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Recommended Methods of Analysis of Pesticide Residues in Foodstuffs

REPORT BY THE JOINT DIMETHOATE RESIDUES PANEL

SET UP JOINTLY BY THE SCIENTIFIC SUB-COMMITTEE ON POISONOUS SUBSTANCES USED IN AGRICULTURE AND FOOD STORAGE, THE ANALYTICAL METHODS COMMITTEE OF THE SOCIETY FOR ANALYTICAL CHEMISTRY AND THE ASSOCIATION OF BRITISH MANUFACTURERS OF AGRICULTURAL CHEMICALS

The Determination of Dimethoate Residues in Fruits and Vegetables

THE Panel was set up by the Scientific Sub-Committee of the Advisory Committee on Pesticides and other Toxic Chemicals, the Association of British Manufacturers of Agricultural Chemicals and the Analytical Methods Committee of the Society for Analytical Chemistry to establish by collaborative study an accurate and reproducible method for the determination of dimethoate residues in crops. German, Italian and United States' workers also joined the Panel. The Panel first met at the end of 1964 and undertook a series of collaborative studies, at first with Chilwell and Beecham's method[1] and then with Frehse's method (H. Frehse, private communication). This report describes the findings of the Panel and recommends Frehse's method for determining dimethoate residues in apples, pears, cauliflowers, peas, cabbages, blackcurrants, olives and oranges. A semi-quantitative identification procedure is also described.

Members of the Panel are listed in Appendix III.

LITERATURE SURVEYED BY THE PANEL—

At its first meeting the Panel carefully considered the methods available for the determination of dimethoate residues in fruits and vegetables and also an assessment, subsequently published by Smart,[2] of three of the more important methods. He found that Laws and Webley's general method[3] was satisfactory for residues of dimethoate in sprouts, lettuces and apples, but not in peas. Chilwell and Beecham's method was also satisfactory at the 1 or 2 p.p.m. level of added dimethoate in cabbages, lettuces, apples and peas. Giang and Schechter's method,[4] however, gave variable results. Fukel'man[5] also investigated Chilwell and Beecham's method and proposed some modification. de Pietri-Tonelli[6] has reviewed the methods available for dimethoate residues analysis. Since this review, several further relevant papers have been published and were considered by the Panel. George, Walker, Murphy and Giang[7] described a method involving reaction with 1-chloro-2,4-dinitrobenzene in methanolic sodium hydroxide, and used it for determinations on a range of plants and vegetables. Engst and Kubel[8] used thin-layer chromatography for the quantitative determination of dimethoate residues, and Mitsui and Suzuki[9] also published a method for the thin-layer chromatographic separation and colorimetric analysis of dimethoate residues. Abbott, Bunting and Thomson[10] used multi-band chromatoplates for the determination of residues of dimethoate in a variety of crops. Smart and Hill[11] described paper and thin-layer chromatographic separations of some polar organophosphorus insecticides extracted and cleaned-up by Frehse's method.

Ashworth[12] has described a gas - liquid chromatographic method for determining dimethoate residues in flue-cured tobacco.

P=S dimethoate is the active ingredient of formulated dimethoate and is gradually metabolised *in vivo* to the more toxic P=O dimethoate. Thus, as residues of dimethoate on a crop decrease with time, the toxicity of the residue per microgram of insecticide may increase, although the weathered residue rarely contains more than 10 to 20 per cent. of the total dimethoate residue in the form of the oxygen analogue. Data, both published and unpublished, on the toxicity and residues of P=O dimethoate were scrutinised closely by the Panel. The Panel considered that a strong case could not be made out for a method to

determine P=O dimethoate separately from P=S dimethoate, and hence the recommended method determines both P=S and P=O dimethoates together. Evidence for the presence or absence of P=S and P=O dimethoate individually by a semi-quantitative identification procedure is also described.

Chilwell and Beecham's method

After considering the available methods, the Panel selected Chilwell and Beecham's micro distillation procedure for initial collaborative study. The method had given good reproducible blank and recovery values in the hands of several workers and was robust, standing up well to Youden's test.[13] The dilute tin(II) chloride solution used for developing the molybdenum-blue colour was stabilised by addition of hydrazinium sulphate.

Table I

Recovery of added dimethoate from apples and cabbages by Chilwell and Beecham's method

