

DAMINOZIDE (104)

EXPLANATION

Daminozide was reviewed by the JMPR in 1977 but as an ADI was not estimated, only Guideline Levels were recorded. Further work on analytical methodology and the nature of the terminal residue was deemed desirable and data on residues in foods in commerce were also lacking. Following a review of the toxicology of daminozide by the 1983 JMPR, further consideration of the compound was deferred.

Information on current use patterns, data from residue trials and monitoring surveys and some information on the hydrolysis and photolysis of daminozide were provided to the Meeting.

USE PATTERN

Daminozide is used as a growth regulator on apples and pears and also, to a much smaller extent, on other fruit crops such as cherries, seed potatoes, field beans, and glasshouse ornamentals. Its principal use is to reduce biennial bearing of apple and pear trees and also to induce fruiting of young trees, enhance fruit colour and prevent fruit drop. Recommended treatment levels using 85% SP are given in Table 1 (United Kingdom, 1989).

Table 1. Use patterns of daminozide in the United Kingdom

Crop	Max. no. applic.	Max. rate (kg ai/acre)	Latest time of application
Apples	3	a) 1.9	King fruit blossom goes down.
		b) 2.9 4 x 0.36 or 6 x 0.21	Four weeks after petal fall. Four weeks before harvest.
		c) 2.9 or 3.8	Seven weeks before harvest. Ten weeks before harvest.
Pears	2	a) 2.9	At 80% petal fall.
		b) 1.2	Ten weeks before harvest.
Seed potatoes	1	0.85	Five weeks before harvest.
Field beans	1	0.85	Five weeks before harvest.

Information on use patterns of 64% and 85% wettable powders in The Netherlands was also provided and is summarized in Table 2 (Netherlands, 1989).

A summary of the application rates for 85% formulations and minimum pre-harvest intervals in a number of other countries is given in Table 3 (Uniroyal, 1989).

Table 2. Uses of daminozide in The Netherlands

Commodity	Purpose	Applic.rate, kg/ha	Treatment	PHI (weeks)
Apples & pears	Growth regulation of young non-bearing trees	0.85-4.0	1-2 applic. at a shoot length of about 15 cm	
“	Prevention of early fruit drop	1.3-4.0	1 applic. at end of blossoming	-
“	Stimulation of fruit-bud formation	1.3-2.5	1 or more applic. until first week in July	6
Apples	Enhance colour	0.85-2.0	1 applic. 7-6 weeks before harvest	6
“	Fruit scald treatment	1.6-2.6	1 applic. 10-6 weeks before harvest	6
Potatoes (for seed)	Promotion of uniform size and increased yield	1.3	1 applic. at start of tuber formation	-

Table 3. Use patterns of daminozide in various other countries

Country	Commodity	Application rate	Min. PHI (days)
Belgium	Apples, pears	0.15-0.3% in 1000 l/ha	42
Bulgaria	Apples	100-200 g/dha	40
Canada	Apples	2.2-9.0 kg/ha	--
Denmark	Apples, pears	0.1-0.2%	56
France	Apples, pears	150-300 g/hl	60
	Cherries	250-300 g/hl	60
	Peaches	150-300 g/hl	60
Greece	Apples	150 g/hl	--
Ireland	Apples, pears	Rates as given for the UK	UK
Norway	Apples	200 g/100 l of water	49
Romania	Apples	150-200 g/hl	-
USA	Apples	2-4 lbs/acre	-

In several of these countries, daminozide is also used on ornamental crops at rates depending on the species, variety and effect required.

However, at a late stage in the Meeting, information was provided by the major manufacturer that, as from October 1989, all registered uses of daminozide on food crops were to be withdrawn (Uniroyal, 1989). It was understood that formulations of daminozide were available from other sources.

RESIDUES RESULTING FROM SUPERVISED TRIALS

Residue data presented in the 1977 Evaluations were obtained using an insensitive colorimetric analytical method (Lane, 1967 or Edgerton *et al.*, 1967). Data from supervised trials in the United Kingdom obtained using a gas-chromatographic method (Abdel-Kader, 1984) have now been made available to the Meeting. Results are given in Table 4 (Uniroyal, 1989).

