0.85	0.84	0.87

NA = not applicable.

Total dichlobenil radioactive residues appeared to reach a plateau in milk after 1.5 days of dosing.

Concentrations of ^{14}C-dichlobenil total residue in plasma and whole blood peak around 2-3 hours after each dose.

Table 4 Total radioactive residues in goat tissues after dosing of dichlobenil at 250 ppm

Tissue	Residue (mg eq./kg)			
	Goat 10P	Goat 7M	Goat 8M	Mean
Plasma (15 hours after final dose)	1.04	0.76	0.64	0.81
Whole blood (15 hours after final dose)	0.80	0.49	0.45	0.58
Liver	32.4	25.0	24.7	27.4
Kidney	4.91	3.82	3.47	4.07
Fat	1.77	3.40	1.98	2.38
Muscle	0.58	0.35	0.34	0.42
GI tract tissue	1.08	1.65	1.65	1.46
GI tract contents	6.52	11.5	12.0	10.0
Carcass	1.40	1.18	1.09	1.22

Residues in milk and tissues of goats 7M and 8M from the main study were further characterised. To provide additional material to aid identification, the samples from the goats treated with unlabelled compound were co-processed, except for muscle, for which no unlabelled samples were available. Instead, the muscle from the pilot study goat was used.

The samples were homogenised, with water in the case of liver, kidney and milk, and with blank skim milk in the case of fat and muscle, and then filtered and centrifuged to remove solid material. The samples were then freeze concentrated if necessary to reduce the volume, prior to HPLC with on-line clean-up using a pre-column. Metabolic profiles of the sample extracts were analysed by HPLC with gradient elution (mobile phases were 3 g/L ammonium hydrogen phosphate and methanol) and radiodetection with fraction collection. Isolated fractions were further purified by passing them through the HPLC system a second time, and by thin layer chromatography.

The proportions of residue extracted were relatively low, and variable, at 65–70% of TRR in milk, 65% in liver, 26% in fat, 13% in muscle, and 28% in kidney. It is noted that no further characterisation was carried out for residues not extracted by the initial homogenisation with water or skim milk.

The metabolites were identified using mass spectrometry and ^{1}H NMR, and the data are presented in tables 5 and 6 below. Radioactivity present at each step of the work up process was reported as a percentage of the TRR. Significant losses were noted in several of the sample work-ups. It is noted that no attempt was made to characterise residues in the post-extraction solids. The metabolic profile was determined at different stages during the extraction and purification processes, with the relative proportions remaining consistent, indicating that losses were non-selective.

Components identified in the milk and tissues of lactating goats given ^{14}C-phenyl dichlobenil were: parent compound, the hydroxylated cysteine conjugate of deschloro dichlobenil, and the subsequent metabolites of the cysteine conjugate (without hydroxylation), 6-chloro-2-methylthiobenzonitrile (tentatively identified on the basis of comparison of retention times with a standard) and 6-chloro-2-methylsulfonylbenzonitrile. Parent compound was only present in significant quantities in fat. The cysteine conjugate was the most significant component of the liver residue, and was also noted in the milk samples, which contained higher proportions of this and other polar metabolites, such as 2-chloro-6-methylsulfonylbenzonitrile. 2-Chloro-6-methylthiobenzonitrile, a cleavage product of the cysteine conjugate, was found in significant proportions in muscle and kidney and the later milk samples, consistent with its position further down the metabolic pathway. The key metabolic pathway for dichlobenil in goats is cysteine conjugation at one of the chlorine positions on the phenyl ring, followed by cleavage and oxidation of the cysteine side chain, together with hydroxylation at the 3-position which may occur in parallel with the cysteine conjugation.

Table 5 Relative amounts of dichlobenil metabolites chromatographed in milk centrifugate (pooled milk of goats 7M and 8M)

Metabolite	0-48 h		48-56 h		56-72 h	
	%TRR	mg eq. /kg	%TRR	mg eq. /kg	%TRR	mg eq. /kg
Dichlobenil	-	-	0.7	0.01	-	-
Unknown 1	12	0.12	3.9	0.05	7.2	0.06
S-(3-Chloro-2-cyano-6-hydroxyphenyl)-cysteine	20	0.20	21	0.29	11	0.09
2-chloro-6-methylsulfonylbenzonitrile (tentative)	38	0.37	20	0.28	14	0.12
2-Chloro-6-methylthiobenzonitrile (tentative)	-	-	20	0.27	33	0.28
Total extracted	70	0.69	65	0.90	65	0.55
TRR	100	0.98	100	1.38	100	0.85

Table 6: Identification of metabolites in tissues

Component	Liver		Fat		Muscle		Kidney	
	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg
Dichlobenil	-	-	16	0.42	-	-	-	-
S-(3-Chloro-2-cyano-6-hydroxyphenyl)-cysteine	62	15.3	-	-	0.9	0.003	1.1	0.04
2-Chloro-6-methylsulfonylbenzonitrile	3	0.81	10	0.28	-	-	1.4	0.05
Unidentified component	-	-	-	-	1.3	0.005	2.5	0.09
2-Chloro-6-methylthiobenzonitrile: tentative	-	-	-	-	11	0.038	23	0.84
Total extracted	65	16.1	26	0.70	13	0.046	28	1.0
TRR	100	24.9	100	2.69	100	0.35	100	3.65

Laying hens: dichlobenil

Metabolism of ^{14}C-phenyl labelled dichlobenil was investigated in laying hens (Cameron and Dunsire, 1988 and Ruijten *et al*, 1988). Eight birds were dosed orally twice daily by capsule for three consecutive days, with radiolabelled material at 9.8-10.2 mg/kg bw/day (mean = 10.0 mg/kg bw/day). Feed consumption was not recorded. Based on an estimated dry matter consumption figure of 7.5% of the body weight per day, these doses would equate to a mean of 133 ppm in feed.

The hens were kept in metabolism cages during the feeding phase of the study. Fresh drinking water and layer pellets were available *ad libitum*. The dose was administered orally twice daily at approximately 0900 and 1700 using gelatin capsules. .

Eggs, excreta and cage washings were collected twice daily prior to administration of each dose, and 15 hours after the final dose. Blood samples were taken from all birds just prior to sacrifice. Two of the hens additionally had blood samples taken and plasma separated prior to doses 1 and 5, and at 1, 3 and 6 hours after these doses. The other six hens were sacrificed 15 hours after the final dose, and samples of liver, kidney, breast muscle, abdominal fat, gastrointestinal (GI) tract and contents, and the remaining carcass were collected.

Total radioactive residues in liquid samples (cage wash and plasma) were mixed with scintillation cocktail and analysed by liquid scintillation counting (LSC). Prior to combustion and LSC of the captured $^{14}CO_2$, excreta and residual carcass samples were homogenised with water; and tissue and GI tract samples were finely chopped.

The majority of the administered dose was excreted (64-73%) with 0.17–0.59% found in eggs, 2.8–16% in the GI tract at sacrifice, and 0.9–11% in the carcase. The recoveries of the total administered radioactivity were 74–92%.

Table 7 Total radioactive residues in eggs

Sampling interval (hours)	Concentration (mg eq./kg)						
	Hen 6P	Hen 7P	Hen 1M	Hen 2M	Hen 3M	Hen 4M	Mean
Pre-dose	0	0	0	0	0	0	0
Post-dose 1	0	NS	0	0	0	0	0
Post-dose 3	0.40	0.13	0.25	0.32	0.43	0.48	0.34
Post-dose 5	NS	1.25	1.15	1.30	1.18	1.37	1.25
Post-dose 6	2.11	1.90	1.64	NS	1.83	2.18	1.93

NS = no sample.

Total dichlobenil radioactive residues do not appear to have reached a plateau in eggs after 3 days of dosing. Concentrations of ^{14}C-dichlobenil total residue in hen plasma peak around 3 hours after each dose.

Table 8 Total radioactive residues in hen tissues

Tissue	Residue (mg eq./kg)						
	Hen 6P	Hen 7P	Hen 1M	Hen 2M	Hen 3M	Hen 4M	Mean
Plasma (15 hours after final dose)	2.4	2.5	1.4	1.7	1.0	2.0	1.8
Whole blood (15 hours after final dose)	1.2	1.3	0.68	1.0	0.65	1.3	1.0
Liver	6.4	6.1	6.6	9.2	18.4	8.5	9.2
Kidney	3.6	3.1	3.5	4.5	3.9	5.4	4.0
Fat	34.8	4.1	15.1	19.0	12.0	14.7	16.7
Muscle	0.46	0.29	0.32	0.33	1.07	0.42	0.48
GI tract	8.7	9.3	10.1	22.8	10.3	33.6	15.8
Carcass	1.3	0.89	3.2	3.9	1.8	1.9	2.2

The highest levels of total radioactive residue are found in fat and the GI tract, with significant levels also being observed in the liver.

Residues in eggs and tissues of hens from the study were further characterised. The samples were homogenised, with water in the case of liver, kidney and eggs, and with skim milk in the case of fat and muscle, and then filtered and centrifuged to remove solid material. The samples were then freeze concentrated if necessary to reduce the volume, prior to HPLC with on-line cleanup using a pre-column. Metabolic profiles of the sample extracts were analysed by HPLC with gradient elution (mobile phases were 3 g/L ammonium hydrogen phosphate and methanol) and radio-detection with fraction collection. Isolated fractions were further purified by passing them through the HPLC system a second time, and by thin layer chromatography.

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.

The metabolites were identified using mass spectrometry and ^{1}H NMR, and the data are presented in table 9 below. Significant losses were noted in several of the sample work-ups. It is noted that no attempt was made to characterise residues in the post-extraction solids.

Components identified in the eggs and tissues of laying hens given ^{14}C-phenyl dichlobenil were: parent compound, the hydroxylated cysteine conjugate of deschloro dichobenil, and the subsequent metabolites of the cysteine conjugate, 6-chloro-2-methylthiobenzonitrile and 6-chloro-2-methylsulfonylbenzonitrile. Parent was the only component identified in the more hydrophobic matrices, fat and eggs, with parent also being found in smaller proportions in the other tissues. A metabolite tentatively identified as 2-chloro-6-methylthiobenzonitrile was the predominant component of the residue in liver and muscle, while kidney showed the greatest number of residue components, and a higher proportion of the more water soluble metabolites, the hydroxylated cysteine conjugate, and its oxidative cleavage product, 2-chloro-6-methylsulfonylbenzonitrile. The key metabolic pathway for dichlobenil in hens is cysteine conjugation at one of the chlorine positions on the phenyl ring, followed by cleavage and oxidation of the cysteine side chain, together with hydroxylation at the 3-position.

Table 9 Identification of metabolites in tissues and eggs

Metabolite	Eggs (day 2)		Liver		Fat		Muscle		Kidney	
	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg	%TRR	mg eq./kg
Dichlobenil	60	0.22	5.4	0.58	15	2.3	1.6	0.01	9.8	0.43
Unknown 1	-	-	-	-	-	-	-	-	1.6	0.07
Unknown 2	-	-	-	-	-	-	-	-	1.2	0.05
S-(3-Chloro-2-cyano-6-hydroxyphenyl)-cysteine	-	-	-	-	-	-	-	-	17	0.74
2-Chloro-6-methylsulfonylbenzonitrile)	-	-	-	-	-	-	-	-	5.1	0.22
2-Chloro-6-methylthiobenzonitrile: tentative	-	-	85	9.1	-	-	6.4	0.03	4.3	0.18
Total characterised	60	0.22	90	9.6	15	2.3	8	0.04	39	1.7
Total residue	100	0.37	100	10.7	100	15.2	100	0.54	100	4.33

Overview of metabolism of dichlobenil in animals

The major metabolic pathway for dichlobenil in food producing animals is conjugation, substituting cysteine for one of the chlorine atoms accompanied by hydroxylation of the phenyl ring. Cleavage and oxidation of the cysteine side chain of the unhydroxylated conjugate also takes place, giving 6-chloro-2-methylthiobenzonitrile and 6-chloro-2-methylsulfonylbenzonitrile.

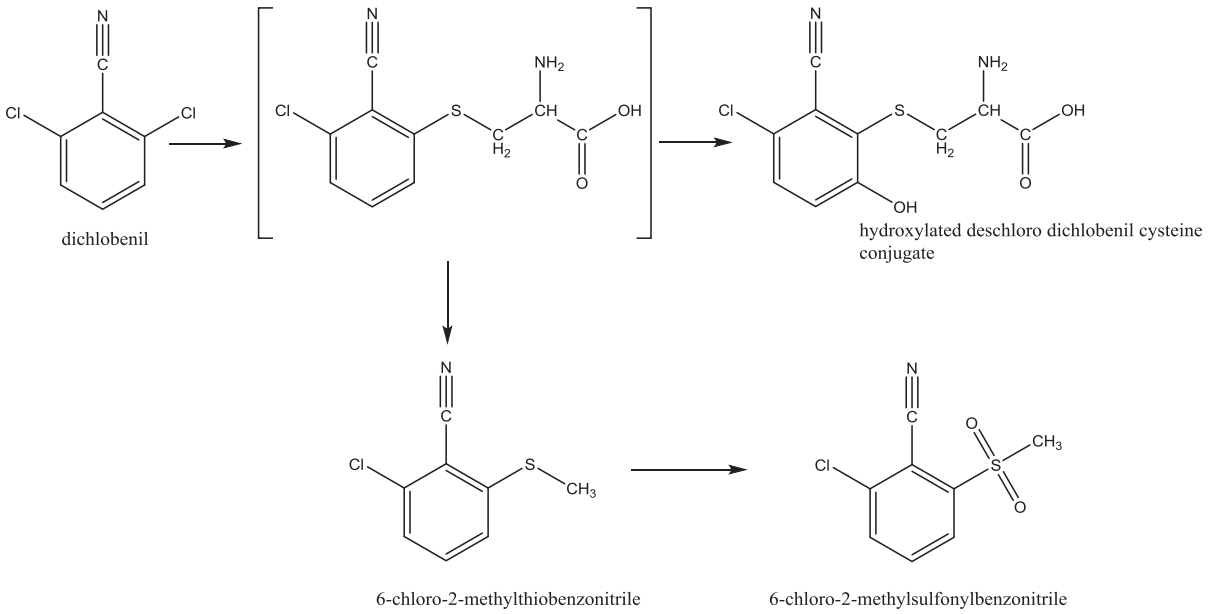


Figure 2: Metabolism of dichlobenil in animals

There are strong similarities to the metabolism of dichlobenil in rats. In rats, hydroxylation at the 3-position also takes place, as well as substitution of one of the chlorine atoms, initially by glutathione, which is subsequently cleaved to give a cysteine moiety. Further cleavage and oxidation of the cysteine substituent to give the methylthio-dichlobenil and methylsulfonyl-dichlobenil also takes place in the rat.

Given that almost the only component of the residue in fruit crops is 2,6-dichlorobenzamide, with no parent being identified, the metabolism of dichlobenil in animals is of secondary importance to the animal metabolism of 2,6-dichlorobenzamide.

Rats: 2,6-Dichlorobenzamide

Metabolism data for 2,6-dichlorobenzamide in rats was not provided to the Meeting.

Lactating goats: 2,6-dichlorobenzamide

Metabolism of ^{14}C-phenyl labelled 2,6-dichlorobenzamide was investigated in lactating goats (Burke, 1994a and Ruijten *et al*, 1994a). Dairy goats were dosed with ^{14}C-2,6-dichlorobenzamide daily at 13 ppm (0.32 mg/kg bw/day) for five days or 252 ppm (7.2 mg/kg bw/day) for three days.