	Collaborative study No. 1				Collaborative study No. 2	
	Net recovery from apples of		Net recovery from sprouts of		Net recovery from apples of	
Laboratory	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.
1	0·24* 0·6*	2·1† 1·5†	— —	— —	0·7 0·35 0·35 0·34	1·8 1·8 1·8 1·7
2	0·44 0·36 0·27	0·86 1·02 1·08	0·13 0·30 0·34	1·26 0·74 1·52	0·48 0·65 0·63 0·54 0·39 0·56	1·85 2·05 1·70 1·73 2·05 1·94
3	0·37 0·51 0·43	1·54 1·69 1·42	0·45 0·28 0·21	1·09 1·01 0·83	0·46 0·28 0·23	1·42 1·46 1·38
4	0·36 0·47	1·42 1·44	0·41‡ 0·39‡ 0·39‡	1·40‡ 1·62‡ 1·65‡	0·58 0·49 0·42	1·69 1·82 1·85
5	0·50	—	—	—	0·45 0·49 0·48	1·83 1·83 1·81
6	0·17 0·23 0·57 0·34	1·08 0·78 1·00	0·29 0·46 0·65	0·96 0·96 1·34 1·02 1·56	0·51 0·42 0·34	1·62 1·46 1·54
7	—	—	—	—	0·80‡ 0·62‡ 0·30‡ 0·28‡	1·2‡ 1·30‡ 1·54‡ 1·54‡
8	—	—	0·40 0·42	1·12 1·10 1·20 1·22 1·30	0·48 0·47 0·40	1·87 1·83 2·05
		1·0 *p.p.m.* level		1·0 *p.p.m.* level		
9	0·33 0·35 0·37	0·88 0·74 0·72	0·34 0·40 0·38	0·73 0·81 0·76	—	—

* Added, 0·45 p.p.m. † Added, 1·80 p.p.m. ‡ By using a modified method.

In the first collaborative study, members of the Panel recovered recrystallised P=S dimethoate from untreated apples and Brussels sprouts at the 0·5 and 2·0 p.p.m. levels.

When studying recovery in this and subsequent collaborative work the pesticide was added before maceration. The results obtained are given in Table I. Reagent and crop-blank values are given in Table II. Although the blank values were generally reasonable, the

TABLE II

BLANK VALUES FOR REAGENTS AND UNTREATED CROPS BY CHILWELL AND BEECHAM'S METHOD

	Collaborative study No. 1			Collaborative study No. 2	
Laboratory	Reagent blank, p.p.m.	Apple blank (*less* reagents), p.p.m.	Sprout blank (*less* reagents), p.p.m.	Reagent blank, p.p.m.	Apple blank (*less* reagents), p.p.m.
					0·29
					0·29
1	0·15	Nil	—	0·30	0·17
	0·14	—	—	0·30	0·27
	0·16	—	—	0·27	0·30
	0·13	—	—	—	0·30
					0·38
					0·47
2	0·14	0·05	0·08	0·14	Nil
		0·08	Nil	0·11	0·14
		0·09	0·07	0·11	0·06
3	0·04	0·10	0·03	0·18	0·22
	0·04	0·09	0·04	0·10	0·13
	0·05	0·08	0·06	0·10	0·05
4	—	<0·02	0·02*	0·04	0·15
			0·08*	0·01	0·09
			0·11*	0·04	0·14
5	0·04	0·04	—	0·03	0·04
	0·02	0·08	—	0·04	0·02
	0·02	0·06	—	0·04	0·03
6	0·20	Nil	Nil	0·50	0·36
	0·12	0·02	Nil	0·58	Nil
	0·12	0·20	0·06	0·41	Nil
	0·20	0·13	0·11	0·46	Nil
		0·07			
		0·15			
7	—	—	—	0·08*	0·12*
				0·08*	0·12*
				0·08*	0·04*
				0·08*	0·04*
8	—	—	—	0·05	0·32
				0·10	0·32
					0·33
					0·32
					0·33
					0·32
					0·53

* By using a modified method.

Panel considered that the recovery values were somewhat disappointing. Several laboratories reported difficulty in filtering the homogenised extract and, for the second collaborative study, an acetone extraction was used, followed by chloroform partition and micro distillation, as in Chilwell and Beecham's original method. It was noted that the reduction of the molybdophosphate complex should be conducted in strongly acidic solution, otherwise the blue colour does not develop properly. In the initial study, some laboratories suggested that the calibration graph was not linear at about 3 p.p.m., or above, and so more reducing agent was used to reduce the molybdophosphate complex subsequently and is advocated in the recommended method. The end of the cold finger should be ground to give greater surface area for condensation of the micro distillate.

The second collaborative study was carried out on apples, again at 0·5 and 2·0 p.p.m. levels, and the results obtained for recoveries are given in Table I, and those for blanks in Table II. The mean recovery of P=S dimethoate from apples at the 0·5 p.p.m. level was 0·46 p.p.m., with a standard deviation of ±0·11 p.p.m., and at the 2·0 p.p.m. level 1·76

p.p.m., with a standard deviation of ±0·19 p.p.m. (neglecting the results from laboratory 7 in which a modified method was used). The Panel considered these recovery values satisfactory for a residue method, but noted the confusing variation in blank values not only between laboratories but sometimes within laboratories. High reagent blanks could in some instances be ascribed to phosphorus in the perchloric acid or to the hydrazine reagent. To avoid "spitting" during the perchloric acid digestion, some members used an asbestos mat on top of the electric hot-plate to counteract uneven heating effects, or micro Kjeldahl flasks with gas burners. One laboratory investigated the modified Chilwell and Beecham procedure on other crops; blank values of 0·05 to 0·10 p.p.m. were obtained with cabbages and 90 to 95 per cent. of added P=S dimethoate was recovered at the 2 p.p.m. level; high blank values with cauliflowers, blackcurrants and peas could be reduced to an acceptable level by treating the chloroform extract with Nuchar C190N before micro distillation; recoveries with the latter crops were 80 to 90 per cent. This laboratory reported 60 per cent. recovery of P=O methoate from apples at the 2 p.p.m. level when using the modified procedure. Another laboratory reported only 20 per cent. recovery of added P=O dimethoate from apples.

