AMITON—A NEW ACARICIDE AND SCALICIDE*

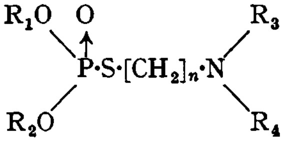
By G. L. BALDIT

The paper deals with the chemistry, biological activity, toxicology and residues in foodstuffs of *OO*-diethyl *S*-2-diethylaminoethyl phosphorothiolate and its salts.

Introduction

The numerous species of spider mites have always given trouble to agriculturalists and horticulturalists throughout the world, and research workers have tried to find a really efficient acaricide to be used at dosages lower than had ever before been considered practicable, which would be sufficiently persistent on foliage so as to avoid the necessity and expense of several applications during the growing season.

The field of organo-phosphorus chemistry seemed to promise success and, in 1952, Ghosh & Newman[1] discovered a novel series of basic organo-phosphorus esters with the general formula



$$(R_1O)(R_2O)\overset{O}{\overset{\uparrow}{P}}\cdot S\cdot[CH_2]_n\cdot N(R_3)(R_4)$$

where R_1, R_2, R_3 and R_4 are alkyl radicals.

Of a large series of compounds tested, *OO*-diethyl *S*-2-diethylaminoethyl phosphorothiolate (all R in the formula above are ethyl and *n* is 2), known originally under the code number R.5158 and which has now been given the common name of *amiton* by the British Standards Institution, was found to be the most efficient.[2]

Chemistry of amiton

Synthesis

Amiton can be prepared by condensing diethyl phosphorochloridothionate (I) with sodium diethylaminoethoxide (II) to give *OO*-diethyl *O*-2-diethylaminoethyl phosphorothionate (III) which isomerizes on heating at 100° to the thiol form, *OO*-diethyl *S*-2-diethylaminoethyl phosphorothiolate (IV). The reaction is shown diagrammatically in Fig. 1.

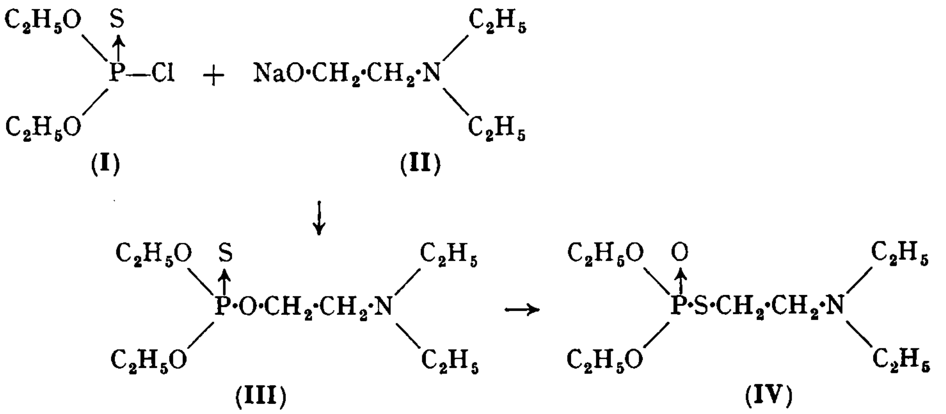


FIG. 1.—*Synthesis of amiton*

Physical properties

Amiton readily forms salts many of which, such as the hydrogen oxalate (code number R.6199) and the toluene-*p*-sulphonate (code number R.6200), are very soluble in water.

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* Read at 4th International Congress of Crop Protection, Hamburg, 1957, and at a meeting of the Pesticides Group, 18th November, 1957

The physical properties of amiton and the hydrogen oxalate and toluene-*p*-sulphonate salts are given in Table I.