The goats were kept in metabolism cages during the feeding phase of the study. Fresh drinking water was available *ad libitum*, while measured quantities of feed (commercially available diet, shredded molasses beet pulp, and hay) were provided each morning and afternoon, with feed consumption being monitored. The dose was administered orally using gelatin capsules. No overt signs of toxicity were observed during the study, and the dosing did not have any adverse effects on the weight of the animals, feed intake, or milk production.

Urine and faeces were collected at 24 hour intervals, with milk being collected twice daily and pooled for each 24 hour period. The cages were rinsed after each collection of excreta and the cage wash was collected. The low dose animal was sacrificed approximately 23 hours after the final dose, while the high dose animal was sacrificed around 4 hours following the final dose. Samples of skeletal muscle (composite of fore and hind quarter), fat (composite of omental and perirenal), liver, and kidney were collected. The tissue and faeces samples were macerated and sub-sampled prior to frozen storage, with the liquid samples being appropriately sub-sampled.

Samples of urine, milk and cage wash were analysed for total radioactivity directly by liquid scintillation counting (LSC), with the tissue and faeces samples being combusted prior to capture and LSC of the liberated $^{14}CO_2$.

In the low dose goat, 62% of the administered dose was recovered in urine, 17% in faeces, 0.3% in milk, and 11% in tissues, with >90% total recovery. For the high dose animal, 48% of the dose was recovered in urine, 18% in faeces, 0.4% in milk and 5% in tissues (>70% total recovery).

Table 10 Total radioactive residues in milk

Sampling interval (hours)	Concentration (mg eq./kg)	
	Low dose animal	High dose animal
0-24	0.033	0.95
24-48	0.045	1.12
48-58	NA	2.88
48-72	0.047	NA
72-96	0.048	NA
96-120	0.045	NA
120-126	0.019	NA

NA = not applicable.

Residues of 2,6-dichlorobenzamide reached a plateau in milk at about day 3 of dosing.

Table 11 Total radioactive residues in goat tissues

Tissue	Residue (mg eq./kg)	
	Low dose animal (12.8 ppm)	High dose animal (252 ppm)
Fat	0.024	2.15
Kidney	1.19	17.9
Liver	9.41	67.6
Muscle	0.25	4.47

Goat tissues and milk were homogenised and uniformly mixed with Lichroprep® 25–40 μm silica, then loaded into a column subjected to online (milk and liver) or off-line (all other matrices) extraction and HPLC analysis using gradient elution with 50 mM ammonium acetate in distilled water adjusted to pH 3 and 50 mM ammonium acetate in methanol, with UV (254 nm) and radio detection with fraction collection. For the off-line extractions, homogenates of muscle and kidney were mixed with the Lichroprep silica, with fat homogenates additionally being mixed with DMSO prior to extraction. An analytical scale of the HPLC method was used for determination of the metabolite profile, with a preparative scale of the same method being used for isolation of the metabolites from the liver of goats from the high dose group. Liver was chosen as it contained the highest amount of total radioactive residue, as well as the greatest number of residue components. The isolated metabolites were further purified using thin layer chromatography and a second HPLC-UV-radio-detection method with fraction collection using 5 g/L ammonium bicarbonate in water and methanol as the mobile phases. Metabolites were identified using a combination of comparison of the HPLC chromatograms with authentic compounds, ^{1}H NMR, and mass spectrometry.

The extraction efficiencies of the on-line HPLC method for the matrices are presented in Table 12 below, together with the composition of the residue in each matrix.

The column extraction methods were effective, with 92% of TRR extracted from liver, 94% from milk, 103% from kidney, 110% from muscle and 121% from fat. The high figures for muscle and fat were attributed to uncertainties in the measurement of small amounts of radioactivity in large extraction volumes.

2,6-Dichlorobenzamide was present in all matrices and was the largest component of the residue in all matrices except liver. Metabolism was most extensive in liver, with three sulphur containing compounds identified, which appear to be degradates of a cysteine conjugate of 2,6-dichlorobenzamide. The largest component of the residue in liver was 6-chloro-3-hydroxy-2-thiobenzamide, present at 6.6 mg eq./kg, or 70% of the TRR. The dichlobenil-cysteine conjugate metabolites 6-chloro-3-hydroxy-thiobenzamide, 6-chloro-3-hydroxy-2-methylthiobenzamide, and 6-chloro-3-hydroxy-2-methylsulfinylbenzamide were found in varying proportions in all matrices. Small amounts of a metabolite found only in muscle and fat were postulated to be dichlobenil on the

basis of matching chromatographic retention times with a standard. However, this assignment should be regarded as tentative, as no structural confirmation was carried out using mass spectrometry or nuclear magnetic resonance.

Cysteine conjugation, together with hydroxylation at the 3-position, followed by cleavage of the cysteine side chain and oxidation of the sulphur therefore forms the main metabolic pathway for 2,6-dichlorobenzamide in goats.

Table 12 Identification of the residues in matrices of goat dosed with 13 ppm 2,6-dichlorobenzamide

Component	Liver		Kidney		Muscle		Fat		Milk (72 h)	
	%TRR	mg eq./kg	%TRR	mg eq./kg		mg eq./kg		mg eq./kg		mg eq./kg
2,6-Dichlorobenzamide	0.98	0.09	63	0.75	77	0.19	40	0.009	80	0.038
Unknown	2.2	0.21	-	-	2.5	0.006	-	-	-	-
6-Chloro-3-hydroxy-2-thiobenzamide	70	6.6	21	0.25	-	-	1.6	< 0.001	13	0.006
6-Chloro-3-hydroxy-2-methylthiobenzamide	17	1.6	19	0.23	-	-	-	-	-	-
6-Chloro-3-hydroxy-2-methylsulfinylbenzamide	1.7	0.16	-	-	4.4	0.01	1.5	< 0.001	-	-
Dichlobenil (tentative)	-	-	-	-	4.4	0.01	24	0.005	-	-
% Extracted	92	9.6	103	1.2	110	0.28	121	0.029	93	0.045
TOTAL	100	9.41	100	1.19	100	0.25	100	0.024	100	0.048

Laying hens: 2,6-dichlorobenzamide

Metabolism of ^{14}C-phenyl labelled 2,6-dichlorobenzamide was investigated in laying hens (Burke, 1994b and Ruijten *et al*., 1994b). A group of five hens was dosed with ^{14}C-2,6-dichlorobenzamide daily for 5 days at a target dose of 10 ppm in feed on a dry matter basis. A second group of five birds was given an exaggerated dose at a target level of 200 ppm in feed daily for 3 days to provide additional samples to facilitate identification of residues. Finally, a single untreated control hen was kept in order to provide material for determination of analytical method LODs and LOQs.

The hens were kept in metabolism cages during the feeding phase of the study. Fresh drinking water was available *ad libitum*, while measured quantities of feed (commercially available diet and grit) were provided each morning and afternoon, with feed consumption being monitored. The dose was administered orally using gelatin capsules. Actual dose levels ranged from 11–14 ppm (mean = 12 ppm), or 0.78–0.92 mg/kg bw/day (mean = 0.86 mg/kg bw/day) and 218–249 ppm (mean value = 236 ppm), or 15–19 mg/kg bw/day (mean value = 17 mg/kg bw/day) for the low and high dose birds respectively. No overt signs of toxicity were observed during the study, and the dosing did not have any adverse effects on the weight of the animals, feed intake, or egg production.

Excreta, and cage debris if required, were collected at 24 hour intervals, with eggs being collected twice daily and pooled for each 24 hour period. Whole eggs were pooled for the low dose birds, with yolks and white being pooled separately for the high dose birds. The cages were rinsed after each collection of excreta and the cage wash was collected. The low dose birds were sacrificed approximately 23 hours after the final dose, while the high dose birds were sacrificed around 4 hours following the final dose. Samples of skeletal muscle (composite of breast and thigh), fat (peritoneal), skin with adhering subcutaneous fat, liver, and kidney were collected. The tissue were macerated and sub-sampled prior to frozen storage, while the egg and excreta samples were homogenised and sub-sampled, the excreta samples after addition of a minimum volume of water, then stored frozen.

Samples of excreta, whole eggs, egg yolks, and tissues were analysed for total radioactivity by liquid scintillation counting (LSC), after combustion and capture of the liberated $^{14}CO_2$. Cage wash and egg whites were added directly to scintillant solutions and LSC was conducted without combustion.

In the low dose hens, 49% of the administered dose was recovered in excreta, 1.5% in cage wash, 3.7% in eggs, and 23% in tissues, with a total recovery of 77%. In the high dose birds, 15% was recovered in excreta, 1.8% in eggs and 30% in tissues (total recovery of 47%).

A good recovery of the total administered radioactive dose was achieved for the low dose birds, while recovery is poor for the high dose group. It is noted that the digestive tract and contents were not analysed, and a significant proportion of the radioactivity could be present there, especially given that sacrifice of the high dose birds took place only 4 hours after the final dose.

Table 13 Total radioactive residues in eggs

Sampling interval (hours)	TRR (mg eq./kg)		
	Low dose birds, whole eggs	High dose birds	
		Egg yolk	Egg white
0-24	0.41	8.35	6.97
24-48	1.01	13.5	10.9
48-52	NA	28.8	21.9
48-72	1.90	NA	NA
72-96	2.09	NA	NA
96-120	2.36	NA	NA

NA = not applicable.

Total radioactive residue concentrations in eggs do not appear to have reached a plateau by the end of the dosing phase, although it is noted that the rate of increase in the whole egg samples from the low dose birds is slowing after three days, indicating that the levels are approaching a plateau.

Table 14 Total radioactive residues in hen tissues

Tissue	Low dose birds, mg eq./kg	High dose birds, mg eq./kg
Fat	1.29	18.0
Skin with attached fat	1.85	25.1
Kidney	5.03	40.4
Liver	8.58	55.3
Muscle	1.93	32.9

Tissues and eggs were homogenised and uniformly mixed with Lichroprep® 25-40 µm silica, then loaded into a pre-column subjected to inline extraction and HPLC analysis using gradient elution with 50 mM ammonium acetate in distilled water adjusted to pH 3 and 50 mM ammonium acetate in methanol, with UV (254 nm) and radio detection with fraction collection. An analytical scale of the HPLC method was used for determination of the metabolite profile, with a preparative scale of the same method being used for isolation of the metabolites from liver from the high dose group of hens, which contained the largest amount of radioactive residue for characterisation. The isolated metabolites were further purified using a second HPLC-UV-radio-detection method with fraction collection using 5 g/L ammonium bicarbonate in water and methanol as the mobile phases. Metabolites were identified using a combination of comparison of the HPLC chromatograms with standard chromatograms, ^{1}H NMR, and mass spectrometry.

The extraction efficiencies of the on-line HPLC method for the matrices are tabulated below, together with the composition of the residue in each matrix. The column extraction methods were essentially quantitative, with 95% of the TRR in kidney extracted, while >99% was extracted from liver, skin, muscle, fat and eggs.

Table 15 Extraction yields and composition of the radioactive residues from hen matrices (low dose birds)

Matrix	% Recovery by extraction and HPLC	2,6-Dichlorobenzamide		Unknown	
		mg eq./kg	%TRR	mg eq./kg	%TRR
Liver	99.3	8.52	99.3	-	-
Kidney	94.6	4.76	94.6	-	-
Skin	99.4	1.84	99.4	-	-
Muscle	99.6	1.87	96.9	0.053	2.7
Fat	99.6	1.28	99.6	-	-
Egg (96 hours)	99.9	1.97	94.3	0.12	5.6

The only component of the residue identified was unchanged 2,6-dichlorobenzamide, with the exception of muscle and egg, in which a single unidentified component was found, comprising 3% and 6% of the radioactivity respectively. 2,6-Dichlorobenzamide is essentially not metabolised by hens, and is largely excreted unchanged.

Overview of metabolism of 2,6-dichlorobenzamide in animals

2,6-Dichlorobenzamide is metabolised in goats in a similar manner to dichlobenil, with the principal metabolic pathway being conjugation with cysteine (replacing one of the chlorine atoms), accompanied by hydroxylation of an adjacent position on the phenyl ring. Subsequently, the cysteine side chain is cleaved and oxidised, giving residues such as 6-chloro-3-hydroxy-2-methylthiobenzamide, 6-chloro-3-hydroxy-2-thiobenzamide, and 6-chloro-3-hydroxy-2-methylsulfinylbenzamide.

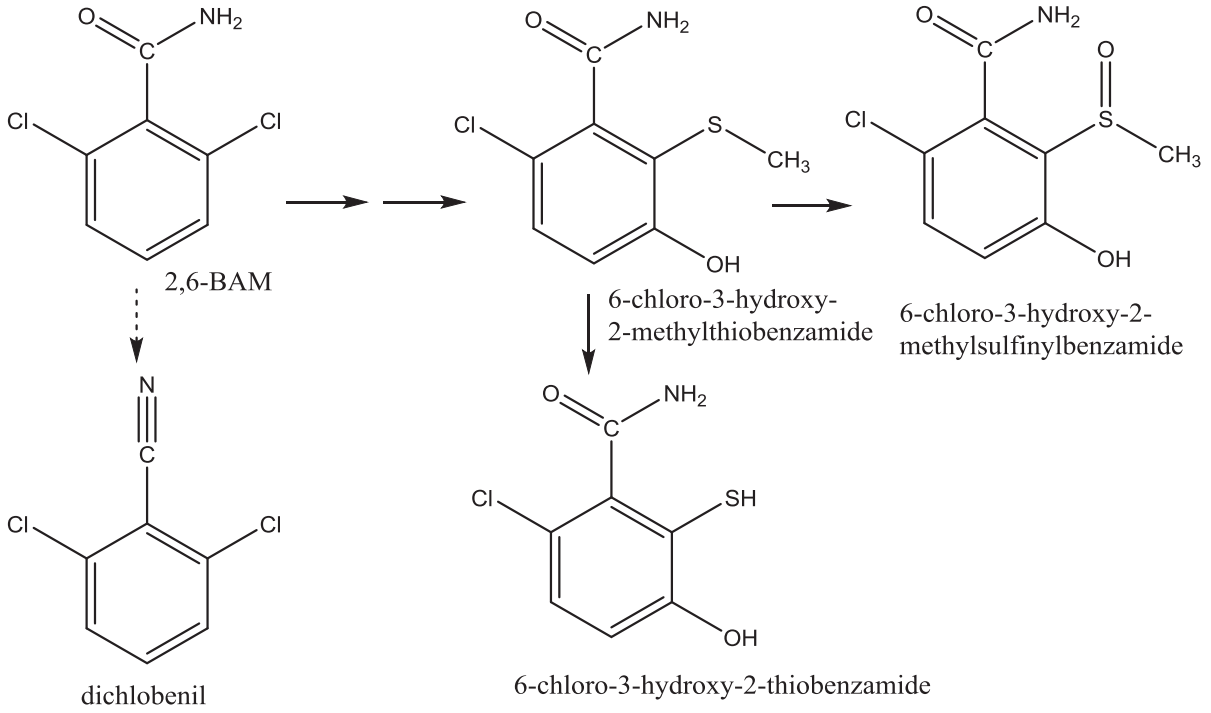


Figure 3 Metabolism of 2,6-dichlorobenzamide in goats

By contrast, 2,6-dichlorobenzamide appears to be excreted largely unchanged by laying hens, with only one other component observed (but not identified) at low levels (3-6% of the TRR) in muscle and eggs.

