compounds 15 and 20 (a or b), were converted to the corresponding chlorides with phosphorus pentachloride. The components of the resulting mixture were separated by column chromatography (alumina eluted with cyclohexane). These compounds had ir spectra identical with the photoproducts of 1.

Photolysis of 1,2,3,4,6,7,8,9,10,10-Decachloropentacyclo[5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{4,8}$]decan-5-ol (21). Compound 21 was prepared from Kepone by reduction with lithium aluminum hydride (Allied Chemical Corp., 1964). A solution of 21 (0.1 g) in 20 ml of acetone was irradiated for 4 hr. The glpc analysis indicated that one product was formed with retention time 0.75 relative to 21 (SE-30, 240°). The mass spectrum of this compound was obtained from a mass spectrometer interfaced with a gas chromatograph (8-ft SE-30, 240°). The mass spectrum included ion clusters for $C_{10}Cl_9H_3O^+$ (parent), for $C_5Cl_6^+$ and $C_5Cl_5H^+$ (from cleavage of the pentacyclic carbon skeleton), and for $C_5Cl_5^+$ ions (dechlorination of the $C_5Cl_6^+$ fragments).

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Analysis of Dimethoate-Treated Grapes for the *N*-Hydroxymethyl and De-*N*-methyl Metabolites and for Their Sugar Adducts

William A. Steller* and William W. Brand

Gas-liquid chromatographic (glc) methods are presented for the determination of de-*N*-methyl dimethoate, *N*-hydroxymethyl dimethoate, de-*N*-methyl dimethoxon, *N*-hydroxymethyl dimethoxon, and the *O*-glucoside of *N*-hydroxymethyl dimethoate in grapes at levels down to 0.05 ppm for each compound. Recovery values for all these compounds are presented. Analyses of field-treated grape samples harvested 28 days after the last application of dimethoate showed no residues of any of these compounds at or above the validated sensitivity limit (0.05 ppm) of the method, indicating that they do not constitute a significant part of the dimethoate-related residues in grapes. Synthesis and isolation procedures used to obtain *N*-hydroxymethyl dimethoate, *N*-hydroxymethyl dimethoxon, de-*N*-methyl dimethoxon, and *N*-hydroxymethyl dimethoate *O*-glucoside are also described.

Dimethoate (I) [*O,O*-dimethyl *S*-(methylcarbamoyl)-methyl phosphorodithioate], the active ingredient in Cygon systemic 25 insecticide (trademark of American Cyanamid Co.), is an organophosphate insecticide effective for the control of a wide variety of pests on crops, including control of Pacific spider mite and grape leafhopper on grapes as well as for house fly control when applied as a residual spray to various surfaces. Previously reported work on the metabolism of dimethoate in plants had shown oxidation to dimethoxon (II) as the only mechanism yielding toxic residues. The main degradative pathways elucidated in this early work were to the nontoxic de-*O*-methyl dimethoate, dimethoate carboxylic acid, and oxy-carboxy dimethoate, as well as to the hydrolysis products resulting from cleavage of the P–S and S–C bonds of the molecule. The metabolism of dimethoate in plants has been reviewed by Lucier and Menzer (1968). Because of this metabolic pattern, the recommended methods of analysis for total toxic residues in crops treated with dimethoate measure only dimethoate and dimethoxon as described by Steller and Pasarela (1972).