Table I

Properties of amiton and its salts

Code No.	R.5158	R.6199	R.6200
Common name	Amiton	Amiton oxalate	Amiton toluene-*p*-sulphonate
Appearance	Colourless mobile liquid	White crystalline powder	
Boiling point	88°/0·04 mm.	—	—
Melting point	—	100°–101° (decomp.)	94°–96° (decomp.)
Density at 25°	1·048 g./ml.	—	—
Viscosity at 25°	4·85 cp.	—	—
Surface tension at 25°	31·2 dynes/cm.	—	—
Vapour pressure, mm. of Hg	$3{\cdot}2 \times 10^{-4}$ at 20° $5{\cdot}4 \times 10^{-4}$ at 25° $6{\cdot}0 \times 10^{-3}$ at 50°	Negligible	
Solubility	Water at 15°—infinitely Water at 25°—sparingly Organic solvents—very soluble	Very soluble in water and polar solvents. Insoluble in non-polar solvents	
Stability	Decomposed rapidly by alkalis and hypochlorite solutions into non-toxic compounds. Decomposed very slowly in acid or neutral solution		

It will be noted that the vapour pressure of amiton is of the same order as that of parathion ($1{\cdot}2 \times 10^{-4}$ mm. Hg at 20°) and about half that of demeton (10×10^{-4} mm. Hg at 33°). The solid salts have negligible vapour pressures so that hazard from the inhalation of vapour when handling is almost non-existent. This, together with the high water solubility of the oxalate and toluene-*p*-sulphonate, makes them very suitable for development as chemicals for crop protection.

The decomposition of amiton and its salts by alkalis and hypochlorite solution is made use of in decontamination of equipment and spillages. A 5% solution of caustic soda will completely decompose amiton and its salts into non-toxic compounds within an hour.

Amiton and its salts are compatible with all crop protection chemicals at present in use, but prolonged contact with alkaline spray chemicals should be avoided.

Analytical methods

No specific analytical methods for amiton and its salts have yet been developed. Infra-red techniques are used to measure P→O, P→S and P—OEt linkages and the oxalate and toluene-sulphonate radicals can be measured by standard methods. Gage[3] has used the high *in vitro* cholinesterase activity of amiton and its salts to measure very small quantities of amiton. This method has been adopted for the determination of residues in foodstuffs, and can also be used to assay amiton and its salts.

Biological results

Most of the field work to date has been carried out with the water-soluble powder, amiton oxalate.

Deciduous fruit

Preliminary results in the United Kingdom showed that amiton oxalate was not only a highly efficient acaricide but it was also highly selective, in that rates as low as 10 p.p.m. would kill fruit tree red spider or European red mite (*Metatetranychus ulmi*) while leaving other insects and predators unharmed.

Extensive trials in the United Kingdom on many varieties of apples showed that the optimum application rate was 2 oz. amiton oxalate/acre (140 g./hectare) applied either at high or low volume at the fruitlet stage. This treatment has given 100% control of red spider, not only until after the fruit has been picked but, from observations made this year, it seems likely that a satisfactory control during a second season can be obtained without further application.

Trials carried out in the U.S.A., South Africa, France and Italy have confirmed the efficacy of amiton oxalate in controlling numerous other mite species on deciduous fruit including *Tetranychus telarius*, *Bryobia praetiosa* and *Amphitetranychus viennensis*.

Tables II–V give some typical results, and compare amiton oxalate with chlorfenson, chlorbenside, demeton and methyl demeton.

Table II

Comparison of amiton oxalate with standard ovicides against the fruit tree red spider (Metatetranychus ulmi) *on apples in the United Kingdom*

Treatment	Rate/acre	Imp. gal./acre	Mites and eggs/100 leaves		
			June	July	September
Amiton oxalate	2¾ oz.	350	4	6	3
50% chlorfenson (applied in June)	9 lb.	350	1144	194	1150
Amiton oxalate	2 oz.	300	452	6	2
20% chlorbenside (applied in July)	6 lb.	300	286	680	1930
Amiton oxalate	2 oz.	300	84	12	1
20% chlorbenside (applied in July)	6 lb.	300	112	218	303
Amiton oxalate	2 oz.	50	—	360	65
50% methyl demeton (applied in Aug.)	20 oz.	50	—	1640	1160