Plant metabolism

The Meeting received studies on the metabolism of dichlobenil in apples and grapes.

Apples

Small outdoor plots of one apple tree each (Gravenstein variety) were treated with ^{14}C-dichlobenil prepared as a liquid formulation at 6.72 kg ai/ha by a single soil application in mid-February (late dormancy; flowering was noted 20 days after application) (Hubert, 1990b). The total radioactive residues in soil and fruit samples were determined by combustion and liquid scintillation counting (LSC) analysis. Homogenised apple was extracted with methanol. Radioactivity in the post-extraction solids was quantified by combustion and LSC. The methanol extract was concentrated and the resulting solid residue filtered out and rinsed then sonicated with methanol. Residues in the methanol extract were further characterised using separate partitions of portions of the extract with ether and toluene. Radioactivity in the various methanol extracts and ether and toluene partitions were analysed by LSC, with residues in the extracts containing sufficient residue (the ether and toluene partitions) being identified with the aid of reference standards using HPLC-UV with fraction collection and LSC, and TLC.

Table 16 Total radioactive residues in apple and soil samples

DALA	Matrix	TRR (mg eq./kg)
-0	Soil 0-7.6 cm	ND
	Soil 7.6-15.2 cm	ND
0	Soil 0-7.6 cm	1.292
	Soil 7.6-15.2 cm	ND
30	Soil 0-7.6 cm	0.494
	Soil 7.6-15.2 cm	ND
77	Soil 0-7.6 cm	0.486
	Soil 7.6-15.2 cm	0.098
107	Soil 0-7.6 cm	0.746
	Soil 7.6-15.2 cm	0.166
137	Soil 0-7.6 cm	0.601
	Soil 7.6-15.2 cm	0.166
77	Fruit	0.012
107	Fruit	0.028
137	Fruit	0.042

The greater amount of the soil residue was found in the upper sections of the cores, consistent with the volatility of dichlobenil. Total residues in mature fruit were low, at 0.042 mg eq./kg. Most of the residue was extracted with methanol, at 81% of the TRR (0.034 mg eq./kg). Around 86% of the methanol extracted radioactivity partitioned into ether, while around 73% partitioned into toluene.

Table 17 Characterisation and identification of residues in apples collected at normal harvest (using the results from the ether partition)

Component/extract	Residue	
	mg eq./kg	%TRR
2,6-Dichlorobenzamide	0.024	56.5
Unidentified residue components in the ether partition	< 0.01 (0.0055)	13.2
Aqueous partition	< 0.01 (0.0057)	16.7
Unextracted residue	< 0.01 (0.0045)	10.7
Sum of identified and extracted/characterised residues	0.039	97.1
Total radioactive residue	0.042	100

The only metabolite identified in apples was 2,6-dichlorobenzamide, at 0.024 mg eq./kg, or 57% of the TRR. Other unidentified components were present at < 0.01 mg eq./kg.

Grapes

A single soil application of ^{14}C-dichlobenil was made to small plots (two plants per plot) of outdoor grown Emperor variety grape vines in mid-February (late dormancy) using a liquid formulation at 6.72 kg ai/ha (Hubert, 1990a). Soil and fruit samples were frozen and homogenised and the total radioactive residues determined by combustion radio-analysis. Homogenised grape samples were

extracted four times with methanol and the extracts combined. The methanol extract was reduced to an aqueous residue and partitioned with dichloromethane. HPLC and TLC analyses were conducted on the aqueous and organic phases. Further characterisation of the aqueous phase was carried out by acid and enzyme (β-glucosidase) hydrolysis followed by LSC analysis.

Table 18 Total radioactive residues in grape and soil samples

DALA	Matrix	TRR (mg eq./kg)
0	Soil 0-7.6 cm	7.924
	Soil 7.6-15.2 cm	ND
61	Soil 0-7.6 cm	1.838
	Soil 7.6-15.2 cm	0.210
159	Soil 0-7.6 cm	0.160
	Soil 7.6-15.2 cm	0.616
190	Soil 0-7.6 cm	0.389
	Soil 7.6-15.2 cm	0.139
222	Soil 0-7.6 cm	0.507
	Soil 7.6-15.2 cm	0.216
159	Immature fruit	0.322
190	Immature fruit	0.286
222	Mature fruit	0.357, 0.392*

*A second combustion radio-analysis of the mature grapes was conducted at the time of extraction and characterisation of the residues, and the %TRRs are based on this second, higher figure.

Nearly all the residue in mature grapes (99% or 0.39 mg eq./kg) was extractable with methanol, with only 7.1% (0.03 mg eq./kg) remaining in the post-extraction solids (PES). Due to the low residue levels, no further characterisation was conducted for the PES. Around 81-85% of the total residue was found in dichloromethane after the initial neutral partition, with a much smaller amount (1.8%) found in dichloromethane after the second partition under acidic conditions.

Table 19 Characterisation and identification of residues in mature grapes

Component/extract	Residue	
	mg eq./kg	%TRR
2,6-Dichlorobenzamide	0.34	86.7
2,6-Dichloro-4-hydroxybenzamide	< 0.01	2.1
Unidentified residue components	0.06	15.3
Unextracted residue	0.03	7.2
Sum of identified and characterised residues	0.43	109.7
Total radioactive residue	0.39	100

The major component of the residue in grapes was 2,6-dichlorobenzamide, which was found at 0.34 mg eq./kg (86.7% of the TRR), when summing the amounts found in the neutral dichloromethane partition and the amounts tentatively identified in other fractions. A trace amount (< 0.01 mg eq./kg) of 2,6-dichloro-4-hydroxybenzamide was tentatively identified in the aqueous partition after acid hydrolysis of the initial aqueous partition.

Plant metabolism summary

The metabolism of dichlobenil in fruit crops is straightforward, with the only significant transformation being hydrolysis to give 2,6-dichlorobenzamide, which comprised 57% of the TRR in apples and 87% of the TRR in grapes. A very minor component of the residue in grapes was 2,6-dichloro-4-hydroxybenzamide, indicating some hydroxylation of the phenyl ring was taking place. 2,6-Dichlorobenzamide is a significant environmental metabolite, indicating that the transformation of the nitrile to the amide may be taking place in soil, with the 2,6-dichlorobenzamide then being taken up by the roots, rather than the hydrolysis taking place within the plant. However, this cannot be confirmed without further data, such as plant metabolism studies for 2,6-dichlorobenzamide itself.

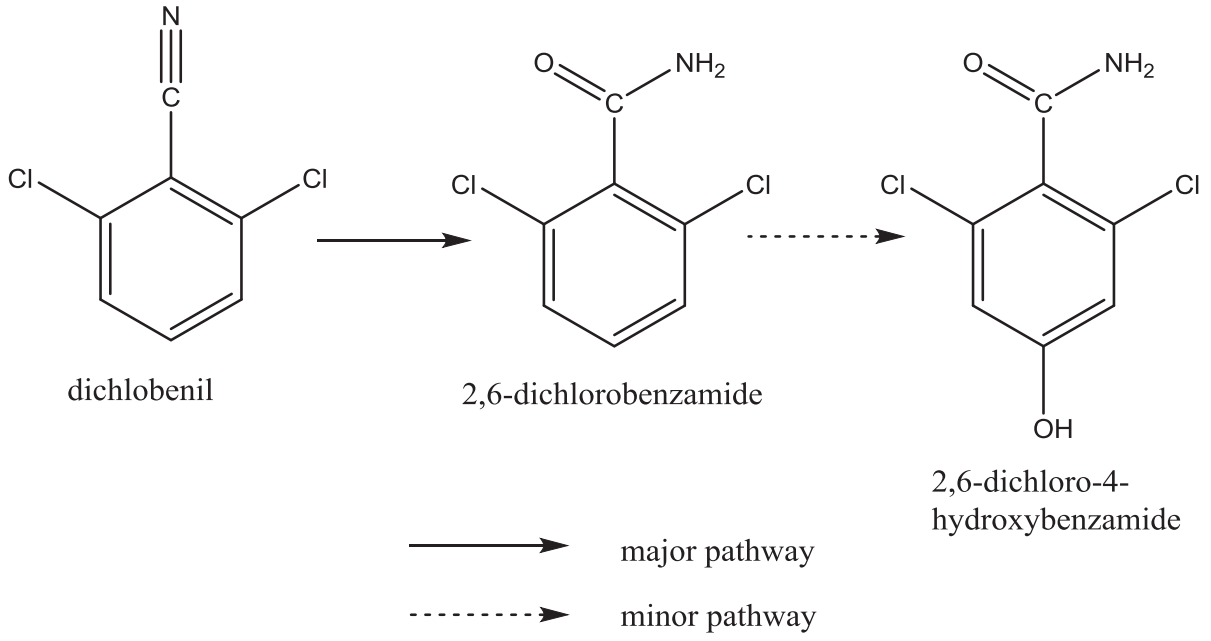


Figure 4 Metabolism of dichlobenil in fruit crops

Environmental fate

Data on aerobic soil metabolism, aqueous photolysis, soil surface photolysis, and aerobic and anaerobic hydrolysis of dichlobenil, and hydrolysis and aqueous photolysis of the soil and plant metabolite 2,6-dichlorobenzamide were supplied.

Based on the environmental data requirements in the 2009 FAO Manual and considering the use pattern of dichlobenil (a soil applied herbicide), the soil metabolism, soil surface photolysis and hydrolysis studies are considered pertinent to the compound. Soil studies are evaluated below, while the results of the hydrolysis studies are summarised in Tables 1a and 1b above.

Aerobic soil metabolism: dichlobenil

Sandy loam soil samples were spiked with ^{14}C-dichlobenil at 2.1 mg/kg (Walstra *et al*., 1989) and incubated in the dark at 24 °C for intervals up to 50 weeks. The biometer sample flasks were equipped with a trap filled with dilute aqueous potassium hydroxide for capturing CO_2 and other volatiles.

Soil samples were extracted twice by homogenising with methanol and twice by refluxing with methanol. The combined extracts were analysed by LSC and HPLC, with the unextracted residues determined by combustion and LSC. Radioactivity in the KOH traps was determined by LSC both before and after partition with dichloromethane. Some of the dichloromethane partitions were also analysed by HPLC.

The composition of the residues extractable from soil and captured in the traps is tabulated below.

Table 20 Degradation of ^{14}C-Dichlobenil in sandy loam soil under aerobic conditions

Sampling interval (weeks)	Volatiles (%TAR)			Extracted (%TAR)				Bound (%TAR)	Total Recovery (%TAR)
	Total	$^{14}CO_2$	dichlobenil	Total	dichlobenil	2,6-dichloro-benzamide	Other metabolites		
0	-	-	-	101	101	< 0.1	< 0.1	< 0.2	101
2	14.8	0.1	13.3	80.7	80.3	0.4	< 0.1	2.2	97.8
5	19.0	0.7	18.0	74.2	71.7	0.8	0.5	5.1	98.2
10	39.2	0.7	37.2	52.9	49.8	1.3	< 0.1	5.8	97.8
20	46.0	1.9	41.9	44.4	35.6	7.3	1.2	8.2	98.5

Sampling interval (weeks)	Volatiles (%TAR)			Extracted (%TAR)				Bound (%TAR)	Total Recovery (%TAR)
	Total	$^{14}CO_2$	dichlobenil	Total	dichlobenil	2,6-dichloro-benzamide	Other metabolites		
30	63.9	1.5	61.0	26.8	20.6	5.2	< 0.6	8.2	98.9
40	62.3	2.7	58.5	26.1	16.2	8.4	0.5	8.0	96.4
50	61.0	2.3	56.8	26.7	11.6	13.1	0.7	10.2	97.9

Over half the applied dichlobenil was observed to dissipate via evaporation due to the high volatility of dichlobenil, mostly as parent compound (up to 61% of the TAR and up to 96% of the volatilised residue), with a small amount (≤2.7% of the TAR) being recovered as $^{14}CO_2$. At 2 weeks incubation, over 99% of the radioactivity extractable from soil was parent compound together with a trace (0.5% of the extractable soil residues) of 2,6-dichlorobenzamide. At 50 weeks, the proportion of dichlobenil had declined to 45% of the soil extractable residues, while 2,6-dichlorobenzamide had risen to 50%. Smaller amounts of the applied radioactivity were bound to the soil (2.2% of the TRR after 2 weeks, rising to 10.2% at 50 weeks).

The calculated DT_{50} for dichlobenil in sandy loam soil is 13 weeks. Most of the loss of dichlobenil in soil is due to volatilisation rather than metabolism.

A second laboratory study of the aerobic degradation of soil (Nimmo, 1985) considered the fate of phenyl ^{14}C-dichlobenil in several soil types, with both sterilised and unsterilised samples. ^{14}C-Phenyl-dichlobenil was uniformly mixed into soil at a target of 1.8 mg/kg. The samples were stored in stoppered Erlenmeyer flasks in the dark at 20 °C. To determine the effect of soil microbes on degradation, some soil samples were sterilised in an autoclave, with the fortification solution being micro-filtered and added to the soil under aseptic conditions. Samples were extracted using ethanol/water, initially at room temperature and then under reflux, and the extracts analysed by LSC. The extracts were isotopically diluted with dichlobenil, 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid , the components separated by recrystallization then analysed by LSC and TLC.

Degradation of dichlobenil, and formation of the metabolites 2,6-dichlorobenzamide and 2,6-dichlorobenzoic acid is shown in the table below for the unsterilised soil samples. Only trace amounts of 2,6-dichlorobenzoic acid were observed (maximum of 0.4% of applied dose), while 2,6-dichlorobenzamide was a much more significant metabolite at up to 57% of the applied dose. The half lives of dichlobenil in the six soil types tested ranged from 4 to 30 weeks.

Table 21 Degradation of dichlobenil in unsterilized soils (percentages of applied dose)

Incubation time (weeks)	Compound	Light clay (pH 6.3, OM 5.9%)	Clay loam (pH 5.6, OM 1.9%)	Silty clay loam (pH 6.7, OM 3.3%)	Sandy peat (pH 5.1, OM 24.4%)	Sandy clay (pH 7.5, OM 1.3%)	Calcareous clay (pH 7.8, OM 1.2%)
0	Parent	94	101	93	95	97	100
2-3	Parent	84	79	70	86	54	91
6-7	Parent	75	65	33	62	28	71
	BAM	-	-	57	-	-	27
	DCBA	-	-	-	-	-	0.2
12-14	Parent	60	39	21	59	-	46
	BAM	31	-	-	41	-	48
	DCBA	0.4	-	-	0.4	-	-
18	Parent	51	-	13	41	-	28
	BAM	-	-	-	-	-	-
	DCBA	-	-	0.4	-	-	-
26	Parent	43	-	-	31	-	-
36	Parent	-	-	-	-	-	19
52	Parent	29	-	-	21	-	-

BAM = 2,6-Dichlorobenzamide. DCBA = 2,6-dichlorobenzoic acid.

Degradation of dichlobenil in the sterilised soil samples was essentially zero, strongly suggesting that soil microbes were largely responsible for the degradation of dichlobenil.

Table 22 DT_{50} values (by soil type)

Soil type	DT_{50} (weeks)
Light clay	30
Clay loam	11
Silty clay loam	6.2
Sandy peat	24
Sandy clay	3.8
Calcareous clay	14

This study is not considered fully representative of the expected fate of dichlobenil in soil under field conditions as the samples were stored in stoppered flasks, preventing losses of parent by volatilisation which was the major route of dissipation in the first study (Walstra *et al*., 1989).