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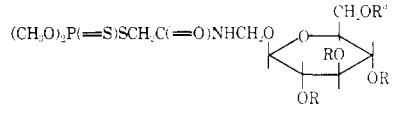
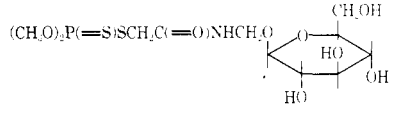
Recently, however, Lucier and Menzer (1970) reported finding trace quantities (0.08 ppm maximum) of de-*N*-methyl dimethoate (III), de-*N*-methyl dimethoxon (V), *N*-hydroxymethyl dimethoate (IV), and *N*-hydroxymethyl dimethoxon (VI) in bean plants treated by foliar application with 15 ppm of dimethoate-*carbonyl*-^{14}C. Six days after treatment 77% of the recoverable radioactivity was present as water-soluble compounds. Acidic and enzymatic hydrolyses of this water-soluble fraction demonstrated the absence of any *N*-hydroxymethyl dimethoate or *N*-hydroxymethyl dimethoxon conjugates in the bean plants as reported by Lucier by private communication (1971). Even though no conjugates were found in bean plants, the possible presence of even small quantities of de-*N*-methyl dimethoate, de-*N*-methyl dimethoxon, *N*-hydroxymethyl dimethoate, and *N*-hydroxymethyl dimethoxon in the bean plants was considered sufficient reason to suggest the possibility of a potential pool of *N*-hydroxymethyl dimethoate sugar adducts in grapes. Giang and Beckman (1968) reported that sugar adducts of two insecticides, Bidrin and Azodrin (with structures similar to dimethoate), were found when these compounds were applied to plants, but Elgar and MacDonald (1966) reported that sugar adducts were found to be absent from fruits (apples and oranges) treated with Bidrin.

Table I

No.	Common name	Structure	Chemical name
I	Dimethoate	$(CH_3O)_2P(=S)SCH_2C(=O)NHCH_3$	*O,O*-Dimethyl *S*-(methylcarbamoyl)methyl phosphorodithioate
II	Dimethoxon	$(CH_3O)_2P(=O)SCH_2C(=O)NHCH_3$	*O,O*-Dimethyl *S*-(methylcarbamoyl)methyl phosphorothioate
III	De-*N*-methyl dimethoate	$(CH_3O)_2P(=S)SCH_2C(=O)NH_2$	*O,O*-Dimethyl *S*-carbamoylmethyl phosphorodithioate
IV	*N*-Hydroxymethyl dimethoate	$(CH_3O)_2P(=S)SCH_2C(=O)NHCH_2OH$	*O,O*-Dimethyl *S*-[(hydroxymethyl)-carbamoyl]methyl phosphorodithioate
V	De-*N*-methyl dimethoxon	$(CH_3O)_2P(=O)SCH_2C(=O)NH_2$	*O,O*-Dimethyl *S*-carbamoylmethyl phosphorothioate
VI	*N*-Hydroxymethyl dimethoxon	$(CH_3O)_2P(=O)SCH_2C(=O)NHCH_2OH$	*O,O*-Dimethyl *S*-[(hydroxymethyl)-carbamoyl]methyl phosphorothioate
VII	*N*-Hydroxymethyl dimethoate *O*-glucoside, tetraacetate		*N*-[(β-D-Glucopyranosyloxy)methyl-2-mercaptoacetamide, tetraacetate, *S*-ester with *O,O*-dimethyl phosphorodithioate
VIII	*N*-Hydroxymethyl dimethoate *O*-glucoside		*N*-[β-D-Glucopyranosyloxy)methyl-2-mercaptoacetamide, *S*-ester with *O,O*-dimethyl phosphorodithioate

[a] R = $CH_3C(=O)$.

The approach chosen to demonstrate the presence or absence of de-*N*-methyl dimethoate, de-*N*-methyl dimethoxon, *N*-hydroxymethyl dimethoate, and *N*-hydroxymethyl dimethoxon and/or sugar adducts in grapes treated with dimethoate was an analytical one almost identical with that previously reported for the analogous metabolites of Bidrin and Azodrin by Giang and Beckman (1968). Grapes treated in the field with dimethoate by commercial application techniques from rate-of-disappearance studies conducted in California were used in this investigation.

MATERIALS AND METHODS

Synthesis of Potential Metabolites. The structures and chemical and common names of the potential metabolites tested in this study are listed in Table I. Nmr spectra were recorded on a Varian A-60 spectrometer. Ir spectra were recorded on a Perkin-Elmer Infracord spectrometer. Mass spectral data were obtained from a Finnigan Model 1015 with C.I. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn., and by American Cyanamid Co., Pearl River, N. Y.