— No assessment

Table III

Comparison of amiton oxalate with demeton against the European red mite (Metatetranychus ulmi) *on apples* in the U.S.A.*

Days after spraying	Mites/100 leaves	
	50% demeton 8 fl. oz./100 U.S. gal.	Amiton oxalate 2 oz./100 U.S. gal.
0	244	788
14	0	6
28	4	2
42	14	8
49	38	0

Application—high-volume—25th July, 1956

* This work was carried out by the Entomology Department, Rutgers University, New Jersey. (unpublished)

Table IV

Effect of amiton oxalate on red spider mite (Tetranychus telarius) *on pears in South Africa*

Days after spraying	Red spider mites/144 leaves	
	Untreated	Dosed with 0·32 oz./100 Imp. gal.
−1	1246	848
13	323	25
27	1769	4
41	1073	13
77	466	8

Application—6th January, 1955—Trees sprayed to run off

Table V

Effect of amiton oxalate on Bryobia mite (Bryobia praetiosa) *on apples in South Africa*

Days after spraying	Bryobia mites/40 leaves		
	Untreated	Dosage, oz./100 Imp. gal.	
		0·32	0·64
−1	1179	1262	1029
21	3506	16	21
35	2420	2	2
49	2026	1	1
63	1838	0	14

Application—25th January, 1955—Trees sprayed to run off

Cotton

Trials carried out in 1956 and 1957 in the cotton belt of the United States have shown that, when applied at 2½–5 oz./acre (180–360 g./ha.), amiton oxalate completely eradicates all mite species (including *Tetranychus bimaculatus*) within 24 hours of treatment and prevents any serious reinfestation for at least 6 weeks.

There is now evidence that at the high concentrations used for aerial spraying of cotton, the following insects are also controlled at the spider mite rate: flea hopper (*Psallus seriatus*), boll weevil (*Anthonomus grandis*), brown leaf worm (*Acontia glacia*), aphids (*Aphis gossypii*), lygus bugs.

Citrus

Although amiton was developed specifically as an acaricide, its powerful scalicide action was demonstrated in 1955 by Metcalf and his co-workers at the University of California, Citrus Experiment Station, Riverside.[4]

In 1956 trials were carried out in the major citrus-growing areas of South Africa, the Mediterranean and the U.S.A. These showed that amiton oxalate, used on a commercial basis, could successfully control the various species of mites and scale insects found on citrus.

Against mites, amiton oxalate gave extremely good control when used high-volume at 40 p.p.m. and low-volume at 400 p.p.m. On scale, high-volume applications at 150 p.p.m. gave complete control for the whole season on large citrus trees. Low-volume application at 400 p.p.m. did not give good control on large trees but on small trees adequate commercial control was obtained.

Some typical results on scale are shown in Tables VI and VII, and results on Texas red mite (*Anychus clarki*) and the rust mite (*Phyllocoptruta oleivora*) in the U.S.A. and Cyprus in Tables VIII–X, while Table XI lists the species of mites and scale insects of citrus which have been controlled by amiton.

Table VI

Effect of amiton oxalate applied low-volume on scale populations on citrus in Texas

Days after treatment	Total active scale population (Californian red scale, chaff scale, glover scale)					
	Total per 1 sq. in. area on each of 100 leaves		Total per 3 sq. in. surface area on each of 10 fruits		Total per 3 in. length on 10 new growth areas	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
−1	194	194	9	9	0	0
69	6	943	4	114	0	96
88	16	1276	3	108	0	117
119	64	2361	12	215	8	176

Application on 5th June, 1956 (4½ months before picking)

Strength of spray: 400 p.p.m. amiton oxalate
Volume of spray per acre: 136 U.S. gal.
Volume of spray per tree: 2½ U.S. gal.
Active ingredient per acre: 7·2 oz. amiton oxalate
Height of tree: 9 ft.