Frehse's method

At this stage Frehse introduced to the Panel his method involving the use of an aluminium oxide column clean-up. It consisted of extraction with acetone, evaporation of the acetone, filtration, extraction of the aqueous extract with chloroform, purification of the chloroform extract by chromatography on a Brockman Grade V aluminium oxide column, followed by evaporation of the eluate, wet ashing the residue and molybdenum-blue determination of phosphorus. The details are essentially those set out in Appendix I. The method had given good recoveries and low blank values with a range of crops in Frehse's laboratory. It was,

Table III

Blank values for reagents and untreated crops by Frehse's method

	Collaborative study No. 3			Collaborative study No. 4		
Laboratory	Reagent blank, p.p.m.	Apples blank (*less* reagents), p.p.m.	Cauliflowers blank (*less* reagents), p.p.m.	Reagent blank, p.p.m.	Peas blank (*less* reagents), p.p.m.	Cabbages blank (*less* reagents), p.p.m.
1	0·32	0·13	0·10	0·32	0·15	—
	0·31	Nil	0·07	0·30	0·08	—
2	0·14	Nil	0·03	—	—	—
	0·10	Nil	0·01			
	0·14	0·02	Nil			
	0·03	0·04	0·16	0·01	0·17	0·12
	0·02	0·06	0·10	0·01	0·17	0·06
	0·02	0·04	0·13	Nil	0·14	0·11
4	0·30	0·01	Nil	—	—	—
	0·19	Nil	Nil			
	0·22	Nil	0·04			
5	0·07	0·15	0·07*	0·07	Nil	0·02
	0·07	0·17	0·10*	0·07	0·05	0·05
	0·05	0·15		0·09	0·01	0·03
7	0·06	0·05	0·15	0·04	0·07	0·07
	0·06	0·05	0·15	0·06	0·07	0·07
	0·06			0·06	0·07	0·09
	0·08				0·09	
	0·08					
	0·09					
8	0·05	0·20	0·05	—	—	—
	0·03	0·24	Nil			
		0·21	Nil			
		0·23				
		0·42				
		0·43				
		0·14				

* By using Super-Cel filtration.

TABLE IV

RECOVERY OF KNOWN AMOUNTS OF ADDED DIMETHOATE FROM APPLES, CAULIFLOWERS, PEAS AND CABBAGES BY FREHSE'S METHOD

	Collaborative study No. 3				Collaborative study No. 4			
	Net recovery from apples of		Net recovery from cauliflowers of		Net recovery from peas of		Net recovery from cabbages of	
Laboratory	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.	0·5 p.p.m., p.p.m.	2·0 p.p.m., p.p.m.
1	0·47	1·7	0·44	1·5	0·38	1·2		
	0·29	1·8	0·29	1·6	0·28	1·4		
2	0·38	1·47	0·43	1·77	—	—	—	—
	0·53	1·83	0·38	1·78				
	0·52	1·83	0·34	1·74				
3	0·39	1·57	0·50	1·48	0·41	1·28	0·36	1·45
	0·35	1·50	0·37	1·46	0·39	1·52	0·39	1·62
	0·42	1·63	0·32	1·60	0·36	1·55	0·36	1·56
4	0·37	1·83	0·40	1·81	—	—	—	—
	0·47	1·70	0·70	1·75				
	0·56	1·63	0·46	1·77				
5	0·47	2·08	0·46*	1·95*	0·51	1·88	0·48	1·95
	0·44	1·97	0·50*	1·97*	0·52	1·97	0·50	1·96
	0·52	2·00	0·48*	2·01*	0·53	2·03	0·47	1·99
7	0·46	1·92	0·44	1·48	0·47*	1·79	0·49†	0·9†
	0·48	1·94	0·44	1·52	0·49*	1·79	0·51†	0·91†
			0·44	1·54	0·51*	1·81	0·53†	0·95†
				1·54	0·51*			0·99†
8	0·50	1·91	0·37	1·93	0·43	2·0	—	—
	0·50	1·73	0·44	1·86	0·41	1·7		
	0·50	1·89	0·63	1·63	0·43	1·7		
Mean	0·46	1·83	0·45	1·73	0·43	1·69	0·44	1·76
Standard deviation ..	±0·06	±0·17	±0·10	±0·18	±0·06	±0·26	±0·06	±0·19