Soil surface photolysis: dichlobenil

The photolytic fate of ^{14}C-phenyl dichlobenil (Kabler and Carpenter, 1990) coated on irradiated soil was studied. Soil samples (sandy loam) were fortified with radio-labelled dichlobenil at a concentration of 10 mg/kg and placed in glass photolysis chambers equipped with water cooled jacketing, traps to capture volatiles, and a constant flow through of CO_2 free water saturated air was maintained. The samples were constantly irradiated using a xenon arc UV lamp at an intensity that resulted in irradiation over a 24-hour period equivalent to a solar day at the spring equinox at 40° N latitude. A number of samples were not irradiated and were wrapped with aluminium foil in order to act as dark controls. Samples of soil were analysed at intervals from 0 to 30 days.

Soil samples were extracted with methanol and the extracts and trapping solutions were analysed using LSC and HPLC. Radioactivity remaining in the extracted soil samples was determined by combustion and LSC.

For the irradiated samples, a DT_{50} of 50 days was calculated, while for the dark control samples, a much shorter DT_{50} of 4.7 days was determined.

Given that the calculated DT_{50} for the irradiated samples were much longer than those for the dark control samples, this study is not considered to be reliable and no conclusion on aqueous photolysis of dichlobenil on the soil surface can be reached.

Aerobic soil metabolism: 2,6-dichlorobenzamide

Studies on metabolism of 2,6-dichlorobenzamide in soil were not provided. However, the EFSA evaluation of dichlobenil reported in-field DT_{50} values of 73–257 days for 2,6-dichlorobenzamide calculated from field dissipation studies in fluopicolide, of which 2,6-dichlorobenzamide is also a metabolite (European Food Safety Authority, 2010). Most losses were through leaching. DT_{50} values of 186-261 days were reported for laboratory experiments with 2,6-dichlorobenzamide in Finnish topsoil samples (Pukkila and Kontro, 2014).

Environmental metabolism summary

Dichlobenil is lost from soil in significant quantities by volatilisation, and is also metabolised in soils, mainly to 2,6-dichlorobenzamide. A laboratory DT_{50} value of 13 weeks was determined for sandy loam soil. Dichlobenil was stable to hydrolysis in buffer solutions.

The key plant and soil metabolite of dichlobenil, 2,6-dichlorobenzamide, was stable to hydrolysis. It is more persistent in soil than dichlobenil, with laboratory DT_{50} values of up to 261 days.

Field dissipation studies for dichlobenil and 2,6-dichlorobenzamide were not available to the Meeting.

Residues in succeeding crops

Uses considered are only for permanent crops and no studies on residues of dichlobenil in rotational crops were made available to the Meeting.

RESIDUE ANALYSIS

Methods of residue analysis

Details of validated analytical methods were supplied for the determination of dichlobenil in plant and animal matrices.

Plant matrices

A GC-ECD method (method number L 3-53-71) was developed and validated for determination of 2,6-dichlorobenzamide in berry fruits, stone fruits and hazelnuts (Ver Hey, 1990). Samples were homogenised with anhydrous sodium sulphate and filter pulp, then extracted with ethyl acetate. An aliquot of the ethyl acetate extract was filtered, evaporated to dryness, and reconstituted in 1:9 v/v acetone/petroleum ether, and cleaned up by solid phase extraction using neutral alumina. The residue was eluted from the column using 3:17 v/v ethanol/petroleum ether, evaporated to dryness, and reconstituted in ethyl acetate, before analysis by GC-ECD. An additional cleanup by partition between acetonitrile and hexane, with the hexane phase being discarded, was incorporated prior to the solid phase extraction step for hazelnut samples to remove oily contaminants.

Quantification was achieved by linear regression using the external standard method. The method linearity was good, with an r^2 value of 0.9978 over a concentration range 1–100 µg/L, or 0.008-0.8 mg/kg in samples (depending on the dilution factor). The method LOQ is 0.01 mg/kg. Method recoveries and precision were generally good, with all recoveries except for those in hazelnuts and prunes in the range 70–120%, and RSD values all <20%.

Table 23 Recovery of 2,6-dichlorobenzamide from fruit and nut matrices after GC-ECD analysis (Ver Hey, 1990)

Matrix	Fortification (mg/kg)	Recovery (%)
Blackberries	0.01	87.0
	0.05	95.8
	0.50	96.2
Blueberries	0.01	94.9
	0.05	90.4
	0.50	94.1
Cranberries	0.01	100.9
	0.05	90.5
	0.50	93.5
Hazelnuts	0.01	64.3
	0.05	58.6
	0.50	68.7
Plums	0.01	103.2
	0.05	97.8
	0.50	93.9
Peaches	0.01	92.2
	0.05	96.4
	0.50	98.2
Raspberries	0.01	104.0
	0.05	97.7
	0.50	98.0

Table 24 Recovery of 2,6-dichlorobenzamide from fruit and nut matrices after GC-ECD analysis using method L-3-53-71 (recoveries from residue trials)

Matrix	Fortification (mg/kg)	Recovery (%)	Reference
Grapes	0.01	92. 93	Chickering, 1991
	0.03	101, 106	
	0.10	94, 96	
	0.30	97, 98	
	1.0	88, 93	
Grape juice	0.01	97	Ver Hey, 1992d
	0.03	86	
	0.05	88	
	0.50	81	
Grape wet pomace	0.01	106	
	0.03	81	
	0.05	91	
	0.50	89	
Grape dry pomace	0.01	117	
	0.03	93	
	0.05	91	
	0.50	93	
Raisins	0.01	104	
	0.03	84	
	0.05	98	
	0.50	91	
Raisin waste	0.05	101	
	0.10	99	
	0.30	98	
	0.50	96	
Plums	0.01	103	Hamilton, 1993
	0.05	88	
	0.50	91	
Prunes	0.01	63	
	0.03	68	
	0.05	74	
	0.50	82	
Raspberries	0.01	91.9, 94.0	Dykstra, 1991c
	0.10	68.9, 95.8	
	0.50	84.0, 97.7	

A second method (study number 2005-046) using GC-ECD and GC-MS/MS as alternative analytical techniques was validated for analysis of both dichlobenil and 2,6-dichlorobenzamide in grapes (Bacher, 2005). Separate extractions and analyses were conducted for the two analytes.

For determination of dichlobenil, grape samples were macerated with anhydrous sodium sulphate and 1:4 v/v ethyl acetate/hexane, then filtered. The extraction was repeated twice more and the extracts were filtered and combined. To an aliquot of the combined extracts, a small amount of 1% decanol in acetone was added, prior to concentration by rotary evaporation using a water bath at 40 °C. Hexane was added, and the volume was reduced again before addition of a second volume of hexane and further evaporation prior to transfer to a sample tube and making up to volume with hexane. The extract was then cleaned up by solid phase extraction using 5% deactivated neutral alumina. The column was rinsed with hexane, with these eluates being discarded, prior to elution of the residue with 2% acetone in hexane. The eluted residue was transferred to an evaporation flask, and a small amount of 1% decanol in acetone was added before concentration by rotary evaporation at 40 °C, prior to adjustment of the extract to volume with hexane. The cleaned up extract was analysed by GC-MS/MS (detection transition was 171 → 136 for dichlobenil, and 173 → 145 for 2,6-dichlorobenzamide), or GC-ECD.

For determination of 2,6-dichlorobenzamide, samples were macerated with ethyl acetate, anhydrous sodium sulphate, and filter pulp. After centrifuging and filtration, an aliquot of the extract

was evaporated to dryness by rotary evaporation followed by a gentle stream of nitrogen, and reconstituted in 1:9 v/v acetone/petroleum ether. The extract was cleaned up by solid phase extraction with 2% deactivated alumina. After loading the extract onto the column, the flask was rinsed with 1:9 v/v acetone/petroleum ether and the rinses added to the column. The initial eluates were discarded, and the 2,6-dichlorobenzamide residues were eluted with 3:17 v/v ethanol/petroleum ether, evaporated to dryness by rotary evaporation at 40 °C followed by a gentle stream of nitrogen, then re-dissolved in ethyl acetate. The cleaned up extract was analysed by GC-MS/MS or GC-ECD.

The method LOQ is 0.01 mg/kg for both analytes. The method linearity was good, with r^2 values >0.99 for both analytes using GC-MS/MS. Linearity was less good for GC-ECD, although r^2 values were >0.98. Method accuracy and precision in grapes were good, with recoveries all in the range 70–120%, and RSD values all <20%.

Table 25 Recovery of dichlobenil and 2,6-dichlorobenzamide from grapes (method 2005-046)

Analyte	Fortification (mg/kg)	n	Recovery, GC-MS/MS determination (%)		Recovery, GC-ECD determination (%)	
			Range	Mean ± RSD	Range	Mean ± RSD
Dichlobenil	0.01	5	86-110	97 ± 8.9	89-109	100 ± 8.2
	0.10	5	82-102	91 ± 9.1	82-98	89 ± 6.5
2,6-Dichlorobenzamide	0.01	5	106-111	109 ± 1.9	103-109	106 ± 2.0
	0.10	5	91-104	98 ± 5.0	95-105	102 ± 3.9

Table 26 Method validation recovery data for method 2005-046 when used for determination of dichlobenil and 2,6-dichlorobenzamide in raw grapes and processed grape commodities (Maselli, 1998)

Matrix	Analyte	Fortification level	Recoveries (%)
Method validation recoveries			
Grapes	Dichlobenil	0.02	70, 76, 84, 88, 90, 90 (mean = 83, RSD = 8.5%)
		0.10	85, 86, 86, 90, 92, 93 (mean = 89, RSD = 0.7%)
		0.20	80, 83, 84, 92, 96, 97 (mean = 89, RSD = 2.3%)
	2,6-Dichlorobenzamide	0.003	89, 99, 101, 106, 111, 118 (mean = 104, RSD = 6.2%)
		0.03	87, 95, 97, 98, 98, 102 (mean = 96, RSD = 5.5%)
		0.30	91, 92, 93, 93, 98, 99 (mean = 94, RSD = 1.1%)
Raisins	Dichlobenil	0.02	76, 77, 79, 79, 86, 89 (mean = 81, RSD = 1.9%)
		0.10	65, 70, 73, 75, 75, 75 (mean = 72, RSD = 5.6%)
		0.20	68, 70, 73, 75, 77, 78 (mean = 74, RSD = 3.4%)
	2,6-Dichlorobenzamide	0.03	83, 87, 87, 89, 89, 90 (mean = 88, RSD = 2.6%)
		0.15	95, 101, 101, 103, 103, 105 (mean = 101, RSD = 3.4%)
		0.30	96, 99, 99, 99, 99, 100 (mean = 99, RSD = 1.8%)
Grape juice	Dichlobenil	0.02	81, 81, 86, 86, 87, 89 (mean = 85, RSD = 3.4%)
		0.10	87, 90, 92, 92, 93, 93 (mean = 92, RSD = 2.8)
		0.20	88, 89, 92, 92, 93, 94 (mean = 91, RSD = 2.3%)
	2,6-Dichlorobenzamide	0.003	137, 138, 142, 146, 147, 147 (mean = 143, RSD = 1.9%)
		0.03	107, 108, 109, 112, 112, 113 (mean = 111, RSD = 0.9%)
		0.30	98, 99, 101, 101, 102, 110 (mean = 102, RSD = 1.5%)

Table 27 Concurrent and method validation recovery data for method based on number 2005-046 for determination of dichlobenil and 2,6-dichlorobenzamide in cherries

Matrix	Analyte	Fortification level	Recoveries	Reference
Concurrent fortification recoveries				
Sour cherry	Dichlobenil	0.08	70, 72, 92, 97 (mean = 83, RSD = 15%)	Maselli, 1996
		0.20	82, 95, 97, 101 (mean = 94, RSD = 8.7%)	
	2,6-Dichlorobenzamide	0.02	98, 99, 102, 104 (mean = 101, RSD = 2.1%)	
		0.20	90, 100, 100, 104 (mean = 99, RSD = 5.9%)	
Sweet cherry	Dichlobenil	0.08	77, 81, 84, 88, 90 (mean = 84, RSD = 4.2%)	Gaydosh, 1996
		0.20	81, 85, 87, 88, 91 (mean = 86, RSD = 3.5%)	
	2,6-Dichlorobenzamide	0.02	97, 100, 100, 120 (mean = 104, RSD = 1.7%)	
		0.20	97, 100, 103, 106 (mean = 102, RSD = 3.0%)	
Method validation recoveries				
Sweet cherries	Dichlobenil	0.02	84, 90, 90 (mean = 88, RSD = 3.9%)	Gaydosh, 1996
		0.04	88, 90, 94 (mean = 91, RSD = 3.4%)	
		0.20	82, 87, 89 (mean = 86, RSD = 4.2%)	
	2,6-Dichlorobenzamide	0.003	110, 111, 111 (mean = 111, RSD = 0.5%)	
		0.03	101, 111, 116 (mean = 109, RSD = 7.0%)	
		0.30	97, 97, 113 (mean = 102, RSD = 9.0%)	

Table 28 Concurrent and method validation recoveries for modified method 2005-046 for determination of dichlobenil and 2,6-dichlorobenzamide in berry fruits (Reibach, 2013)

Matrix	Analyte	Fortification level (mg/kg)	Recoveries (%)
Concurrent recoveries			
Raspberry	Dichlobenil	0.01	113, 186*
		0.20	75, 94
	2,6-Dichlorobenzamide	0.01	80, 111
		0.20	75, 90
Blueberry	Dichlobenil	0.01	82, 92
		0.10	88
		0.20	87
	2,6-Dichlorobenzamide	0.01	80, 127, 127
		0.20	48*, 95, 101, 118
Blackberry	Dichlobenil	0.01	76, 112
		0.20	95, 100
	2,6-Dichlorobenzamide	0.01	83, 118, 120
		0.20	81, 95, 102
Method validation recoveries			
Raspberry	Dichlobenil	0.01	99, 104, 106
		0.20	78, 90, 101
	2,6-Dichlorobenzamide	0.01	95, 111, 135
		0.20	77, 96, 103
Blueberry	Dichlobenil	0.01	91, 102, 102
		0.20	74, 81, 82
	2,6-Dichlorobenzamide	0.01	107, 108, 109
		0.20	86, 88, 91
Blackberry	Dichlobenil	0.01	86, 94, 96
		0.20	78, 84, 88
	2,6-Dichlorobenzamide	0.01	75, 79, 89
		0.20	94, 95, 96

*Rejected due to fortification error.

An independent validation of the method was conducted for both metabolites with no significant modifications to the extraction procedures, with analyses using both GC-ECD and GC-MS rather than GC-MS/MS (Wolf, 2006). Good recoveries were achieved in grapes (70–120%), with an LOQ of 0.01 mg/kg being confirmed for both analytes.

Animal matrices

A GC-MS method (96045) was developed and validated for determination of both dichlobenil and 2,6-dichlorobenzamide in animal matrices (Batorewicz, 1997).

Fat samples were extracted twice by homogenisation with hexane. The hexane extracts were then cleaned up by partitioning twice with acetonitrile, with the hexane being discarded and the acetonitrile phases combined and concentrated by rotary evaporation before addition of the internal standard, adjustment to volume and analysis.