Table VII

Effect of high-volume spraying of amiton oxalate on soft brown scale (Coccus hesperidum) *in South Africa*

Amiton oxalate, oz. per 100 Imp. gal.	Concentration, p.p.m.	% adult scale dead, days after spraying	
		13	22
1·5 oz.	100	39·6	32·3
3·0 oz.	200	89·5	55·6
6·0 oz.	400	92·4	96·9
Control	—	3·2	6·8

Application—10 gal. per tree on 21st November, 1956

Penetration and movement within the plant

The behaviour of ^{32}P-labelled amiton and its salts has been investigated by Metcalf *et al.*[5] using cotton and lemon plants. It was found that the base, amiton, penetrates leaves about ten times as fast as amiton oxalate solid. More recent work has shown that in acid solution amiton oxalate does not penetrate, but at a pH of 7·5 or above, penetration of the cuticle and translocation through the plant takes place readily. This is probably due to the fact that in alkaline solution amiton oxalate readily dissociates, and if the water is hard, the oxalate ion will be precipitated as insoluble calcium oxalate.

Table VIII

Effect of amiton oxalate on rust mite (Phyllocoptruta oleivora) *in Texas. Visual assessment for mites on fruit*

Method of application	Low-volume		High-volume		High-volume	
Strength of spray	400 p.p.m.		400 p.p.m.		40 p.p.m.	
Amiton oxalate, oz./acre	7·2		5·5		0·5	
Height of tree	9 ft.		4 ft.		3 ft.	
Time after treatment	Treated	Untreated	Treated	Untreated	Treated	Untreated
−1 day	A	A	A	A	A	A
1 week	O	S	O	A	A	A
4 weeks	O	S	O	A	A	S
8 ,,	O	M	O	A	A	S
12 ,,	O	H	A	S	S	S
16 ,,	A	H				

O Nil population
A Active stages present
S Slight mite population present
M Medium mite population present
H Heavy mite population present

Table IX

Effect of amiton oxalate on citrus rust mite (Phyllocoptruta oleivora) *on lemons in Cyprus*

Days after treatment	No. mites per 20 fruits			
	Dosage, oz./100 Imp. gal.			Untreated
	0·21	0·42	0·84	
8	12	6	2	562
15	10	0	10	803
25	0	0	0	1096
37	0	0	0	1770
46	0	0	0	2065
53	0	0	0	1355
68	20	30	70	695
81	37	15	158	412

Application—1000–1200 Imp. gal./acre on 15th July, 1955

Table X

Effect of amiton oxalate on Texas red mite (Anychus clarki) *on citrus*

Days after treatment	Adult mites per 100 leaves					
	Grapefruit		Grapefruit		Oranges	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
−1	5584	5584	5584	5584	2906	2906
2	0	—	0	—	—	—
15–18	0	8758	0	8758	0	2563
55–58	86	—	86		16	3462
69–70	94	6435	2nd spray application	6435		
76–78	—	—	0	—		
102–114	386	9564	0	9564		

— No assessment

Application—7·2 oz. amiton oxalate in 136 U.S. gal./acre (400 p.p.m.)

Table XI

Citrus mite and scale species controlled by amiton oxalate

Mites

Paratetranychus citri	Citrus red mite
Anychus (*Eutetranychus*) *clarki*	Texas red mite
Phyllocoptruta oleivora	Citrus rust mite

Scale insects

Aonidiella auranitii	Californian red scale	*Icerya purchasi*	Cottony cushion scale
Aonidiella citrina	Yellow scale	*Coccus hesperidum*	Soft brown scale
Parlatoria pergandii	Chaff scale	*Lepidosaphes gloverii*	Glover scale
Lepidosaphes beckii	Purple scale	*Chrysomphalus ficus*	Circular purple scale
Saissetia oleae	Black scale	*Chrysomphalus dictyospermi*	Dictyospermum scale

This finding probably accounts for a number of otherwise anomalous residue figures which have been obtained. This aspect is being further investigated in all citrus-growing States in the U.S.A. this year.