* By using Super-Cel filtration.
† 0·54 p.p.m. added at 0·5 p.p.m. level and 1·08 p.p.m. at 2 p.p.m. level.

therefore, submitted to collaborative study to compare it with Chilwell and Beecham's method. Recovery of added P=S dimethoate from apples and cauliflowers was first examined in the third collaborative study, and then from peas and cabbages in the fourth collaborative study. Blank values for these studies are given in Table III and recovery values in Table IV. High reagent blanks obtained in two laboratories were ascribed to impurities in the aluminium oxide and in the acetone used. It is important to cool the aqueous extract thoroughly before

TABLE V

RECOVERY OF ADDED DIMETHOATE FROM SUNDRY CROPS BY FREHSE'S METHOD

		Net recovery	
Crop	Total blank	0·5 p.p.m. level, p.p.m.	2·0 p.p.m. level, p.p.m.
Blackcurrants	0·14	0·53	—
	0·11	0·49	
Orange flesh	0·15	—	1·91
	0·14		1·93
Orange peel	0·09	—	1·95
	0·13		1·83
Pears	0·05	0·43	1·82
	0·05	0·43	1·84
	0·05	0·32	1·81
Olives	0·07	0·78	—
	0·10	0·61	
		0·53	

filtering, otherwise high blank values can occur. Results for the method with other crops were obtained in individual laboratories and are set out in Table V. With olives it was necessary to extract the aqueous solution with two 50-ml volumes of hexane before partition with chloroform, otherwise oils interfered seriously with the wet-oxidation stage. Although it took slightly longer to run single determinations with Frehse's method than with Chilwell and Beecham's method, several determinations could more easily be run together, and the Panel considered it to be more reliable than the latter.

The Panel tested the recovery of P=O dimethoate by Frehse's method for the fifth collaborative study. Recovery values, at 0·5 p.p.m. level, are set out in Table VI.

TABLE VI

RECOVERY OF A KNOWN AMOUNT OF P=O DIMETHOATE FROM APPLES BY FREHSE'S METHOD

Laboratory	Net recovery of 0·5 p.p.m. of P=O dimethoate, p.p.m.		
2	0·35	0·40	0·36
3	0·24	0·24	0·24
5	0·30	0·35	0·28
7	0·29	0·31	0·33
8	0·48	0·36	0·41
Mean	0·33		
Standard deviation ..	±0·07		

As a final test of Frehse's method the Panel recovered unknown amounts of P=S dimethoate from locally purchased apples. The amounts of dimethoate found by the collaborating laboratories are set out in Table VII.

TABLE VII

RECOVERY OF UNKNOWN AMOUNTS OF ADDED DIMETHOATE FROM APPLES BY FREHSE'S METHOD

Laboratory	Net recovery of 0·70 p.p.m., p.p.m.	Net recovery of 1·40 p.p.m., p.p.m.
1	0·62, 0·57	0·32, 0·93
3	0·66, 0·61	1·31, 1·36
4	0·61, 0·75	0·88, 0·94
5	0·73, 0·78	1·56, 1·53
7	0·67, 0·69	1·39, 1·41
8	0·94, 0·94	1·39, 1·39
10	0·80, 0·83	1·39, 1·58
11	0·58, 0·78	1·19, 1·13
Mean	0·72	1·29
Standard deviation ..	±0·12	±0·23

A slight modification of the original procedure for evaporating the acetone after extraction was checked and found to be satisfactory, and has been incorporated into the method.

The Panel recommends Frehse's method, set out in detail in Appendix I, for the quantitative determination of dimethoate residues in fruits and vegetables.

SEMI-QUANTITATIVE IDENTIFICATION OF DIMETHOATE

At its initial meeting the Panel considered that if the quantitative recommended method were not specific for dimethoate a semi-quantitative identification step should be recommended for use alongside it to establish any residues determined as dimethoate. A semi-quantitative identification of P=O dimethoate was also considered necessary to give an approximate assessment of the amount of P=O dimethoate in the residue, thus enabling a more accurate toxicological evaluation (P=O dimethoate may be ten times more toxic than P=S dimethoate). It was clearly advantageous if a semi-quantitative identification step for P=O and P=S dimethoates could be worked out following the extraction and clean-up used in the proposed quantitative method, rather than initiate a new extraction and clean-up procedure.

THIN-LAYER CHROMATOGRAPHY—

One laboratory, therefore, investigated various published and unpublished paper and thin-layer chromatographic techniques on the cleaned-up extract from Frehse's method. The problem appeared to be not only one of identifying dimethoate in a sample known to have been sprayed with the insecticide, but also that of identifying P=O and P=S dimethoates in a sample whose spray history was not known. To this end, nearly thirty other organophosphorus insecticides and metabolites were screened to see whether they were likely to interfere with the determinations. Identification of 0·1 p.p.m. insecticides, particularly of the P=O metabolite, was sought. In this laboratory, difficulty was encountered with Abbott's multi-band identification technique; the R_F values were appreciably higher than reported in the literature but clean-up of cabbage extracts was good. Other laboratories, however, had achieved satisfactory results. The silica gel - kieselguhr (1 + 1) system, used in the multi-band plates, provided a good separation in two dimensions, and gave acceptable identification to a first approximation. Alumina plates were not satisfactory as they gave poor separation in the solvents required to move the pesticide from the origin. Steller's method[14] gave a useful separation, but crop interferences were observed.