Kidney, muscle and liver samples were homogenised with water and extracted twice with ethyl acetate. An aliquot of the combined ethyl acetate supernatants was evaporated to dryness and reconstituted in hexane. The hexane extract was then partitioned twice with acetonitrile, with the hexane being discarded and the combined acetonitrile extracts being washed with hexane. The combined acetonitrile extracts were evaporated to about 1 mL by rotary evaporation at 30-40 °C followed by a gentle stream of nitrogen. The internal standard was added and the sample adjusted to volume.

Milk samples were homogenised and then extracted twice with ethyl acetate. Combined aliquots of the ethyl acetate supernatants were evaporated, then reconstituted in hexane. The extract was then cleaned up by solid phase extraction (deactivated Florisil column). The residue was eluted with 1:1 v/v hexane/ethyl acetate and concentrated. The internal standard was added and the extract adjusted to volume.

Samples were analysed by GC-MS using 4-chlorobenzonitrile as an internal standard. Quantification was achieved by linear regression. The method LOQ is 0.01 mg/kg. Method linearity was good, with r^2 values >0.99 for dichlobenil and 2,6-dichlorobenzamide.

Table 29 Recovery of dichlobenil and 2,6-dichlorobenzamide from animal matrices

Analyte	Matrix	Fortification (mg/kg)	n	Recovery (%)	
				Range	Mean ± RSD
Dichlobenil m/z = 136, 171	Beef fat	0.01	3	73-87	79 ± 8.8
		0.05	3	67-74	71 ± 4.8
		0.10	3	70-74	73 ± 3.3
	Cattle kidney	0.01	3	77-96	89 ± 11
		0.05	3	62-79	72 ± 13
		0.10	3	86-90	88 ± 2.4
	Cattle liver	0.01	3	90-98	95 ± 4.2
		0.05	3	90-96	93 ± 3.0
		0.10	3	89-94	91 ± 2.6
	Beef muscle	0.01	3	81-89	84 ± 4.4
		0.05	3	72-84	79 ± 8.2
		0.10	3	80-82	81 ± 0.9
	Milk	0.01	3	65-76	72 ± 8.7
		0.05	3	79-85	82 ± 3.4
		0.10	3	80-83	82 ± 1.7
2,6-Dichlorobenzamide m/z = 173, 189	Beef fat	0.01	3	68-81	76 ± 8.7
		0.05	3	73-83	78 ± 6.3
		0.10	3	70-84	76 ± 9.4
	Cattle kidney	0.01	3	111-128	121 ± 7.2
		0.05	3	97-106	102 ± 4.6
		0.10	3	103-128	112 ± 13
	Cattle liver	0.01	3	92-110	98 ± 10
		0.05	3	75-87	81 ± 7.8
		0.10	3	111-122	115 ± 5.1
	Beef muscle	0.01	3	103-105	104 ± 1.0

Analyte	Matrix	Fortification (mg/kg)	n	Recovery (%)	
				Range	Mean ± RSD
		0.05	3	84-100	93 ± 9.0
		0.10	3	102-121	110 ± 9.0
	Milk	0.01	2	84-108	96
		0.05	3	88-97	92 ± 5.0
		0.10	3	91-112	104 ± 11

Multiresidue methods were not provided by the sponsor.

The USA FDA official multiresidue method PAM I has been successfully validated (complete recoveries, i.e. >80%) (US FDA, 1999). For dichlobenil parent compound this involved using Method 302 (E1-E3, DG1-19), corresponding to acetone extraction followed by solvent partition or removal of the aqueous component using diatomaceous earth, and GC analysis with a low temperature column (detector not specified) and Method 304 E1-E3 + C1-C4 + DG1-19 (extraction with acetone followed by solvent partition or removal of the aqueous component followed by solid phase extraction cleanup and GC analysis). Analysis of 2,6-dichlorobenzamide was successful using Method 302 (E1-E3, DG1-19).

EFSA reported good recoveries from unspecified high water content matrices for dichlobenil parent compound using a QuEChERS method (mean = 94%, RSD = 5.3%, n = 12 at 0.05 mg/kg fortification and mean recovery = 97%, RSD = 10%, n = 25 at 0.1 mg/kg fortification) (European Food Safety Authority, 2013).

Stability of pesticide residues in stored analytical samples

Plant matrices

No separate stability study was submitted for residues of diclobenil or 2,6-dichlorobenzamide in plant matrices. However, stability was verified concurrently with the analyses of the samples in the residue trials.

Table 30 Storage stability data for dichlobenil and 2,6-dichlorobenzamide in sweet cherries at 0.20 mg/kg fortification (Gaydosh, 1996)

Analyte	Storage interval (months)	Stored recovery (%)	Concurrent recovery (%)
Dichlobenil	0	-	90
	0.5	73, 92, 95 (mean = 87, RSD = 14%)	73
	1	99, 105, 113 (mean = 106, RSD = 6.6%)	87
	2	74, 88, 88 (mean = 83, 9.7%)	98
	6 (197 days)	62, 76, 77 (mean = 72, RSD = 12%)	94
2,6-Dichlorobenzamide	0	-	95
	0.5	100, 101, 101 (mean = 101, RSD = 0.6%)	101
	1	93, 94, 95 (mean = 94, RSD = 1.1%)	98
	2	94, 101, 104 (mean = 100, RSD = 5.1%)	102
	8 (246 days)	88, 90, 92 (mean = 90, RSD = 2.2%)	100

The above data for sweet cherries is sufficient to confirm that the samples from the cherry residue trials, which were stored for a maximum of 198 days (dichlobenil analyses), and 251 days (2,6-dichlorobenzamide analyses) before analysis.

Table 31 Concurrent storage stability data for 2,6-dichlorobenzamide in stone fruit and berries (1990s trials)

Matrix	Fortification (mg/kg)	Storage interval for stability samples (days)	Maximum storage interval for trial samples	Stored recovery	Reference
Blackberries	0.01	25-26	35	93, 94	Dykstra, 1991b
	0.10			88, 92	
	0.50			89, 91	
Cranberries	0.01	75-87	89	92, 100	Ver Hey, 1992c
	0.05			78, 88	
	0.50			70, 91	
Grapes	0.10	112	141	93, 93, 95	Chickering, 1991
		119		87, 88, 94	
		119		98, 98, 98	
		133		88, 89, 91	
Grape juice	0.01	0	124-163*	88	Ver Hey, 1992d
		99		96	
	0.03	0		91	
		99		81	
	0.05	0		87	
		99		80	
	0.50	0		88	
		99		82	
Wet pomace	0.01	0		98	
		108		92	
	0.03	0		92	
		108		85	
	0.05	0		90	
		108		91	
	0.50	0		90	
		108		93	
Dry pomace	0.01	111		80	
	0.03	111		96	
	0.05	111		97	
	0.50	111		98	
Raisins	0.01	0		93	
		79		75	
	0.03	0		96	
		79		69	
	0.05	0		93	
		79		56	
	0.50	0		94	
		79		56	
Plums	0.01	29	39	76	Ver Hey, 1992b
	0.05			90	
	0.50			97	
Plums	0.01	35	37	88	Hamilton, 1993
	0.05			94	
	0.50			91	
Prunes	0.01	4	35	50	
	0.03			62	
	0.05			59	
	0.50			68	
Peaches	0.01	50-54	60	95, 114, 124, 130	Dykstra, 1991a
	0.10			90, 94, 97, 99	
	0.50			84, 88, 94, 97	
Peaches	0.01	52-59	60	90, 99	Ver Hey, 1992a
	0.05			95, 94	
	0.50			83, 84	
Raspberries	0.01	7-8	49	92, 94	Dykstra, 1991c

Matrix	Fortification (mg/kg)	Storage interval for stability samples (days)	Maximum storage interval for trial samples	Stored recovery	Reference
		40-41		124, 82	
	0.10	7-8		69, 96	
		40-41		88, 90	
	0.50	7-8		84, 98	
		40-41		89, 9e	

*See also the data in Table 32 below for grapes, raisins and juice.

With the exception of prunes, the data listed in Table 31 above is sufficient to verify the stability of 2,6-dichlorobenzamide residues in relevant stone and berry fruit matrices for the period the samples were stored between collection and analysis for the appropriate residue trials.

Table 32 Residue stability data for dichlobenil and 2,6-dichlorobenzamide in raw and processed grape matrices at a 0.10 mg/kg fortification (Maselli, 1998)

Analyte	Matrix	Storage interval (months)	Stored recovery (%)	Concurrent recovery (%)
Dichlobenil	Grapes	0	-	80, 91
		1	88, 90, 94 (mean = 91, RSD = 3.4%)	87, 89
		3	84, 86, 86 (mean = 85, RSD = 1.4%)	81, 86
		6	79, 79, 82 (mean = 80, RSD = 2.2%)	96, 100
		11 (323 days)	78, 85, 85 (mean = 83, RSD = 4.9%)	88, 90
	Raisins	0	-	79, 96
		1	73, 82, 86 (mean = 80, RSD = 8.3%)	80, 84
		3	69, 70, 74 (mean = 71, RSD = 3.7%)	100, 102
		6	55, 57, 60 (mean = 57, RSD = 4.4%)	97, 98
		9 (273 days)	61, 75, 76 (mean = 71, RSD = 12%)	78, 79
	Grape juice	0	-	83, 89
		1	89, 89, 91 (mean = 90, RSD = 1.3%)	81, 82
		3	78, 81, 86 (mean = 82, RSD = 4.9%)	94, 99
		6	68, 79, 85 (mean = 77, RSD = 11%)	96, 97
		11 (322 days)	78, 82, 83 (mean = 81, RSD = 3.3%)	83, 80
2,6-Dichlorobenzamide	Grapes	0	-	100, 101
		1	90, 91, 94 (mean = 92, RSD = 2.3%)	108, 108
		3	88, 89, 93 (mean = 90, RSD = 2.9%)	99, 101
		6	82, 92, 94 (mean = 89, RSD = 7.2%)	103, 103
		11 (345 days)	95, 99, 101 (mean = 98, RSD = 3.1%)	104, 111
	Raisins	0	-	97, 100
		1	105, 110, 112 (mean = 109, RSD = 3.3%)	70, 92
		3	97, 99, 100 (mean = 99, 1.5%)	100, 102
		6	101, 105, 108 (mean	93, 94

Analyte	Matrix	Storage interval (months)	Stored recovery (%)	Concurrent recovery (%)
			=105, RSD = 3.4%)	
		9 (266 days)	97, 104, 107 (mean = 103, RSD = 5.0%)	100, 110
	Grape juice	0	-	96, 98
		1	101, 101, 102 (mean = 101, RSD = 0.6%)	103, 111
		3	89, 92, 95 (mean = 92, RSD = 3.3%)	102, 107
		6	87, 96, 97 (mean = 93, 5.9%)	105, 107
		11 (343 days)	102, 103, 105 (mean = 103, RSD = 1.5%)	91, 99

The above results show 2,6-dichlorobenzamide residues to be stable for at least 9 months of frozen storage in raisins, and for at least 11 months in raw grapes and grape juice. Residues of dichlobenil parent compound are stable for at least 11 months in raw grapes and grape juice and for 3 months in raisins, with lower recoveries being observed in raisins after 6 months of storage.

Animal matrices

Storage stability data for residues of dichlobenil and 2,6-dichlorobenzamide in cattle matrices (milk, muscle, fat, liver and kidney) was generated concurrently with the cattle feeding study (Batorewicz, 1998). Storage stability for 2,6-dichlorobenzamide residues was acceptable in all matrices over the period for which trial samples were stored, as well as for dichlobenil in muscle, milk and fat. Poor stability was noted for dichlobenil residues in kidney and particularly liver samples. However, based on the plant metabolism studies and field trial data, residues in plant commodities are expected to consist only of 2,6-dichlorobenzamide, with finite residues of dichlobenil not being expected in livestock feed. Further, as the animal metabolism studies for 2,6-dichlorobenzamide show that dichlobenil is not a significant animal metabolite of 2,6-dichlorobenzamide, parent dichlobenil is not expected to be found in animal commodities.

USE PATTERN

Dichlobenil is registered in the USA in stone fruit (cherries, both sweet and sour varieties,) and berries and other small fruits (blueberries, blackberries, cranberries, grapes and raspberries).

Table 33 Registered uses of dichlobenil in the USA relevant to the evaluation

Crop	Country	Application						PHI (days)	Comments
		Form.	Method	Spray conc. (kg ai/hL)	Volume (L/ha)	Rate (kg ai/ha)	No.		
Stone fruits									
Cherries (Sweet and Sour)	USA	CS	Ground based soil surface spray.			2-4	1	-	[1]
Cherries	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.8-6.7	1	-	[2.]
Cherries	Canada	G	Broadcast or banded soil application.	-	-	4.4-7	1	-	[3.]
Peaches	Canada	G	Broadcast or banded soil application.	-	-	4.4-7	1	-	[3]
Plums	Canada	G	Broadcast or banded soil application.	-	-	4.4-7	1	-	[3.]
Berries and other small fruits									
Blueberries	USA	CS	Ground based soil surface spray.			2-4	1	-	[4.]
Blueberries	USA	G	Soil surface application	-	-	4.5-6.7	1	-	[5.]

Crop	Country	Application						PHI (days)	Comments
		Form.	Method	Spray conc. (kg ai/hL)	Volume (L/ha)	Rate (kg ai/ha)	No.		
			followed by shallow incorporation as needed.						
Blueberries (Highbush)	Canada	G	.	-	-	7-9	1	-	6.
Blackberries	USA	CS	Ground based soil surface spray.			2-4	1	-	1.
Blackberries	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.5	1	-	2.
Cranberries	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.48	1	-	7..
Cranberries	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.5	1	-	8.
Cranberries	Canada	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.4	1	-	9.
Cranberries	Canada (BC only)	G		-	-	3.6-4.4	2	-	10.
Grapes	USA	CS	Ground based soil surface spray.			2-4	1	-	4.
Grapes	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.5-6.7	1	-	2.
Grapes	Canada	G	Soil surface application.	-	-	4.4.-7	1	-	11.
Grapes	Canada	G	Soil application.	-	-	7-9	1	-	12.
Raspberries	USA	CS	Ground based soil surface spray.			2-4	1	-	4..
Raspberries	USA	G	Soil surface application followed by shallow incorporation as needed.	-	-	4.5	1	-	2.
Raspberries	Canada	G		-	-	7	1	-	2.

Comments:

1. Spray prior to weed emergence or when emerged weeds are less than 2 inches tall. Apply from late fall through to early spring.

2. Apply as a soil surface treatment for perennial weeds in late fall (15 November to 15 February). Apply as an incorporated treatment for perennial weeds in late fall or very early spring prior to 1 May. For control of annual weeds, apply in early spring before germination of weeds or after cultivation. Incorporation or irrigation recommended..

3. Apply to well prepared weed free soil in early spring or late fall before annual weed seeds have germinated. Do not apply more than the recommended amount per season. Application in spring should be made well before bud burst.

4. Spray prior to weed emergence or when emerged weeds are less than 2 inches tall. Apply from late fall through to early spring.

5. For control of annual weeds, apply in early spring before germination of weeds or after cultivation. Incorporation or irrigation recommended.

6. Apply in the dormant period (late winter).

7.. Apply in early spring while perennial weeds are still dormant and annual weeds have not started to germinated or in late fall after crop has been harvested. Apply pre-budbreak or postharvest. Thoroughly incorporate granules into the surface through watering in.

8. Apply in early spring when perennial weeds are still dormant and annual weeds have not started to germinate or in late fall after the crop has been harvested. Thoroughly incorporate through watering in. Do not apply more than 4.5 kg ai/ha in a 12-month period.