Effect of amiton oxalate on predators

The insecticidal activity of amiton oxalate is much lower than its activity against mites. At acaricidal concentrations, amiton oxalate produces little disturbance of predator populations. The probable reason for this is that amiton oxalate has no fumigant action, and its contact toxicity is low. The slowly liberated free amine which is formed in solution possesses both fumigant and contact effect, but it passes readily through the plant cuticle and so is less dangerous to the non-plant-feeding predator insect.

Field observations have been made on several beneficial predators and detailed laboratory tests have been carried out in California on the following insects:

Stethorus picipes (Coccinellid)—major predator of citrus red spider
Metaphycus luteolus (Hymenoptera)—parasite of soft brown scale
Hippodamia ambiqua (Coccinellid)—general predator
Orius insidiosus (*Homoptera*)—predator of mites, thrips, etc., on cotton and lucerne

It was found that amiton oxalate at 200 p.p.m.—i.e. five times the concentration needed to kill mites—had no effect on *Hippodamia ambiqua*, gave less than 50% kill of *Stethorus picipes* but was slightly more harmful to *Metaphycus luteolus* and *Orius insidiosus*.

Toxicology

Most of the toxicological work on amiton and its salts has been carried out by the Industrial Hygiene Research Laboratories of Imperial Chemical Industries, Ltd., and will be reported in detail elsewhere.

In common with other organo-phosphorus compounds, amiton is toxic to mammals by virtue of its ability to inhibit cholinesterase. Excessive absorption is attended by a reduction of this enzyme in the blood and nervous system, leading to symptoms typical of parasympathetic stimulation. There is no evidence that it possesses any other type of toxic action.

The *acute oral toxicity* to rats ranges from 3·3 mg./kg. for females to 7·0 mg./kg. for the larger males. These figures indicate that the oral toxicity of amiton is about double that of parathion.

The LD_{50} to rats by *intraperitoneal injection* is 0·5 mg./kg., compared with about 4 mg./kg. for parathion reported by Du Bois *et al.*[6]

By *intratracheal injection* the LD_{50} to rats is about 0·1 mg./kg., but since the oxalate and toluene-*p*-sulphonate salts of amiton are solids with negligible vapour pressures, there is little risk of inhalation of their vapours.

The most likely routes of absorption of pesticides into the human body are through the eyes and skin. When a solution of amiton oxalate is instilled into the *conjuctival* sac of a rabbit's eye, the LD_{50} is in the region of 0·25 mg./kg. but a dose of 0·1 mg./kg. produces miosis lasting several hours. For a human being weighing 70 kg. it is probable that just under 0·2 g. of amiton oxalate in the eye would be fatal. This amount would correspond with about half a litre of spray fluid at low-volume spraying strength for deciduous fruit.

Experiments on percutaneous *absorption* show clearly that the salts of amiton are less hazardous than the base itself. The LD_{50} to rats when a strong aqueous solution of amiton oxalate is applied to the skin is about 1·3 mg./kg. For amiton in toluene the figure is about 0·2 mg./kg. This compares with 9·6 mg./kg. for neat demeton applied in the same way. When more dilute solutions of amiton oxalate are maintained in contact with the skin of rats for 6 hours, a concentration of 500 p.p.m. is lethal, while 90 p.p.m. produces no symptoms other than a slight inhibition of blood cholinesterase. The toxicity of the dry crystals in contact with the skin is very much lower, but owing to variations in absorption, no definite figure can be given.

When considering the possible harmful effect of residues of amiton in foodstuffs, the results of long-term feeding of sub-acute doses are more important than acute toxicity figures.

Rats fed for three months on a diet containing 20 p.p.m. of amiton oxalate showed inhibition of blood and brain cholinesterase, but no abnormality was revealed by a post-mortem histopathological examination. Dogs fed for several months on diets containing 0·04 p.p.m. of amiton oxalate show no inhibition of red cell cholinesterase, but a slight depression of the plasma enzyme.