Modifications, involving both paper and thin-layer chromatography, of Bates'[15] formamide-impregnated systems were worked out and gave good identification of both P=O and P=S dimethoates in a wide range of crops. Some further clean-up was, however, necessary with some crops. Adequate sensitivity was obtained with the 4-(*p*-nitrobenzyl)pyridine reagent of Watts.[16] This work was subsequently published by Smart and Hill.[11]

The use of polyamide plates was also investigated and adequate separation achieved by using a two-dimensional technique, but cauliflowers and peas, even after further clean-up, did not give satisfactory chromatograms.

As the silica gel - kieselguhr (1 + 1) and formamide-impregnated, silica gel thin-layer chromatographic systems were the most successful, these were examined collaboratively by the Panel. Laboratories investigated the methods on several crops and found them generally satisfactory, except that in some instances overloading was contributing to some distortion of R_F values of the pesticides extracted from crop materials compared with standards run at the sides of the plates. Smaller aliquots (corresponding to 5 g of crop material) of cleaned-up extracts were, therefore, taken for the eighth collaborative study on the same thin-layer chromatographic systems, and the effect of brief drying of the plates at 110° C between runs to regularise R_F values was also investigated.

Distortion of R_F values and interferences were less with the smaller samples, and 0·1 p.p.m. (0·5 μg) of P=S and P=O dimethoates was detectable on silica gel - kieselguhr plates. The formamide-impregnated plates were somewhat less sensitive; 0·2 p.p.m. (1 μg) of the P=S and P=O dimethoates was generally identifiable, but crop constituents were nearly absent. Baking the silica gel - kieselguhr plates for 5 minutes at 100° C between developments was considered advantageous. The Panel therefore recommends the silica gel - kieselguhr semi-quantitative identification system as first action, but that if crop interferences confuse the identification the formamide-impregnated system should be used. Details of the identification procedures are set out in Appendix II.

If an aliquot of the chloroform extract is used for thin-layer chromatography before being cleaned-up on the alumina column, the identification is not so clear-cut and in some instances may not be possible.

Approximate R_F values to be expected with the recommended chromatographic systems are—

(*a*) With silica gel - kieselguhr plates: P=S dimethoate 0·6 in chloroform - acetone (9 + 1) and 0·8 in chloroform - acetone (2 + 1); and P=O dimethoate 0·2 in chloroform - acetone (9 + 1) and 0·4 in chloroform - acetone (2 + 1).

(*b*) With formamide-impregnated plates: P=S dimethoate 0·5 to 0·6 in 1,2-dichloroethane - benzene (2 + 1) and 0·7 in *cis*-1,2-dichloroethylene; and P=O dimethoate: 0·05 to 0·07 in 1,2-dichloroethane - benzene (2 + 1) and 0·1 to 0·15 in *cis*-1,2-dichloroethylene.

GAS - LIQUID CHROMATOGRAPHY—

The Panel also considered the application of gas - liquid chromatography to the determination of dimethoate. Several laboratories looked at the problem independently by using the Aerograph phosphorus detector.

One laboratory had investigated[17] Apiezon on Chromosorb G, allowing identification of a fraction of a nanogram of P=S dimethoate and about 10 ng of P=O dimethoate, but obtained better results with an X.E. 60 cyanosilicone-impregnated column. The latter showed a sensitivity of 0·2 to 0·3 ng of P=S dimethoate and 2 ng of P=O dimethoate, the peaks having retention times of 95 and 75, respectively, with reference to 100 for parathion. Butane-1,4-diol succinate also showed promise. Another laboratory had used the X.E. 60 cyanosilicone-impregnated column with extracts of fortified crops obtained by the recommended method. With no alumina column clean-up, peas had given a 0·04 p.p.m. blank, 76 per cent. recovery of P=S dimethoate and 107 per cent. of P=O dimethoate. With column clean-up, recoveries were 50 per cent. for P=S dimethoate and 80 per cent. for P=O dimethoate. A column temperature of 150° to 200° C was used for P=S dimethoate and 120° C for P=O dimethoate.

One laboratory reported that a successful column for P=S and P=O dimethoates was 2 per cent. diethylene glycol succinate on 80 to 100-mesh Gas Chrom Q. The retention times of P=S and P=O dimethoates, relative to parathion (100), were 135 and 108.

Another laboratory used an S.E.30 silicone-impregnated column for identifying P=S dimethoate in acetone extracts of cherries and plums. The response of the detector was linear in the range 1 to 10 ng. Apiezon L (0·5 per cent.) was more promising for detection of P=O dimethoate.