9. Do not make a spring application after application the previous fall.

10. Apply two equal applications in early spring with an interval of 3 to 6 weeks between each application. Use the higher rate only every second year. Do not apply after flowering. Do not make fall applications

11. For seedling weeds apply this rate in early spring or fall.

12.. For perennial weeds apply this rate in the fall.

RESIDUES RESULTING FROM SUPERVISED RESIDUE TRIALS

The Meeting received information on dichlobenil supervised field residue trials for stone fruit (peaches, plums and cherries) and berries and other small fruits (blueberries, blackberries, cranberries, grapes and raspberries).

Trials were well documented with laboratory and field reports. The laboratory reports included method validation with determination of recoveries at the method LOQ and one other fortification level. Dates of sample collection and analysis were provided, allowing determination of storage intervals. At all trials, a granular formulation of dichlobenil was applied using either a hand spreader (e.g. shaker can), cyclone spinner applicator, or by a tractor-mounted granular spreader. A single plot was treated at each trial site. Plot areas and sample sizes were adequate. Samples were frozen within 6 hours of collection and stored frozen until analysis, with the exception of plums from the processing study (Hamilton, 1993) where the unprocessed fruit sample was frozen the day after collection. Generally, a single untreated control sample and duplicate or triplicate treated plot samples were collected.

In some studies, only residues of 2,6-dichlorobenzamide have been determined, while for others, both 2,6-dichlorobenzamide and dichlobenil have been tested for. Residues were not found in untreated control samples except where noted. Residues and application rates have generally been rounded to two significant figures. The tabulated results have not been corrected for recovery. When residues were not detected, they are shown as below the LOQ, and the LOQ value was used for maximum residue level determination and dietary intake assessment. Where replicate samples were analysed, results for the individual samples are reported together with the mean result, with the mean results used for maximum residue level estimation and dietary risk assessment.

The residue data is presented in the following tables:

Crop group	Commodity	Table
Stone fruits	Cherries	34
	Peach	35
	Plum	36
Berries and other small fruits	Blueberries	37
	Blackberries	38, 39
	Cranberries	40
	Grapes	41, 42
	Raspberries	43, 44

Stone fruits

Cherries

A series of residue trials was conducted in both sour and sweet varieties of cherries (Maselli, 1996 and Gaydosh, 1996 respectively). A single banded or broadcast soil application of a 4% w/w granular formulation of dichlobenil was made at a target application rate of 6.7 kg ai/ha when the fruit was at mid maturity, followed by irrigation with 0.6-1.3 cm of water (this was not required at some sites due to adequate rainfall being received shortly after application). Mature fruit was harvested at a single interval from each site, approximately 28 days after application.

Samples were analysed for residues of both dichlobenil and 2,6-dichlorobenzamide using a GC-ECD method very similar to study number 2005-046. Residues were determined on the edible portion (minus stones). Concurrent and method validation recoveries for dichlobenil and 2,6-dichlorobenzamide in sweet and sour cherries were within 70–120%. Sour cherry samples were stored frozen for up to 198 and 226 days between collection and analysis of dichlobenil and 2,6-dichlorobenzamide respectively, while sweet cherry samples were stored for up to 184 and 251 days respectively. A concurrent storage stability study was conducted for sweet cherries as part of the residue study, with mean recoveries of 72% for dichlobenil after 197 days of frozen storage, and 90%

for 2,6-dichlorobenzamide after 246 days of frozen storage. The samples are therefore unlikely to have been adversely affected by storage.

Table 34 Residues of dichlobenil in cherries (mature fruit)

Location, Trial no., Year (Variety)	Application				DAT	Residue (mg/kg)		Reference
						Dichlobenil	2,6-Dichlorobenzamide	
	Date	Growth stage	Application method	Rate (kg ai/ha)				
Hart, MI, USA, JGC-95008, 1995 (Montmorency)	14/06/95	immature fruit 0.95-1.3 cm dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Hart, MI, USA, JGC-95013, 1995 (Gold)	14/06/95	immature fruit 1.3 cm dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
Cedar, MI, USA, JGC-95009, 1995 (Montmorency)	18/06/95	immature fruit 0.95-1.3 cm dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
North Rose, NY, USA, RGC95RES05, 1995 (Mahaleb)	19/06/95	0.95 cm green-yellow fruit	banded soil	7.6	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Conklin, MI, USA, JGC-95007, 1995 (Montmorency)	13/06/95	immature fruit 1.3 cm dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Conklin, MI, USA, JGC-95012, 1995 (Sam's)	02/06/95	immature fruit 0.95-1.3 cm inch dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
Dix, IL, USA, JGC-95010, 1995 (Montmorency grafted on Mahaleb)	17/05/95	0.3 cm fruit diameter	broadcast to area under each tree	7.4	28	< 0.02 (< 0.02, < 0.02)	0.004 (0.004, 0.005)	Maselli, 1996
Paw Paw, MI, USA, JGC-95011, 1995 (Montmorency grafted on Mahaleb)	09/06/95	immature fruit 1.3 cm dia.	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Perry, UT, USA, RCP-95006, 1995 (Montmorency grafted on Mazzard rootstock)	08/07/95	orange immature fruit	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Hillsboro, OR, USA, DNJ-95106, 1995 (Montmorency)	14/06/95	immature green to yellow fruit	soil broadcast	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Maselli, 1996
Manteca, CA, USA, RCP-95002, 1995 (Bing)	21/04/95	mid maturity fruit	banded soil	7.5	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
Hughson, CA, USA, RCP-95003, 1995 (Bing)	12/04/95	small fruit	banded soil	7.6	28	< 0.02 (< 0.02, < 0.02)	0.004 (0.005, < 0.003)	Gaydosh, 1996
Zillah, WA, USA, DNJ-95102, 1995 (Bing)	26/05/95	mid maturity green fruit	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
Buena, WA, USA, DNJ-95103, 1995 (Bing)	26/05/95	mid maturity green fruit	banded soil	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
Gaston, OR, USA, DNJ-95104, 1995 (Royal Anne)	30/05/95	immature fruit 0.64-1.3 cm dia.	soil broadcast	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996
The Dalles, OR, USA, DNJ-95105, 1995 (Royal Anne)	31/05/95	immature fruit 0.64-0.85 cm dia.	soil broadcast	7.4	28	< 0.02 (< 0.02, < 0.02)	< 0.003 (< 0.003, < 0.003)	Gaydosh, 1996

No residues were found above the LOQ (0.02 mg/kg for dichlobenil and 0.003 mg/kg for 2,6-dichlorobenzamide) in the untreated control samples.

Peaches

Four residue trials were conducted in peaches in South Carolina and California during 1990 and 1991 (Dykstra, 1991a and Ver Hey, 1992a). A single banded or broadcast soil application of dichlobenil was made using a 4% granular formulation at a target application rate of 6.72 kg ai/ha. Mature fruit was sampled at varying intervals (20–68 days) after application.

Peach samples were analysed for 2,6-dichlorobenzamide residues using a GC-ECD method (method number L 3-53-71), evaluated in detail above. It is not clear whether the residues have been calculated on a whole fruit basis (including stones), or only on the edible portion. Concurrent recoveries of 2,6-dichlorobenzamide in peach samples were within 70–120%. Samples were stored frozen for up to 60 days before analysis. Untreated control peach samples were fortified with 2,6-dichlorobenzamide and stored frozen for up to 59 days: mean recoveries after storage were all within the range 70–120%, indicating that the peach samples are unlikely to have been adversely affected by storage.

Table 35 Residues of dichlobenil in peaches (mature fruit)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)	Reference
	Date	Growth stage	Application method	Rate (kg ai/ha)			
Madera, CA, USA, C 201 50 020, 1990 (Springcrest)	05/05/90	5.0-7.5 cm diameter fruit	Banded soil	6.8	20	< 0.01 (< 0.01, < 0.01, < 0.01)	Dykstra, 1991a
Kline, SC, USA, C 201 50 021, 1990 (Winblo)	26/04/90	not stated	soil broadcast	7.1	47	0.01 (0.01, 0.01, < 0.01)	Dykstra, 1991a
Fresno, CA, USA, C 201 50 031, 1991 (Flavorcrest)	22/04/91	2.5 cm diameter fruit	Banded soil	7.7	63	0.023 (0.027, 0.021, 0.02)	Ver Hey, 1992a
Kline, SC, USA, C 201 50 032, 1991 (Winblo)	17/04/91	not stated	soil broadcast	7.4	68	0.037 (0.038, 0.035, 0.039)	Ver Hey, 1992a

No residues were found above the LOQ (0.01 mg/kg) in the untreated control samples.

Plums

Two trials were conducted in plums in Idaho during 1991 and in Michigan during 1992 (Ver Hey, 1992b and Hamilton, 1993). A single soil broadcast or banded application of a 4% granular formulation of dichlobenil was made at a target rate of 6.72 kg ai/ha. Mature fruit was sampled around 4-5 months after treatment.

Plum samples were analysed for 2,6-dichlorobenzamide residues using a GC-ECD analytical method (method number L 3-53-71, evaluated in detail above). For the Michigan trial, residues were determined on the edible portion while for the Idaho trial it is not clear whether the residues have been calculated on a whole fruit basis (including stones), or only on the edible portion. Samples were stored frozen for up to 41 days prior to analysis. Concurrent recoveries of 2,6-dichlorobenzamide from plum samples were within the range 70–120%, as were those in fortified samples stored frozen for up to 35 days, indicating that the plum samples are unlikely to have been adversely affected by storage.

The results from the Parma, Idaho trial are anomalously high when compared with other trial results in stone fruit.

Table 36 Residues of dichlobenil in plums (mature fruit)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)	Reference
	Date	Growth stage	Application method	Rate (kg ai/ha)			
Parma, ID, USA, C 201 50 034, 1991 (Empress)	19/04/91	not stated	soil broadcast	7.9	154	0.39 (0.37, 0.40)	Ver Hey, 1992b
Conklin, MI, USA, C 201 50 048, 1992 (Stanley)	15/04/92	not stated	banded soil	6.7	139	< 0.01 (< 0.01, < 0.01, < 0.01)	Hamilton, 1993

Residues were not found above the LOQ (0.01 mg/kg) in the untreated control samples.

Berries and other small fruits

Blueberries

Four trials in blueberries were reported (Reibach, 2013). A single broadcast soil application of dichlobenil was made using a 4% w/w granular formulation at a target rate of 4.48 kg ai/ha. Mature fruit was sampled, typically around 50–90 days after application.

Samples were analysed for both dichlobenil and 2,6-dichlorobenzamide residues using a GC-ECD method, a modification of the method in study number 2005-046 evaluated above, without the solid phase extraction cleanup. Mean and individual method validation recoveries and mean concurrent recoveries were within the range 70–120%. Samples were stored frozen for up to 90 days prior to analysis.

Table 37 Residues of dichlobenil in blueberries (mature fruit)

Location, Trial no., Year (Variety)	Application			DAT	Residue (mg/kg)	
					Dichlobenil	2,6-Dichlorobenzamide
	Date	Application method	Rate (kg ai/ha)			
Lehigh, PA, USA, 2012-01-PA, 2012 (Blueray)	07/05/12	soil broadcast	4.3	53	< 0.01 (< 0.01, 0.01)	0.015 (0.016, 0.014)
Bucks, PA/NJ, USA, 2012-02-PA/NJ, 2012 (Bluecrop)	08/05/12	soil broadcast	4.3	51	< 0.01 (< 0.01, 0.01)	< 0.01 (< 0.01, 0.01)
Ottawa, MI, USA, 2012-03-MI, 2012 (Draper)	10/05/12	soil broadcast	4.3	56	< 0.01 (< 0.01, 0.01)	< 0.01 (< 0.01, 0.01)
Washington, OR, USA, 2012-04-OR, 2012 (Bluecrop)	14/05/12	soil broadcast	4.3	58	< 0.01 (< 0.01, 0.01)	< 0.01 (< 0.01, 0.01)

No residues were found in the untreated control samples above the LOQ (0.01 mg/kg for each analyte).

Blackberries

A single residue study in blackberries was conducted in Oregon, USA during 1990 (Dykstra, 1991b). A single broadcast soil application of dichlobenil was made using a granular formulation at a target application rate of 4.48 kg ai/ha. Mature fruit was harvested 3 months after application.

Blackberry samples were analysed for 2,6-dichlorobenzamide using a GC-ECD method (method number L 3-53-71, evaluated in detail above). Mean and individual method validation recoveries in blackberries were within 70–120%. Storage stability recoveries for samples of blackberries fortified with 2,6-dichlorobenzamide and stored frozen for 26 days were within 70–

120%. Samples were stored frozen for up to 35 days between collection and analysis, and are unlikely to have been adversely affected by storage.

A further four trials in blackberries were reported (Reibach, 2013). A single broadcast soil application of dichlobenil was made using a 4% w/w granular formulation at a target rate of 100 pounds per acre (4.48 kg ai/ha). Mature fruit was sampled, typically around 50–90 days after application.

Samples were analysed for both dichlobenil and 2,6-dichlorobenzamide residues using a GC-ECD method, a modification of the method in study number 2005-046 evaluated above, without the solid phase extraction clean-up. Mean and individual method validation recoveries and mean concurrent recoveries in blackberries were within the range 70–120%. Samples were stored frozen for up to 70 days before analysis.

Table 38: Residues of dichlobenil in blackberries, mature fruit (Dykstra, 1991b)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)		
Cornelius, OR, USA, 1990, C 201 50 009 (Marion)	19/04/90	not stated	soil broadcast	4.7	89	0.01 (0.01, 0.01, 0.01)

Residues were not found at levels above the LOQ (0.01 mg/kg) in the untreated control samples.

Table 39: Residues of dichlobenil in blackberries, mature fruit (Reibach, 2013)

Location, Trial no., Year (Variety)	Application			DAT	Residue (mg/kg)	
					Dichlobenil	2,6-Dichlorobenzamide
	Date	Application method	Rate (kg ai/ha)			
Bucks, PA/NJ, USA, 2012-05-PA/NJ, 2012 (Chester)	08/05/12	soil broadcast	4.3	78	< 0.01 (< 0.01, < 0.01)	0.056 (0.049, 0.062)
Washington, OR, USA, 2012-06-OR, 2012 (Marion)	14/05/12	soil broadcast	4.3	57	< 0.01 (< 0.01, < 0.01)	< 0.01 (< 0.01, < 0.01)
Washington, OR, USA, 2012-07-OR, 2012 (Chester)	14/05/12	soil broadcast	4.3	88	< 0.01 (< 0.01, < 0.01)	0.035 (0.035, 0.034)
Washington, OR, USA, 2012-08-OR, 2012 (Evergreen blackberry)	22/06/12	soil broadcast	4.3	53	< 0.01 (< 0.01, < 0.01)	0.031 (0.029, 0.032)

No residues were found in the untreated control samples above the LOQ (0.01 mg/kg for each analyte).

Cranberries

Two residue studies in cranberries were conducted in Massachusetts and Wisconsin during 1991 (Ver Hey, 1992c). A single broadcast soil application of dichlobenil was made during spring using a granular formulation at a target application rate of 4.48 kg ai/ha. Mature fruit was harvested around 5-6 months after application.

Cranberry samples were analysed for residues of 2,6-dichlorobenzamide using a GC-ECD method (method number L 3-53-71, evaluated in detail above). Mean and individual method validation recoveries in cranberry samples were within the range 70–120%. Samples were stored frozen between collection and analysis for up to 94 days. Mean and individual storage stability recoveries for untreated control samples of cranberries fortified with 2,6-dichlorobenzamide and stored frozen for up 87 days were within the range 70–120%; the residue trial samples are therefore unlikely to have been adversely affected by storage.