Table 4. Residues of daminozide in apples, pears and field beans (UK)

Commodity	Year	<u>Application</u>		PHI (days)	Daminozide residues (mg/kg)
		Rate, kg ai/ha	No.		
Apples	1985	1.43	1	0	1.59,0.94
				7	2.16,1.18
				14	1.74,1.27
				21	1.74,0.50
				28	2.07,1.77
Apples	1985	0.36 + 0.95	4 + 1	7	2.05
				14	2.09
				21	1.82
				28	1.91
Apples	1986	0.42 0.47 0.47	4 4 4	76	1.53,1.26
				72	3.56,3.20
				72	3.74,1.81,0.37
Apples	1987	1.87	1	0	2.62,2.59,0.69
				3	2.79,2.65
				4	3.87
				7	9.75,2.29
				14	2.65,0.56,0.46
				21	3.35
				28	5.58,1.28,1.26
				47	1.58
				49	1.85
68	4.74				
Apples	1987	0.467	4	0	1.76,1.03
				3	2.61,1.17
				7	0.99
				14	2.32,1.61
				28	0.93
				47	1.01,0.81
				70	0.18
Apples	1987	1.87	1	59	0.78,0.76
				63	2.15,2.14,1.17
				94	2.13,2.03,1.12,0.57
				128	0.62
				135	6.52,1.83,1.16,0.57,0.45

Table 4. (cont.)

Commodity	Year	Application		PHI (days)	Daminozide residues (mg/kg)
		Rate, kg ai/ha	No.		
Apples	1987	0.467	4	59	2.76,1.66,0.98,0.73
				63	1.89,1.89,1.81,0.38
				71	2.91
Apples	1987	1.445	1	71	7.04
Apples	1987	1.445	1	49	2.74
				98	0.19
Apples	1987	1.87	1	70	4.58
				95	0.64
				97	0.94
				98	0.14
				129	0.27
Apples	1987	1.02	1	91	0.12
Apples	1987	0.476	4	55	0.26
Pears	1987	1.445	1	122	0.37
				133	2.84
Pears	1987	0.357	4	49	5.37
				70	5.05
Field beans (seed)	1985	0.43	1	88	1.19,1.06
				100	0.69,0.62
				122	0.87,0.75
Field beans (seed)	1985	0.85	1	88	1.28,1.06
				100	1.25,1.19
				122	0.87,0.80
				127	1.12,1.06,1.00
Field beans (seed)	1986	0.85	1	69	3.38,2.46,1.97,1.88, 1.88,1.29,1.19,0.71
				78	5.10,0.46
				84	2.79,2.49,1.88,1.82, 0.95,0.71
				89	0.62
				90	4.61,2.56

Some data on residues of daminozide in seed potatoes were submitted to the Meeting (Netherlands, 1989). Treatment at three different sites with the 85% wettable powder at a rate of 1.3 kg ai/ha gave residues ranging from 0.2 to 0.8, 0.6 to 3.3, and 1.5 to 3.2 mg/kg respectively, using the colorimetric method of Lane (1967); the corresponding mean values were 0.5, 2.0 and 2.4 mg/kg.

## FATE OF RESIDUES

### In soil

Daminozide (75 mg/kg) was applied to 5-g samples of a sandy loam soil at pH 5.6 and exposed to ultraviolet light for the equivalent of 30 days of 12 hours of sunlight (Lengen, 1985). Aliquots were removed at intervals for quantitation of radioactivity and characterisation by HPLC of the degradation products after extraction with 1% formic acid for 1 hour. <sup>14</sup>C residues not extracted by the formic acid were determined by dry combustion to CO<sub>2</sub>. A similar series of control samples was incubated in darkness.

An overall loss of radioactivity occurred in the control soils, and this loss was only slightly accelerated in the irradiated soils. Levels of unextractable <sup>14</sup>C increased in both types of sample. The decrease in the radiocarbon balance was probably due to the formation of CO<sub>2</sub>, shown to occur when daminozide is added to aerobic soils (Dannals *et al.*, 1972), while the increase in unextractable material may indicate the incorporation of this CO<sub>2</sub> into the soil matrix.

Analyses of the soil extracts showed that the daminozide concentration decreased in both sets of samples. The half-lives were calculated as 50.1 and 43.6 days for the control and treated soils respectively, thus indicating that there is no significant photolysis of daminozide on soil. The degradation products noted were cyclic daminozide, formaldehyde and 1,1-dimethylhydrazine, the same as those found in the aqueous photolysis study.

### In water

A report of a hydrolysis study was made available to the Meeting (Lengen *et al.*, 1984). Carbon-14-labelled daminozide (200 mg/l) was incubated in the dark under aseptic conditions for up to 30 days at 25°C in buffered aqueous solutions at pH 1, 5, 7 and 9, aliquots being removed at intervals for quantitation of radioactivity. The resulting solutions were analysed for parent compound and hydrolysis products by HPLC. At pH 5, 7 and 9, no measurable hydrolysis was observed over the 30-day test period. At pH 1, daminozide was hydrolysed rapidly (half-life 41 days), the major hydrolysis products being 1,1-dimethylhydrazine and cyclic daminozide (N-dimethylaminosuccinimide), with limited evidence for the presence of dimethylamine and formaldehyde; hydrazine, detection limit 0.01 mg/kg, was not observed.