Della Bella & Rota[7] fed apples which had been sprayed 10 days previously with amiton oxalate* to rabbits in amounts corresponding to 50 and 100 g./kg. body weight. After ten days, no diminution in serum cholinesterase activity was observed.

These toxicological results indicate that, although amiton and its salts must be regarded as highly toxic, the handling of the concentrate in the field in the form of solid amiton oxalate can be accomplished with no greater hazard than accompanies the use of established organophosphorus pesticides. Since the compound is used only once a season, it can be argued that risks are very small. Nevertheless, the commonsense precautions which are advocated for parathion and demeton must be observed.

Residues in foodstuffs

The extensive biological work which has been carried out with amiton oxalate has been accompanied by a corresponding amount of analytical work to determine the residues left in foodstuffs.

Although no specific method of analysis for amiton is available, its high *in vitro* cholinesterase activity has allowed methods developed by Gage[3] to be used with a high degree of precision—less than 1 μg. of amiton can be detected.

So far, work has been concentrated on apples, citrus and cotton, but information on residues in grapes, peaches and hops is now being collected.

Apples

Residue tests have been run on numerous varieties of apple in several countries. When sprayed at the recommended rate of 2 oz. of amiton oxalate/acre (140 g./hectare) at the fruitlet stage, the residues at harvest 2½–3 months later are of the order of 0·04 p.p.m.

Fig. 2 shows how the amounts of residue fall after spraying.

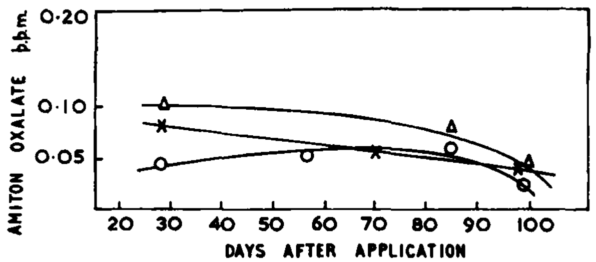

FIG. 2.—*Change of amiton oxalate residues on apples with time*

○ ¾ oz. of amiton oxalate/acre
× 1½ oz. of amiton oxalate/acre
△ 3 oz. of amiton oxalate/acre

Citrus

Information on the residues on citrus has been obtained in the U.S.A. and Italy. In the U.S.A., trials carried out on oranges, grapefruit, tangerines and lemons involving 800 analyses showed that, applied at the spider rate of 3 oz. per acre, amiton oxalate residues fell to below 0·01 p.p.m. after 60 days. When used at rates up to 60 oz. per acre, amiton oxalate residues were less than 0·012 p.p.m. after 90 days. These levels of residue were reached irrespective of the method of application or type of fruit.

Some typical results indicating the fate of the residues on processing citrus are given in Table XII.

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* Although not stated in the paper, the amiton oxalate was used at 200 g./hectare.

Table XII

Residues of amiton oxalate on Texas citrus

Portion of fruit	Amiton oxalate, p.p.m.	
	Grapefruit	Oranges
Whole washed fruit	0·010	0·005
Peel	0·034	0·006
Pulp	0·004	0·004
Dried peel	0·076	0·048
Canned peel	0·011	No sample
Juice	0·008	0·005
Molasses	0·011	0·038

Fig. 3 shows how amiton oxalate residues in growing oranges vary with time after application. The oranges were washed before analysis. The effect of a long drought which caused dehydration of the fruit while growing is clearly shown.

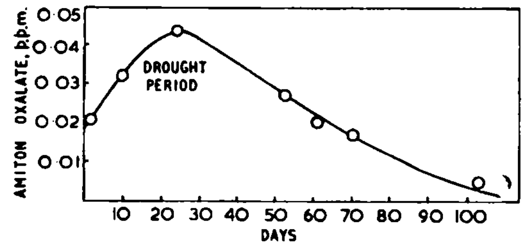

Fig. 3.—*Change in amiton oxalate residues in whole washed oranges with time*

Application : 7·2 oz. of amiton oxalate in 150 U.S. gal./acre (Texas)

Further extensive trials are in progress so that information on residues in citrus under a variety of climatic conditions can be obtained. Results obtained in Italy on mandarins and oranges give residues of the same order.