In the co-ordinating laboratory a 2 foot × $\frac{1}{8}$-inch stainless-steel column packed with 5 per cent. S.E.30 silicone gum supported on 80 to 100-mesh Chromosorb W and pre-treated with hexamethyldisilazane was used. To avoid decomposition of dimethoate it was necessary to treat the column by repeated injection of dimethoate initially, and then daily before use with 1 μg of dimethoate. The oven temperature was 215° C, when 0·1 ng of P=S dimethoate was detectable. Menazon, vamidothion, vamidothion sulphoxide, demeton-S-methyl sulphoxide and sulphone did not interfere. Untreated apples, peas, cauliflowers, blackcurrants and cabbages extracted and cleaned-up by the recommended method gave little or no response, and recoveries of 0·1 to 0·3 p.p.m. of P=S dimethoate were usually 70 to 120 per cent. Pears needed additional clean-up. P=O dimethoate could probably be determined at 150° to 160° C at 0·3 p.p.m. Formamide from impregnated thin-layer chromatographic plates interfered when spots on such plates were scraped and eluted. Spots from silica gel - kieselguhr plates needed to be extracted by using a Soxhlet apparatus.

The Panel was unable to recommend a gas - liquid chromatographic method at this stage.

Appendix I

RECOMMENDED METHOD FOR QUANTITATIVE DETERMINATION OF DIMETHOATE RESIDUES IN FRUITS AND VEGETABLES

APPARATUS—

Macerator.

Chromatographic column—A column, with internal diameter about 2 cm and length 30 cm, with tap and fitted with detachable reservoir, was used.

Absorption spectrophotometer and 4-cm cells.

REAGENTS—

All reagents should be of analytical-reagent grade when possible.

Acetone—Re-distillation may be necessary.

Chloroform.

Carbon tetrachloride.

Aluminium oxide—Neutral, Brockman Grade V (with 15 per cent. of water).

Perchloric acid solution, 72 per cent., aqueous, sp. gr. 1·70.

Nitric acid, sp. gr. 1·42.

Ammonium molybdate solution, 5 per cent., aqueous.

Sulphuric acid, 0·6 N.

Tin(II) chloride - hydrazinium sulphate solution—Dissolve 2·0 g of hydrazinium sulphate in 1 litre of 0·6 N sulphuric acid, cool to about 10° C and add 1·0 g of tin(II) chloride, from a recently opened bottle. This solution becomes clear after about 12 hours in a refrigerator and can be kept for 2 weeks.

Isobutyl alcohol.
Ethanol.
Potassium dihydrogen orthophosphate solution (for preparation of calibration graph)—Dissolve 43·9 mg of potassium dihydrogen orthophosphate in 100 ml of water and dilute 100-fold to give a solution containing 1 μg of phosphorus per ml.

PREPARATION OF CALIBRATION GRAPH—

By pipette introduce a suitable volume of standard potassium dihydrogen orthophosphate solution into a 100-ml separating funnel. Add 2 ml of perchloric acid and make up to 30 ml with water. Add 5 ml of 5 per cent. ammonium molybdate solution and 15 ml of isobutyl alcohol. Shake the mixture for 90 seconds, allow the layers to separate and discard the lower aqueous layer. Wash the isobutyl alcohol with 15 ml of 0·6 N sulphuric acid, shaking the mixture for 60 seconds. Allow to separate and discard the lower layer. Wash the isobutyl alcohol into a 50-ml calibrated flask with ethanol, add 0·5 ml of hydrazinium sulphate - tin(II) chloride solution and make up to 50 ml with ethanol, mixing thoroughly. Read the optical density at 735 nm in a 4-cm cell against water in the reference cell.

EXTRACTION—

Macerate a 250-g sample of crop with 500 ml of acetone for 1 minute and filter through a Buchner funnel. Again macerate the "cake" that is left with 250 ml of acetone for 1 minute and filter through the Buchner funnel. Combine the acetone extracts and make up to 1 litre with acetone. Take 200 ml (corresponding to 50 g of crop material) and evaporate off the acetone under reduced pressure at a temperature not higher than 60° C. Cool the flask thoroughly under the cold water tap. Filter the residue and wash the filter-paper with water. Transfer to a separating funnel, making the final volume up to 150 to 200 ml. Extract three times with 200 ml of chloroform. (Emulsions are rarely encountered if the volume of chloroform is equal to, or greater than, the volume of the aqueous extract.) Any semi-solid interface should be left with the aqueous phase at each extraction. Combine the chloroform extracts and filter through Whatman No. 1 paper. Evaporate just to dryness under reduced pressure at a temperature not higher than 60° C.

If the method cannot be completed the same day, solutions containing crop extracts should be stored at 0° C.