In a later study (Lengen, 1985), 200 mg/l of daminozide in neutral aqueous solution was exposed to ultraviolet light for a period equivalent to 30 days of 12 hours of sunlight and the products formed were compared with control samples, maintained in darkness, by means of reverse-phase HPLC, GLC, and GC-MS. The data indicated that ultraviolet light caused a significant loss in daminozide concentration. Decomposition products were characterized as 1,1-dimethylhydrazine, cyclic daminozide and formaldehyde, suggesting that the route of degradation was hydrolytic in nature.

### In processing

In the USA, about 60% of all apples are processed into either juice or sauce. Apples destined for processing may be treated with daminozide to prevent crop losses due to pre-harvest fruit drop and to increase yield. Daminozide is especially important to the growers of McIntosh apples in the north-eastern states where it is one of the major varieties grown and where most of the treated apples are likely to be processed.

Samples of fresh McIntosh apples from orchards known to have been treated with daminozide were processed commercially into juice or sauce (Smilo *et al.*, 1984). Samples

were taken from the processing line and analysed for residues of daminozide (colorimetric method of Lane, 1967) and UDMH (isotope dilution method of Moody et al., 1983). The results obtained are shown in Table 5. None of the samples of apples contained more than 3 mg/kg of daminozide; one sample contained 0.017 mg/kg of UDMH but all other samples were below 0.002 mg/kg. Residues of UDMH were highest in the apple sauce and pasteurized apple juice, reaching levels of 0.089 and 0.123 mg/kg respectively; this is evidently due to decomposition of the daminozide during the heat treatment of these products.

Table 5. Daminozide and UDMH residues in apples and apple products

Commodity	Daminozide (mg/kg)				UDMH (mg/kg)			
Fruit	2,	2	<1	2	<0.001	0.001 -	-----,	0.004
Pomace	3,	3,	3,	2	0.002,	0.002,	0.001,	0.004
Sauce	2,	2,	3,	1	0.032,	0.063,	0.089,	0.041
Fruit	2,	1,	2,	1	<0.001	<0.001,	0.017,	<0.001
Pomace	2,	1,	1,	1	<0.001,	0.001,	0.049,	<0.001
Juice - raw	2,	1,	2,	1	<0.001,	0.007,	0.001,	0.002
Juice - pasteurized	1,	1,	2,	<1	0.010,	0.007,	0.123,	0.007

RESIDUES IN FOOD IN COMMERCE OR AT CONSUMPTION

Data on residues of daminozide and UDMH in samples of some fruits and processed products obtained through normal commercial channels were provided to the Meeting (Canada, 1989). Tables 6 and 7 show the results which were obtained by the GLC method of Newsome and Collins (1988) or a slight modification of it.

Table 6. Daminozide and UDMH in fruits and fruit products (1985-89)

Commodity & Source	No. of samples	Daminozide		No. with residues	UDMH Residues (mg/kg)
		No. with residues	Residues mg/kg		
<u>Canadian</u>					
Apples	15	9	0.05-4.1	0	-
Apples	177	70	0.01-3.7	-	-
Apple sauce	6	6	0.03-0.92	3	0.02,0.05,0.05
Apple juice	10	9	0.01-0.53	2	0.03
Cherries	3	2	0.05	-	-
Grapes	10	7	0.11-0.90	-	-
Peaches	4	4	-	-	-
Pears	9	8	0.09	-	-
<u>Imported</u>					
Apples	11	2	0.6,2.3	2	0.02
Apple juice	2	2	0.20,0.47	0	-
<u>Total diets</u>					
Apples, raw & cooked	7	6	0.10-0.68	0	-
Apple juice	7	7	0.10-0.93	2	0.007,0.02
Apple sauce	7	7	0.13-0.41	1	0.01
Apple pie	7	7	0.01-0.16	1	0.005

Table 7. UMDH residues in apple and cherry products

Commodity	Type	No. of samples	Residues (mg/kg)	Mean (mg/kg)
<u>Apples</u>				
Juice	Infant	3	<0.001-0.012	0.01
	Regular	11	<0.001-0.014	0.008
Sauce	Infant	8	<0.001-0.033	0.024
	Regular	13	<0.001-0.093	0.030
Pie filling	-	4	0.068-0.082	0.043
Jelly	-	1	<0.001	-
Fresh fruit	-	3	<0.001-0.003	-
<u>Cherries</u>				
Canned	-	4	<0.001-0.065	-
Pie filling	-	3	0.010-0.070	0.035
Jam	-	2	<0.001	-
Maraschino	-	1	<0.001	-