Table 40: Residues of dichlobenil in cranberries, mature fruit (Ver Hey, 1992c)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)		
East Wareham, MA, USA, C 201 50 037, 1991 (not specified)	19/04/91	mid spring	soil broadcast	5.7	158	0.020 (0.018, 0.024, 0.018)
Warrens, WI, USA, C 201 50 038, 1991 (not specified)	19/04/91	mid spring	soil broadcast	4.8	166	0.019 (0.015, 0.023, 0.020)

Residues were not found at levels above the LOQ (0.01 mg/kg) in the untreated control samples.

Grapes

Four trials were conducted in the USA (Chickering and Koch, 1991). A banded soil application was made to grapevines using a granular formulation (40 g/kg dichlobenil) at a target rate of 6.73 kg ai/ha in mid to late spring. Approximately 2.5-3.8 cm of irrigation was applied by sprinkler shortly after application at the Biola and Fresno trial sites. Mature grapes were sampled around 4-5 months after application.

Residues of 2,6-dichlorobenzamide in grapes were determined using a GC-ECD method based on method number L 3-53-71, evaluated in detail above. Individual and mean method validation and concurrent recoveries were within the range 70–120%. Grapes were analysed within 196 days of harvest. Fortified grape samples were stored frozen for 112-133 days, and showed individual and mean recoveries within the range 70–120%. Given that residues of dichlobenil and 2,6-dichlorobenzamide were shown to be stable for 323 and 345 days of frozen storage respectively during the Maselli, 1998 study (see below), the grape samples are unlikely to have been adversely affected by storage.

Additional residue data for grapes is available from the field component of a processing study in grapes (Maselli, 1998). Field trials were conducted at two sites in California during 1996. A single banded soil application of a 4% w/w granular formulation of dichlobenil was made around the end of dormancy at a target application rate of 8.75 kg ai/ha. At least 6.4 mm of water was applied within 48 hours of application, either by rainfall or irrigation if rainfall was insufficient to prevent sublimation of the test material. Mature grapes were sampled around 6 months after application.

Residues of dichlobenil and 2,6-dichlorobenzamide in raw and processed grape samples were determined using a GC-ECD method very similar to method number 2005-046 (evaluated in more detail above). Acceptable individual and mean method validation and concurrent recoveries in the range 70–120% were achieved in grapes, for both dichlobenil and 2,6-dichlorobenzamide. Analyses of dichlobenil were completed within 289 days of sample collection, while those for 2,6-dichlorobenzamide were completed within 124 days. A concurrent storage stability study was conducted, showing recoveries of dichlobenil and 2,6-dichlorobenzamide remaining within the range 70–120% after storage for 323 and 345 days respectively.

Table 41 Residues of dichlobenil in grapes, mature berries (Chickering and Koch, 1991)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)		
Biola, CA, USA, C 201 50 014, 1990 (Thompson Seedless)	30/04/90	15 cm bunch length	Banded soil	7.1	119	0.041 (0.042, 0.037, 0.043)
Fresno, CA, USA, C 201 50 015, 1990 (Thompson Seedless)	30/04/90	15 cm bunch length	Banded soil	6.8	119	0.057 (0.061, 0.047, 0.062)

Location, Trial no., Year (Variety)	Application				DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)		
Madera, CA, USA, C 201 50 016, 1990 (Thompson Seedless)	01/05/90	Shot berry	Banded soil	6.8	120	0.032 (0.031, 0.026, 0.040)
Phelps, NY, USA, C 201 50 017, 1990 (Catawba)	27/04/90	Not stated	Banded soil	6.7	158	0.041 (0.041, 0.053, 0.029)

Except where otherwise noted, residues were <LOQ in untreated control samples.

Table 42: Residues of dichlobenil in grapes, mature berries (Maselli, 1998)

Location, Trial no., Year (Variety)	Application				DAT	Residues (mg/kg)	
	Date	Growth stage	Application method	Rate (kg ai/ha)		Dichlobenil	2,6-Dichlorobenzamide
Madera, CA, USA, CEJ-96001, 1996 (Thompson seedless)	02/03/96	end of dormancy	Banded spray	8.1	177	< 0.02	0.029
Fresno, CA, USA, CEJ-96002, 1996 (Thompson seedless)	01/03/96	end of dormancy	Banded spray	8.3	178	< 0.02	0.004

No residues were found in untreated control samples above the LOQ (0.02 mg/kg for dichlobenil, and 0.003 mg/kg for 2,6-dichlorobenzamide).

Raspberries

Two residue trials in raspberries were conducted in Oregon and Washington during 1990 (Dykstra, 1991c). A single broadcast soil application of dichlobenil was made during spring using a granular formulation at a target application rate of 4.48 kg ai/ha.

Raspberry samples were analysed for 2,6-dichlorobenzamide using a GC-ECD method (method number L 3-53-71, evaluated in detail above). Individual and mean method validation recoveries were within the range 70–120%. Samples were analysed within 49 days of collection. Mean storage stability recoveries for untreated control samples of raspberries fortified with 2,6-dichlorobenzamide were within the range 70–120% after 41 days storage. The residue trial samples are therefore unlikely to have been adversely affected by storage.

A further four trials in raspberries were reported (Reibach, 2013). A single broadcast soil application of dichlobenil was made using a 4% w/w granular formulation at a target rate of.48 kg ai/ha. Mature fruit was sampled, typically around 50–90 days after application.

Samples were analysed for both dichlobenil and 2,6-dichlorobenzamide residues using a GC-ECD method, a modification of the method in study number 2005-046 evaluated above, without the solid phase extraction cleanup. Mean method validation and quality control recoveries in raspberries were within the range 70–120%. Samples were stored frozen for up to 79 days between collection and analysis.

Table 43 Residues of dichlobenil in raspberries (Dykstra, 1991c)

Location, Trial no., Year (Variety)	Application				Sample	DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)			
Banks, OR, USA, C 201 150 022, 1990 (Willamette)	20/04/90	mid spring	soil broadcast	4.8	Mature berries	60	< 0.01 (< 0.01, < 0.01, < 0.01)

Location, Trial no., Year (Variety)	Application				Sample	DAT	2,6-Dichlorobenzamide residue (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)			
Vancouver, WA, USA, C 201 150 023, 1990 (Willamette)	20/04/90	mid spring	soil broadcast	4.9	Mature berries	60	0.020 (0.021, 0.021, 0.018)

No residues were found at levels above the LOQ (0.01 mg/kg) in the untreated control samples.

Table 44 Residues of dichlobenil in raspberries, mature fruit (Reibach, 2013)

Location, Trial no., Year (Variety)	Application			DAT	Residue (mg/kg)	
					Dichlobenil	2,6-Dichlorobenzamide
	Date	Application method	Rate (kg ai/ha)			
Lehigh, PA, USA, 2012-09-PA, 2012 (Boyne)	07/05/12	soil broadcast	4.3	44	< 0.01 (< 0.01, < 0.01)	0.067 (0.054, 0.080)
Ottawa, MI, USA, 2012-10-MI, 2012 (Nova)	10/05/12	soil broadcast	4.3	50	< 0.01 (< 0.01, < 0.01)	0.021 (0.026, 0.016)
Washington, OR, USA, 2012-11-OR, 2012 (Willamette)	14/05/12	soil broadcast	4.3	51	< 0.01 (< 0.01, < 0.01)	< 0.01 (< 0.01, < 0.01)
Washington, OR, USA, 2012-12-OR, 2012 (Coho)	14/05/12	soil broadcast	4.3	57	< 0.01 (< 0.01, < 0.01)	< 0.01 (< 0.01, < 0.01)

No residues were found in the untreated control samples above the LOQ (0.01 mg/kg for each analyte).

FATE OF RESIDUES IN PROCESSING

Plums

A processing trial for plums was conducted in Michigan (Hamilton, 1993). After a single banded soil application during spring of a 4% w/w granular formulation of dichlobenil at a target rate of 6.7 kg ai/ha, mature plums were harvested and shipped unfrozen for processing. Processing commenced the day after harvest. An unwashed raw plum sample was set aside, before processing the remaining plums into prunes using a batch process. Plums were dried to a target moisture content of 18-22% over a period of approximately 24 hours at temperatures from 66-82 °C. Samples were frozen within a few hours of completion of processing.

Frozen whole plums and prune samples were shipped to the laboratory for analysis of 2,6-dichlorobenzamide residues using a GC-ECD method (method number L 3-53-71, evaluated in detail above). The mean concurrent recovery for 2,6-dichlorobenzamide from prunes were within the range of 70–120%, although it is noted that some individual concurrent recovery values and the recoveries after only four days of frozen storage were < 70%. The plum samples showed storage stability recoveries within the 70–120% range after 35 days of storage. Plum and prune samples were analysed 39–40 days after harvest/processing, and prune samples may therefore have been adversely affected by storage.

Table 45 Residues of dichlobenil in plums and prunes

Location, Trial no., Year (Variety)	Application				Sample	DAT	2,6-Dichlorobenzamide residue (mg/kg)	Reference
	Date	Growth stage	Application method	Rate (kg ai/ha)				
Conklin, MI, USA, C 201 50 048, 1992 (Stanley)	15/04/92	not stated	banded soil	6.7	plums	139	< 0.01 (< 0.01, < 0.01, < 0.01)	Hamilton, 1993
					prunes		0.01 (0.01, 0.01, 0.01)	

No residues were found in untreated control samples above the LOQ (0.01 mg/kg).

Residues of 2,6-dichlorobenzamide were observed to concentrate slightly in plums.

Grapes

Residue trials for generating processing data for grapes were conducted at two sites in California (Maselli, 1998). Plots of grapevines were treated with a single banded spray application at a target rate of 8.8 kg ai/ha of 4G dichlobenil formulation. The application was made just as the dormancy period was finishing. Grapes were sampled at maturity from the treated plots and untreated control areas, with separate samples for analysis of unprocessed grapes, and for processing into juice and raisins being collected. Unprocessed grapes were frozen within 4 hours of collection, while grapes for processing were kept at ambient temperature, with processing into raisins commencing on the day of harvest, and processing into juice 3 days after harvest.

For juice processing, grapes were crushed and destemmed using a commercial crusher-stemmer, and the wet mash was loaded into a hydraulic press for juicing. Juice samples were collected and stored frozen until analysis, while the wet pomace was discarded.

Grapes were processed into raisins by sun drying on paper trays for approximately 3 weeks, with the drying grapes being turned twice. Untreated control grapes were kept well separated from the treated grapes during drying. After drying to approximately 20% of the fresh weight, the stems were removed using simulated commercial equipment, involving first passing over a screen shaker to remove loose impurities such as leaf and stem fragments, then destemming followed by passing over a vacuum unit to remove remaining stem fragments and substandard raisins. The raisins were then passed through a sizing shaker to separate the smaller raisins normally used for baking or breakfast cereals, with the remaining raisins being passed over two further vacuum units to remove any remaining substandard raisins, and C-grade, which are discarded. The combined A- and B-grade raisins were washed and then rehydrated to commercial specification using a 'riffle' washer, followed by cleaning and visual inspection. A sample of raisins was collected and placed in frozen storage prior to analysis.

Residues of dichlobenil and 2,6-dichlorobenzamide in raw and processed grape samples were determined using a GC-ECD method very similar to method number 2005-046 evaluated in more detail above. Acceptable individual and mean method validation and concurrent recoveries in the range 70–120% were achieved in grapes, for both dichlobenil and 2,6-dichlorobenzamide. Analyses of dichlobenil in grapes were completed within 289 days of sample collection, those for raisins were completed within 277 days, and those for grape juice were completed within 277 days. Analyses of 2,6-dichlorobenzamide in grapes were completed within 124 days, those for raisins were completed within 276 days, and those for grape juice were completed within 275 days. A concurrent storage stability study was conducted, showing recoveries of dichlobenil and 2,6-dichlorobenzamide remaining within the range 70–120% after storage for 323 and 345 days respectively for grapes. In raisins, a storage stability trial was carried out over 273 days for dichlobenil, and 266 days for 2,6-dichlorobenzamide, with mean recoveries remaining within 70–120% at the end of storage. In grape juice, fortified samples were stored for 322 days (dichlobenil) and 343 days (2,6-dichlorobenzamide),

with mean recoveries remaining within 70–120%. The storage stability data shows that the raw and processed grape residue trial samples are unlikely to have been adversely affected by storage.

A second processing study was conducted in the USA (Ver Hey, 1992d). At a site in California, a single application of dichlobenil was made using a granular formulation at a target rate of 150 pounds of a 4% w/w formulation per treated acre (6.72 kg ai/ha), with the application made prior to flowering . The vines were irrigated (5.0-7.5 cm) on the day of application. Mature grape samples were collected for processing into juice and raisins.

Processing of grapes into juice and raisins commenced on the day of harvest. Limited information on processing was provided. Processing was conducted using domestic kitchen equipment rather than simulated commercial processes. The grapes were juiced using a food processor, and the juice was decanted. The remaining solids were collected as the wet pomace fraction, and a portion was placed in a cloth bag and the moisture squeezed to generate dry pomace. Raisins were sun dried over 19 days, and separated into raisins and raisin waste (stems and debris). Samples were frozen within 6 hours of collection.

Samples of grape juice, raisins, raisin waste, and wet and dry grape pomace were analysed for residues of 2,6-dichlorobenzamide using a GC-ECD method (method number L 3-53-71, evaluated above). Concurrent and stored recoveries (after 99, 108, 111, 79 and 101 days for juice, wet pomace, dry pomace, raisins and raisin waste respectively) were within the range 70–120%, with the exception of stored recoveries for raisins (mean stored recovery = 64%). Samples were analysed within 124–163 days of harvest of the grapes. Given the storage stability results for this and the Maselli study, with the possible exception of raisins, it is unlikely that the samples have been adversely affected by storage.

Samples were analysed in triplicate. The results are tabulated below. However it is noted that residues were not reported for raw grapes from the field site, so processing factors cannot be calculated.

Table 46: Processing study in grapes (Maselli, 1998)

Location, Trial no., Year (Variety)	Application				Sample	DAT	Residues (mg/kg)	
	Date	Growth stage	Application method	Rate (kg ai/ha)			Dichlobenil	2,6-Dichlorobenzamide
Madera, CA, USA, CEJ-96001, 1996 (Thompson seedless)	02/03/96	end of dormancy	Banded soil	8.1	Mature berries	177	< 0.02	0.029
					Raisins		< 0.02	0.082
					Juice		< 0.02	0.027
Fresno, CA, USA, CEJ-96002, 1996 (Thompson seedless)	01/03/96	end of dormancy	Banded soil	8.3	Mature berries	178	< 0.02	0.0041
					Raisins		< 0.02	< 0.03
					Juice		< 0.02	0.0059

No residues were found in untreated control samples above the LOQ (0.02 mg/kg for dichlobenil, and 0.003 mg/kg for 2,6-dichlorobenzamide).