COLUMN CHROMATOGRAPHY—

Prepare a chromatographic column of 10 g of aluminium oxide in a liquid phase consisting of a mixture of chloroform and carbon tetrachloride (1 + 1). Dissolve the residue from the chloroform extraction in 10 ml of chloroform - carbon tetrachloride (1 + 1), introduce the solution into the column, and allow it to run at a rate of about one drop per second. When the level of the solvent has reached the top of the alumina, add a further 10 ml of chloroform - carbon tetrachloride (1 + 1), rinsing out the flask. When this has reached the top of the alumina, elute the column with 120 ml of the chloroform - carbon tetrachloride mixture. After about 50 ml of solvent have run through the column, its rate of flow can be increased to two drops per second. Evaporate the solvents and, by using chloroform, transfer the residue to the flask for wet oxidation.

WET ASHING—

Evaporate the chloroform, add 5 ml of distilled water, five drops of concentrated nitric acid, 2 ml of 72 per cent. perchloric acid and a small glass bead to the residue in the flask and heat to dense white fumes for several minutes. Cool, add a few millilitres of water, and also a few drops of nitric acid if the solution is coloured, and again heat to white fumes. Cool and transfer to a 100-ml separating funnel with water, making the volume up to 30 ml.

COLORIMETRIC DETERMINATION—

Add 5 ml of ammonium molybdate solution and 15 ml of isobutyl alcohol. Shake the mixture for 90 seconds, allow the layers to separate and discard the lower aqueous layer. Wash the isobutyl alcohol layer with 15 ml of 0·6 N sulphuric acid, shaking it for 60 seconds. Allow to separate and discard the lower layer. Wash the isobutyl alcohol layer into a 50-ml

calibrated flask with ethanol, add 0·5 ml of hydrazinium sulphate - tin(II) chloride solution and make the volume up to 50 ml with ethanol, mixing thoroughly. Read the optical density at 735 nm in a 4-cm cell against water in the reference cell.

Read the corresponding amount of phosphorus from the calibration graph and multiply it by 7·40 to express the results as dimethoate.

Appendix II

RECOMMENDED METHOD FOR SEMI-QUANTITATIVE IDENTIFICATION OF DIMETHOATE RESIDUES IN FRUIT AND VEGETABLES

REAGENTS—

Silica gel G (Merck, for chromatography).
Kieselguhr G (Merck, for chromatography).
Silica gel HF (Merck, for chromatography).
Formamide—General-purpose reagent.
1,2-*Dichloroethane.*
cis-1,2-*Dichloroethylene*—Obtained from Ralph N. Emanuel, 3–4 Leather Market, London, S.E.1.
4-(p-*Nitrobenzyl*)*pyridine*—Obtained from Ralph N. Emanuel. Prepare a 2 per cent. solution in acetone.
Tetraethylenepentamine, 10 per cent. in acetone.
Active carbon, Nuchar C190N.
Acetonitrile, redistilled.
Hexane, redistilled.
Magnesium oxide—Chromatographic grade.
Standard solutions of P=S and P=O dimethoates—These were prepared in chloroform and should be stored at 0° C in the dark.

THIN-LAYER PLATES—

(*a*) *Silica gel - kieselguhr plates*—Slurry 20 g of silica gel G, 20 g of kieselguhr G and 80 ml of water for 1¾ minutes and spread the mixture over five 20 × 20-cm plates by using a spreader set at 0·25 mm. After initial air-drying, dry the plates in the oven at 110° to 120° C for at least 1 hour and allow them to stand in a desiccator cabinet to cool.

(*b*) *Formamide-impregnated silica gel plates*—Slurry 30 g of silica gel HF with 100 ml of 20 per cent. formamide in ethanol for 1 minute, allow to stand for 1 minute to remove air bubbles and spread the mixture over four 20 × 20-cm plates by using a spreader set at 0·5 mm. With some batches of silica gel HF it is necessary to vary the amount of formamide solution to obtain a slurry of suitable consistency. After spreading, air-dry the plates for 5 to 10 minutes and then oven-dry them at 50° C for 10 minutes before storing in a desiccator cabinet.

(*a*) *Further clean-up for silica gel - kieselguhr plates*—Evaporate an aliquot, corresponding to 5 g of fruit or vegetable, of the eluate from the alumina column of the recommended quantitative method (Appendix I) nearly to dryness on a water-bath, with a gentle stream of air. Take up the residue in 25 ml of acetonitrile and extract four times with 10 ml of hexane, shaking the mixture for 1 minute on each occasion. Discard the hexane extracts. Add 0·2 g of Nuchar C190N and 0·4 g of magnesium oxide to the acetonitrile solution and shake it for 5 minutes. Filter through a Whatman 2V (No. 12) paper and concentrate the filtrate in a stream of air just to dryness on a water-bath. (A test-tube with a drawn-out lower end is suitable for this stage.) Take up in 20 to 25 μl of chloroform for chromatography. With apples, pears, cabbages and blackcurrants there is no need for acetonitrile - hexane partition, or magnesium oxide treatment, and the chloroform - carbon tetrachloride eluate can be shaken directly with 0·2 g of Nuchar C190N, filtered and concentrated for chromatography.