A "market basket" survey was carried out in the USA in three phases between March 1986 and February 1987. In all, 730 samples of apples, apple products, cherry pie filling, grape juice, peanuts and peanut butter were taken at normal retail outlets in 72 different locations and were analysed for daminozide and UDMH by means of a modified version of the method of Conditt et al., (1988). There was a general decline in residue levels across the three phases. Results are summarized in Table 8 (Uniroyal, 1989). Of the 730 samples examined, 577 contained less than 1 mg/kg of daminozide and 575 contained less than 0.025 mg/kg of UDMH. Of 250 apple samples, only 9 contained more than 5 mg/kg of daminozide.

Table 8. Residues of daminozide and UDMH (mg/kg) in various samples from a market basket study

Product		Phase 1			Phase 2			Phase 3		
		No.	Damin.	UDMH	No.	Damin.	UDMH	No.	Damin.	UDMH
Apples	Mean	88	1.61	0.003	77	1.01	0.002	85	0.46	0.001
	Max.		12.0	0.023		4.61	0.012		9.92	0.008
Apple juice	Mean	39	0.51	0.020	36	0.49	0.026	35	0.22	0.021
	Max.		2.83	0.112		1.80	0.112		1.40	0.099
Apple sauce	Mean	39	0.41	0.025	36	0.57	0.037	35	0.36	0.028
	Max.		2.37	0.177		3.20	0.192		2.30	0.228
Cherry filling	Mean	23	1.64	0.150	24	1.87	0.110	23	0.81	0.070
	Max.		4.15	0.403		11.0	0.495		5.40	0.518
Grape juice	Mean	24	0.03	0.002	24	0.02	0.001	-	-	-
	Max.		0.06	0.004		0.04	0.001		-	-
Peanuts	Mean	24	0.56	0.019	24	0.90	0.023	23	1.16	0.032
	Max.		4.43	0.077		2.80	0.054		3.62	0.054
Peanut butter	Mean	24	0.63	0.008	24	0.51	0.012	23	0.35	0.012
	Max.		2.58	0.020		1.63	0.042		1.99	0.036

Additional data from monitoring studies carried out in the USA were also made available to the Meeting (FDA, 1989). Analyses were carried out using the colorimetric method given in PAM 11 (1987) or a slight modification of it; the results obtained are summarized in Table 9.

Table 9. Residues of daminozide in various fruit products, 1981-1989

Commodity	Year	No. of samples	No. with residues	Range (mg/kg)	Mean (Mg/kg:)
Apples	1981	27	14	<0.01-8.2	2.07
Apples	1982	19	10	<0.01-7.1	1.96
Apples	1985	20	12	<0.01-5.05	1.81
Apple juice	1985	267	7	0.19-0.83	0.31
Apples	1986	100	28	0.14-3.17	1.34
Apple juice	1986	16	13	<0.01-1.10	0.52
Apple sauce	1986	15	9	<0.01-0.80	0.22
Cherries (frozen or canned)	1986	12	11	<0.01-5.90	1.63
Apples	1987	11	9	0.42-2.60	1.00
Apples	1988	96	34	<0.01-3.69	0.68
Peanuts	1988	20	4	0.54-1.29	0.92
Apples	1989	26	3	1.88-2.51	2.14

Fuller details of the studies carried out in 1986 have been published by Saxton *et al.* (1989). This paper also includes results obtained from some analyses for UDMH. They found that the level of UDMH relative to that of daminozide varied considerably from one product to another, indicating that UDMH residues are dependent not only on the daminozide concentration present in the product but also on the type of product and/or the amount of heat processing that occurs. Apart from two samples of canned cherries (0.6 and 0.3 mg/kg), residues of UDMH did not exceed 0.07 mg/kg.

#### METHODS OF RESIDUE ANALYSIS

For the daminozide residue determinations previously reviewed (FAO/WHO, 1977b), analyses carried out for Uniroyal Chemical used the colorimetric methods of Lane (1967) or Edgerton *et al.* (1967), in which the UDMH released by hydrolysis was reacted with trisodium pentacyanoamine ferroate or phosphomolybdic acid, respectively. The 1977 Meeting indicated that a more specific GLC or HPLC method would be desirable.

The more recent analyses have been carried out using such methods. In that of Abdel-Kader (1984), daminozide is extracted from apple homogenates with 0.01 N HCl and derivatized with acetic anhydride to form cyclic daminozide (N-dimethylaminosuccinimide) which, after extraction into dichloromethane, is determined by GLC with a nitrogenspecific detector.