Table 47 Processing study in grapes (Ver Hey, 1992d)

Location, Trial no., Year (Variety)	Application				Sample	DAT	2,6-Dichlorobenzamide residues (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)			
Fresno, CA, USA, C 201 50 043, 1991 (Thompson seedless)	19/04/91	Pre-flowering	banded soil	7.9	Grapes	118	Not analysed for this site

Location, Trial no., Year (Variety)	Application				Sample	DAT	2,6-Dichlorobenzamide residues (mg/kg)
	Date	Growth stage	Application method	Rate (kg ai/ha)			
					Juice		0.030 (0.031, 0.031, 0.028)
					Wet pomace		0.057 (0.061, 0.056, 0.055)
					Dry pomace		0.11 (0.085, 0.13, 0.11)
					Raisins		0.010 (0.012, 0.008, 0.009)
					Raisin waste		0.28 (0.28, 0.28, 0.28)

Residues in untreated control samples were all <LOQ (0.01 mg/kg).

Table 48 Processing factors for 2,6-dichlorobenzamide in grape products (Maselli, 1998)

Product	Processing factor
Raisins	<1, 2.8
Juice	0.93, 1.4

Moderate concentration of residues of 2,6-dichlorobenzamide is noted in both grape juice and raisins.

RESIDUES IN ANIMAL COMMODITIES

Farm animal feeding studies

Lactating cattle

A feeding study for the major plant metabolite of dichlobenil, 2,6-dichlorobenzamide (BAM), was conducted in lactating cattle (Batorewicz, 1998).

Sixteen lactating Holstein cattle were purchased and placed in quarantine for 7 days followed by a further 14 days of acclimatisation. Twelve cattle aged between 3-6 years and weighing 534-758 kg (mean milk production during the feeding phase was 17.9 L/animal/day, while mean feed intake was 18.9 kg dry matter/animal/day) were selected for the study on day 6 of acclimatisation from the group of sixteen. Three animals were randomly selected for each of the four groups: group I (untreated control), group II (low dose, 1× at a level of 0.6 ppm), group III (mid dose, 3×, at a level of 1.6 ppm), and group IV (high dose, 5×, at a level of 5.3 ppm). BAM was administered to the animals in groups II-IV orally once daily after the morning milking by capsule for 28 days. The control animals received blank capsules at the same time as the treated animals. The cattle were kept in outdoor pens with overhead shelter, with the different feeding groups being separated. *Ad libitum* access was allowed to fresh drinking water and a total mixed ration containing alfalfa hay, corn silage, rolled corn, cotton seed and various seed and grain by-products and mineral supplements. No adverse effects on cattle weight, feed consumption or milk yield were noted during the trial.

Milk samples were collected twice daily, and weighted pooled 200 gram subsamples (PM and the following AM) from selected days were frozen and submitted for analysis. The animals were sacrificed with 6 hours of the final dose and samples of liver, kidney, muscle (thigh and loin composite) and fat (composite of mesenteric and perirenal) were collected for analysis and frozen. Frozen tissue samples were processed by grinding in the presence of liquid nitrogen. The liquid nitrogen was allowed to evaporate in the freezer, and subsamples were collected for submission for analysis (250 grams for fat and 500 grams for other tissue types).

Samples were analysed for both dichlobenil and 2,6-dichlorobenzamide using a GC-MS method (method number 96145, evaluated in detail above). Mean concurrent recoveries in fortified control samples were generally within 70–120%.

Samples were stored frozen for up to 62 days (fat), 66 days (kidney), 70 days (liver), 63 days (muscle), and 66 days (milk) between collection and analysis. Storage stability was checked at fortification level of 0.05 mg/kg for both dichlobenil and 2,6-dichlorobenzamide. Acceptable storage stability was observed for 2,6-dichlorobenzamide in fat (62 days), kidney (67 days), liver (70 days), muscle (63 days), and milk (71 days), and for dichlobenil in muscle (63 days), fat (62 days), kidney (52 days), and milk (71 days). After 67 days storage, the mean stored recovery in kidney had dropped to 62%, although after correction for concurrent recovery, a corrected recovery of 70% was determined. Stability of dichlobenil in liver was poor, with mean recoveries of 43% after 55 days, and 31% after 70 days.

Residues of dichlobenil were not found above the LOQ (0.01 mg/kg) in any of the control or treated milk or tissue samples. Finite residues were found in tissue samples from all treatment groups, and in milk sample from the mid- and high-dose groups.

Table 49 Residues of BAM in milk

Dose level (ppm dry weight in feed)	Study day	2,6-dichlorobenzamide residues (mg/kg)
0.6	0	Not analysed.
	4	
	8	
	15	
	22	
	27	
1.6	0	Not analysed.
	4	
	8	
	15	
	22	0.008, 0.009, 0.008 (0.008)
	27	0.010, 0.013, 0.011 (0.011)
5.3	0	< 0.01, < 0.01, < 0.01 (< 0.01)
	4	0.019, 0.013, 0.019 (0.017)
	8	0.014, 0.016, 0.021 (0.017)
	15	0.018, 0.019, 0.031 (0.023)
	22	0.025, 0.029, 0.040 (0.031)
	27	0.016, 0.022, 0.015 (0.018)

Residues of 2,6-dichlorobenzamide were not found above the LOQ (0.01 mg/kg) in the control milk samples.

Table 50 Residues of dichlobenil and 2,6-dichlorobenzamide in tissues

Tissue	Dose level (ppm dry weight in feed)	2,6-dichlorobenzamide residues (mg/kg)
Liver	0	< 0.01
	0.6	0.038, 0.062, 0.059 (0.053)
	1.6	0.054, 0.137, 0.065 (0.085)
	5.3	0.263, 0.024, 0.422 (0.31)
Kidney	0	< 0.01
	0.6	0.008, 0.012, 0.013 (0.011)
	1.6	0.022, 0.037, 0.025 (0.028)
	5.3	0.035, 0.027, 0.046 (0.036)
Muscle	0	< 0.01
	0.6	0.007, 0.010, 0.010 (0.009)
	1.6	0.018, 0.029, 0.021 (0.023)
	5.3	0.060, 0.053, 0.065 (0.059)
Fat	0	< 0.01
	0.6	Not analysed.
	1.6	0.010, 0.010, 0.009 (0.010)
	5.3	0.023, 0.017, 0.020 (0.020)

Residues of 2,6-dichlorobenzamide were not found above the LOQ (0.01 mg/kg) in the control milk samples.

Samples of fat and milk from the 1× treatment group were not analysed, as residues were either not detected or only found at the LOQ in samples from the 3× group. Residues of 2,6-dichlorobenzamide in milk peaked at or around day 22 of dosing. A clear linear relationship between feeding level and tissue residue was observed in liver and muscle ($r^2 > 0.95$), while the relationship was less clear for kidney ($r^2 = 0.61$).

Table 51: Relationship between dosing level and maximum tissue residue

Tissue	Regression equation (forced through origin)	r^2 value
Liver	y = 0.0804x	0.9974
Kidney	y = 0.01x	0.6008
Muscle	y = 0.0128x	0.9651

Table 52: Relationship between dosing level and mean tissue residue

Tissue	Regression equation (forced through origin)	r^2 value
Liver	y = 0.0584x	0.993
Kidney	y = 0.0078x	0.6108
Muscle	y = 0.0114x	0.9855

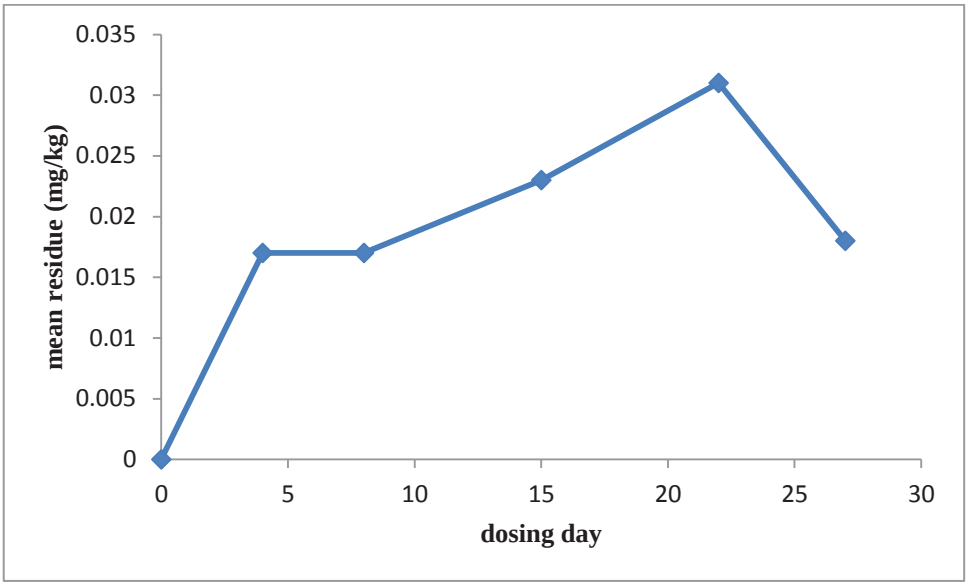


Figure 5 Mean milk residues over time in the 5 ppm feeding group

Poultry

A poultry feeding study was not provided to the Meeting.

APPRAISAL

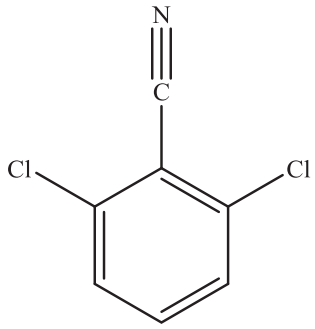
Dichlobenil is a benzonitrile herbicide for use by soil application in fruit crops, including stone fruit, grapes, and other berry crops. Dichlobenil was scheduled at the Forty-fifth Session of the CCPR in 2013 for evaluation as a new compound by the 2014 JMPR. Data was provided on physicochemical properties, 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.

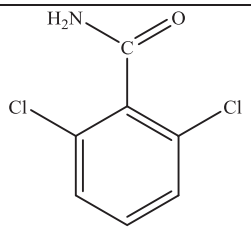
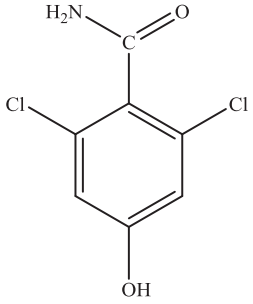
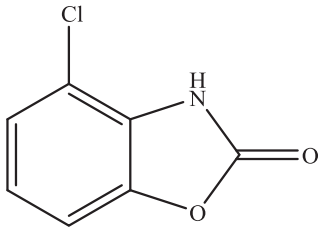
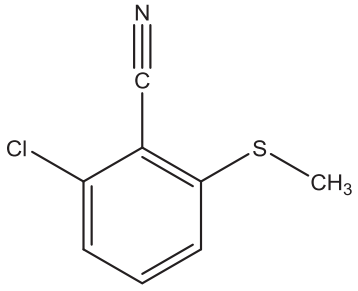
Processing factors calculated for the processed commodities for the above raw agricultural commodities, including previously estimated, are shown in the table below. STMP-Ps was calculated for processed commodities of strawberry, onion and peas for which maximum residue levels were estimated.

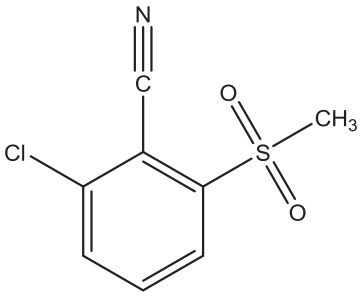
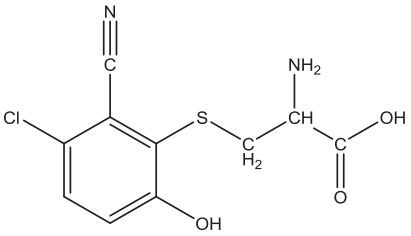
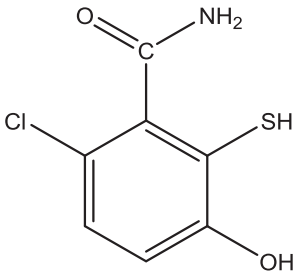
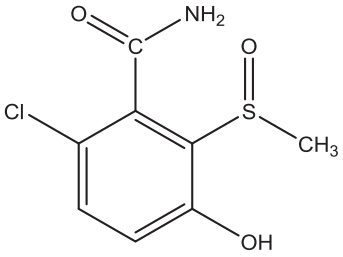
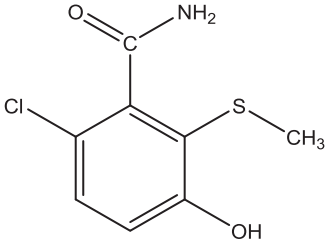
Chemical name and structure

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.

The Meeting estimated the maximum residue levels, STMR and HR values below:

CCN	Commodity name	Recommended maximum residue level, mg/kg		STMR (P), mg/kg	HR (P), mg/kg
		New	Previous		
VB 0040	Brassica vegetables	0.05 (FL)	-	0.01	0.04
FB 2005	Caneberries	0.2 (D)	-	0.034	0.13
VS 0624	Celery	0.07 (FL)	-	0.01	0.04
GC 0080	Cereal grains	0.01* (FL)	-	0.01	-
DF 0269	Dried grapes (= Currants, Raisins and Sultanas)	0.15 (FL)	-	0.028	0.11
MO 0105	Edible offal (mammalian)	0.04 (FL)	-	0.01	0.031
PE 0112	Eggs	0.03 (FL)	-	0.01	0.019
VC 0045	Fruiting vegetables, Cucurbits	0.01* (FL)	-	0.01	0.01
VO 0050	Fruiting vegetables, other than Cucurbits (except sweetcorn and mushrooms)	0.01* (FL)	-	0.01	0.01
	Tomato juice	-	-	0.01	-
	Tomato purée	-	-	0.01	-
	Tomato paste	-	-	0.01	-
JF 0269	Grape juice	0.07 (FL)	-	0.014	0.056
FB 0269	Grapes	0.05 (FL)	-	0.01	0.04
VL 0053	Leafy vegetables	0.3 (FL)	-	0.07	0.19
MF 0100	Mammalian fats (except milk fats)	0.01* (FL)	-	0.01	0.01
MM 0095	Meat (from mammals other than marine mammals)	0.01* (FL)	-	0.01	0.01
ML 0106	Milks	0.01* (FL)	-	0.01	-
VA 0385	Onion, Bulb	0.01* (FL)	-	0.01	0.01
VA 0387	Onion, Welsh	0.02 (FL)	-	0.01	0.01
HS 0444	Peppers, Chili, dried	0.01* (FL)	-	0.01	0.01
PO 0111	Poultry, edible offal of	0.1 (FL)	-	0.014	0.081
PF 0111	Poultry fats	0.02 (FL)	-	0.01	0.012
PM 0110	Poultry meats	0.03 (FL)	-	0.01	0.018
VD 0070	Pulses (FL)	0.01* (FL)	-	0.01	-
AS 0081	Straw and fodder (dry) of cereal grains (FL)	0.4 (FL)	-	-	-

D = indicates values estimated to accommodate residues of 2,6-dichlorobenzamide arising from dichlobenil use.
FL = indicates values estimated to accommodate residues of 2,6-dichlorobenzamide arising from fluopicolide use.

CCN	Commodity name	Median residue, mg/kg	Highest residue, mg/kg
	Cereal forage (as received) (FL)	0.01	0.02
	Cereal hay (FL)	0.04	0.24
	Cereal straw (FL)	0.011	0.034
	Grape pomace (FL)	0.028	-
	Legume animal feeds (as received) (FL)	0.02	0.10
	Tomato pomace (FL)	0.01	-

FL = indicates values estimated to accommodate residues of 2,6-dichlorobenzamide arising from fluopicolide use.

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.

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