Cotton

As cottonseed oil is used in the manufacture of margarine, mayonnaise, etc., and cottonseed cake is fed to animals, information on the residues of amiton oxalate in cotton was obtained.

Cotton in the Rio Grande Valley of Texas was sprayed by aeroplane and ground machines with rates from 2 to 5½ oz. of amiton oxalate in 2–25 U.S. gal./acre, one to two months before bolls opened.

The *maximum* amounts of amiton oxalate found in the various portions of the ginned seed were: linters 0·075, raw oil 0·020 and cake 0·003 p.p.m.

Typical results are given in Table XIII.

Table XIII

Amiton oxalate residues in cotton in Texas

Application details		Sampling details		Residues—p.p.m. of amiton oxalate				
Rate per acre	Air or ground	Position of bolls	Days after application	Raw oil	Cake	Linters (calc. on whole undelinted seed)	Whole delinted seed	Whole undelinted seed
2·2 oz./25 U.S. gal.	Ground	Bottom	32	0·003	0·002	0·071	0·002	0·073
		Mid upper	49	N.D.	0·001	0·020	<0·001	0·021
		Top	56	N.D.	N.D.	N.D.	N.D.	N.D.
2·6 oz./5 U.S. gal.	Air	Bottom	24	0·014	N.D.	0·015	0·001	0·025
		Middle	24	<0·001	N.D.	0·035	Trace	0·035
		Mid upper	37	N.D.	0·002	0·001	0·002	0·003
		Top	45	0·001	N.D.	N.D.	<0·001	<0·001
2·6 oz./2 U.S. gal.	Air	Middle	37	N.D.	0·0025	0·011	0·003	0·013
5·3 oz./2 U.S. gal.	Air	Middle	37	<0·001	N.D.	0·033	Trace	0·033
		Mid upper	51	0·003	0·002	0·059	<0·001	0·060
		Top	56	<0·001	N.D.	N.D.	Trace	Trace

N.D. = None detected

It has further been established that 75% of the amiton oxalate in the cake and over 90% of that in the oil is destroyed by the normal commercial process of ' cooking ' and alkali refining.

Commercial form of amiton

This year, amiton is being sold in the form of the oxalate. The commercial product which contains 75% of amiton oxalate (equivalent to 56% amiton) is a dark violet-coloured, non-dusty solid which dissolves very rapidly in water. The colour is due to a dyestuff which is incorporated in order to make spillages etc. readily detectable.

Glossary

Throughout this paper the common names of pesticides as approved by the British Standards Institution[8] are used. The correct chemical equivalents are given below:

amiton:	*OO*-diethyl *S*-2-diethylaminoethyl phosphorothiolate
chlorbenside:	*p*-chlorobenzyl *p*-chlorophenyl sulphide
chlorfenson:	*p*-chlorophenyl *p*-chlorobenzenesulphonate
demeton:	a mixture of diethyl 2-ethylthioethyl phosphorothionate and *OO*-diethyl *S*-(2-ethylthioethyl) phosphorothiolate
methyl demeton:	a mixture of 2-ethylthioethyl dimethyl phosphorothionate and *S*-2-ethylthioethyl dimethyl phosphorothiolate
parathion:	diethyl *p*-nitrophenyl phosphorothionate

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THE TOCOPHEROL CONTENTS OF SOME AUSTRALIAN CEREALS AND FLOUR MILLING PRODUCTS

By E. L. MASON and W. L. JONES

A modification is proposed of the two-dimensional chromatographic procedure of Green *et al.* in order to achieve uniform and replicate separation of the tocopherol spots. The proposed method is more suitable than the previous procedure for control purposes in a small laboratory. Results are recorded for a number of Australian cereals.