Newsome (1980) described a method in which UDMH, obtained by the hydrolysis of daminozide, is isolated on a cation-exchange column and reacted with pentafluorobenzoyl chloride, the product being cleaned up on silica and determined by electron-capture GLC. This procedure has been developed further and improved by Newsome and Collins (1988), including confirmation by GC-MS. A similar procedure has been described by Breakall (1987).

In a variation of this method, proposed by Allen (1980), the UDMH formed by hydrolysis is allowed to react with salicylaldehyde to form a stable derivative that is amenable to GLC using a thermionic nitrogen detector for quantification. Results of the analysis of sweet cherries by this method were in close agreement with those obtained by the colorimetric method of Edgerton *et al.* (1967) and its modifications by Lynch (1969) and Dicks (1971). Although both types of method gave "complete recovery" when daminozide was used as the standard material, recoveries based on standard UDMH solutions showed recoveries of only about 25%. However, as this was a factor in the hydrolysis step it applied to both procedures and it is thus likely to be a feature of all methods based on this general approach.

This GLC procedure was adapted by Conditt *et al.* (1988) for the analysis of highprotein foods and a slightly modified version (Uniroyal, 1986) was used in the "market basket" study referred to above. This used hydrolysis in boiling aqueous 50% NaOH to release UDMH which was distilled off and reacted with salicylaldehyde to form salicylaldehyde dimethylhydrazone which, after partitioning into dichloromethane, was determined by GC-MS using selected ion monitoring of key ions in the fragmentation pattern. The limit of quantification is quoted as 0.1 mg/kg on a 50 g sample of peanuts or peanut butter.

Wright (1987) proposed a method for the determination of residues of UDMH in apple and pears. After extraction with aqueous L-ascorbic acid, reaction with 2-nitrobenzaldehyde forms the corresponding hydrazone which is then determined by GLC using an electron-capture detector. Recoveries were close to 100% at levels from 0.001 to 0.01 mg/kg.

An isotope dilution GC-MS procedure for the determination of residues of UDMH in apple juice and sauce has been reported by Moody *et al.* (1983), based on the method of Parkins *et al.* (1983). The samples to be analysed were fortified with a known amount of UDMH-[2-<sup>15</sup>N], derivatized with pentafluorobenzoyl chloride and extracted into dichloromethane for analysis by GC-MS with negative ion detection and selected ion monitoring at m/e = 404 and 405, the base peaks in the mass spectra of the <sup>14</sup>N and <sup>15</sup>N derivatives respectively. The UDMH concentration was determined by comparison of the ratio of the ions so detected with a calibration curve generated from samples of known <sup>14</sup>N/<sup>15</sup>N ratio. This technique had the advantages of an ability to cope with low and irreproducible recoveries, high sensitivity, freedom from interference and ease of sample preparation.

#### NATIONAL MAXIMUM RESIDUE LIMITS

The following MRLs were reported to the Meeting.

<u>Country</u>	<u>Commodity</u>	<u>MRL (mg/kg)</u>
The Netherlands	Pome fruit	5
	Other food	0* (0.5)

\*At or about the limit of determination.

The MRL applies to "Sum of daminozide and 1,1-dimethylhydrazine expressed as daminozide".

## APPRAISAL

Daminozide has uses as a growth regulator on apples and pears and also, to a much lesser extent, on other fruit crops such as cherries, seed potatoes, field beans and glasshouse ornamentals. Latest use patterns are more closely circumscribed than those presented in the 1977 Evaluations (FAO/WHO, 1978b) and residue levels now reported are appreciably lower.

The Meeting was informed by the major manufacturer that, as from October 1989, all registered uses of daminozide on food crops were to be withdrawn (Uniroyal, 1989). However, it was understood that formulations of daminozide were available from other sources.

## RECOMMENDATIONS

In view of the changed use pattern and corresponding lower residue levels observed, the Meeting withdrew all of the Guideline Levels recorded in 1977. The following maximum residue levels are estimated to meet recent use pattern requirements. As an ADI has now been estimated, they are suitable for the establishment of MRLs.

Definition of residue: sum of daminozide and 1,1-dimethylhydrazine expressed as daminozide.

Commodity	MRL (mg/kg)	PHI on which recommendations are based (days)
FP 0226 Apple	5 (prev. 30, GL)	42
FP 0230 Pear	5 (prev. 30, GL)	42

## FURTHER WORK OR INFORMATION

### Desirable

1. Further information on the conversion of daminozide to UDMH during processing.
2. Quantitative data on the conversion of daminozide to UDMH in domestic animals.

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