If the method cannot be completed the same day, solutions containing crop extracts should be stored at 0° C.

(*b*) *Further clean-up for formamide-impregnated plates*—The details of the procedure for further clean-up for formamide-impregnated plates are identical with those for silica gel - kieselguhr plates, except that magnesium oxide is omitted when the 0·2 g of Nuchar C190N is added to the acetonitrile solution, and the same provision applies with apples, pears, cabbages and blackcurrants.

CHROMATOGRAPHY (TWO DIMENSIONAL)—

(*a*) *With silica gel - kieselguhr plates*—With a suitable capillary, carefully apply the solution in successive 2 to 3-μl amounts at a spot 2·5 cm from the edges of two adjacent sides. Also apply P=S and P=O dimethoates (say 1 μg of each) 2 cm from the far ends of the two adjacent sides and 2·5 cm from the edges. Develop in chloroform - acetone (9 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the top edge. Dry at 100° C for 5 minutes. Develop at right angles in chloroform - acetone (2 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the far edge. Allow the plate to dry in the air. Spray with 15 to 20 ml of 4-(*p*-nitrobenzyl)pyridine solution in acetone. Heat for 10 minutes at 110° C in an oven. Spray with tetraethylenepentamine solution, whereupon the pesticides appear as blue spots on a white background. Compare the position and intensity of the sample spots with the standards.

(*b*) *With formamide-impregnated plates*—With a suitable capillary, carefully apply the solution in successive 2 to 3-μl amounts at a spot 2·5 cm from two adjacent sides. Also apply P=S and P=O dimethoates (say 1 μg of each) 2 cm from the far ends of the two adjacent sides and 2·5 cm from the edges. Develop in 1,2-dichloroethane - benzene (2 + 1), in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the top edge. Allow to dry in the air at room temperature. Develop at right angles, in *cis*-1,2-dichloroethylene, in a tank that has been allowed to equilibrate, until the solvent front is 3 cm from the far edge. Allow to dry in the air. Spray with 15 to 20 ml of 4-(*p*-nitrobenzyl)pyridine solution in acetone. Heat for 10 minutes at 110° C in the oven. Spray with tetraethylenepentamine solution, whereupon the pesticides appear as blue spots on white background. Compare the position and intensity of the sample spots with the standards.

Appendix III

MEMBERSHIP OF THE PANEL

The Panel consisted of D. C. Abbott (Chairman), the late E. D. Chilwell, W. B. Chapman, H. Crossley (until 23rd May, 1966), H. Frehse, N. Gandolfo, K. A. Lord, A. F. Machin, A. Müller, R. A. Savidge (from 4th October, 1966), N. A. Smart (Secretary), R. W. Storherr (from 16th September, 1966), R. P. Tew and B. Vasta (until 6th September, 1966).

REFERENCES

1. Chilwell, E. D., and Beecham, P. T., *J. Sci. Fd Agric.*, 1960, **11**, 400.
2. Smart, N. A., *Analyst* 1966, **91**, 621.
3. Laws, E. Q., and Webley, D. J., *Ibid.*, 1961, **86**, 249.
4. Giang, P. A., and Schechter, M. S., *J. Agric. Fd Chem.*, 1963, **11**, 63.
5. Fukel'man, L. M., *Trudy Vses. Inst. Zashch. Rast.*, 1964, No. 2, pt 4, 61.
6. de Pietri-Tonelli, P., Bazzi, B., and Santi, R., *in* Gunther, F. A., *Editor*, "Residue Reviews," Springer-Verlag, Berlin, Göttingen and Heidelberg, 1965, Volume 11, p. 60.
7. George, D. A., Walker, K. C., Murphy, R. T., and Giang, P. A., *J. Agric. Fd Chem.*, 1966, **14**, 371.
8. Engst, R., and Kubel, D., *Z. Lebensmittelunters. u. Forsch.*, 1966, **113**, 149.
9. Mitsui, K., and Suzuki, S., *Botyu-Kagaku*, 1966, **31**, 103.
10. Abbott, D. C., Bunting, J. A., and Thomson, J., *Analyst*, 1966, **91**, 94.
11. Smart, N. A., and Hill, A. R. C., *J. Chromat.*, 1967, **30**, 626.
12. Ashworth, R. J., *Tob. Sci.*, 1967, 186.
13. Youden, W. J., *J. Ass. Off. Agric. Chem.*, 1963, **46**, 55.
14. Steller, W. A., and Curry, A. N., *Ibid.*, 1964, **47**, 645.
15. Bates, J. A. R., *Analyst*, 1965, **90**, 453.
16. Watts, R. R., *J. Ass. Off. Agric. Chem.*, 1965, **48**, 1161.
17. Ruzicka, J., Thomson, J., and Wheals, B. B., *J. Chromat.*, 1967, **30**, 92.

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