Synthesis of O,O-Dimethyl S-[(Hydroxymethyl)carbamoyl]methyl Phosphorodithioate (IV). A mixture of 19.6 g (0.10 mol) of potassium *O,O*-dimethyl phosphorodithioate and 12.4 g (0.10 mol) of *N*-hydroxymethylchloroacetamide (Einhorn and Ladisch, 1905) was heated to reflux for *ca.* 40 min in 80 ml of a 1:1 methylene chloride–water mixture. The organic layer was separated, washed with water, dried, and concentrated *in vacuo* to give 14.1 g of solids, mp 71–77°. Recrystallization from benzene gave 12.2 g of colorless crystals, mp 79.0–80.6°: ir 1550, 1660, and 3250 (secondary amide), 3340 cm^{-1} (OH); nmr ($CDCl_3$) δ 8.2 (br, *ca.* 1 H, NH), 4.76 (d, J = 6 Hz, 2 H, NCH_2O), 4.4 (br, *ca.* 1 H, OH), 3.80 (d, J = 15 Hz, 8 H with δ 3.62 d, CH_3OP), 3.62 (d, J = 16 Hz, $PSCH_2$). *Anal.* Calcd for $C_5H_{12}NO_4PS_2$: C, 24,49; H, 4.90; N, 5.71; P, 12.63; S, 26.15. Found: C, 24.69; H, 4.86; N, 5.80; P, 12.44; S, 26.30.

Synthesis of O,O-Dimethyl S-Carbamoylmethyl Phosphorothioate (V). A mixture of 18.0 g (0.10 mol) of potassium *O,O*-dimethyl phosphorothioate and 9.4 g (0.10 mol) of chloroacetamide in 100 ml of acetone was heated at reflux overnight. The solids were filtered off and the filtrate was concentrated to an oily solid mixture containing as contaminants chloroacetamide and a simple phosphate ester—presumably *O,O*-dimethyl *S*-methyl phosphorothioate. The residue remaining (2.7 g) after several extractions of the oil with hot cyclohexane and hot benzene was chromatographed on a 0.5 in. × 7 in. column of silica gel (E. Merck; 30–70 mesh) collecting 30-50-ml fractions of methylene chloride eluent. The sixth fraction (50–75 ml) was concentrated, remixed with chloroform, and stirred with diatomaceous earth and anhydrous sodium sulfate. Filtration, followed by concentration of the filtrate, afforded 0.92 g of product as a colorless oil: ir 1660, 3100, 2350 (primary amide), 1240 cm^{-1} (P=O); nmr ($CDCl_3$) δ 7.3 and 6.7 (br s, 2 H, NH_2), 3.86 and 3.58 (d, J = 13 and 18 Hz for CH_3OP and $PSCH_2$, respectively, 8 H total). *Anal.* Calcd for $C_4H_{10}NO_4PS$: C, 24.13; H, 5.06; N, 7.03; P, 15.55; S, 16.10. Found: C, 23.84; H, 5.20; N, 7.04; P, 15.51; S, 16.12.

Synthesis of O,O-Dimethyl S-[(Hydroxymethyl)carbamoyl]methyl Phosphorothioate (VI). To a mixture of 18.0 g (0.10 mol) of potassium *O,O*-dimethyl phosphorothioate in 50 ml of saturated salt solution was added 16.8 g (0.10 mol) of *N*-hydroxymethyl-2-bromoacetamide (Einhorn and Ladisch, 1905) in 150 ml of acetone. After 24 hr, the acetone layer was separated and the aqueous phase was extracted again with acetone. The combined acetone mixtures, after standing over anhydrous sodium sulfate, were concentrated *in vacuo* with portions of benzene being added to remove residual water. The residual oil was only partially soluble on remixing with fresh acetone, 5.3 g being recovered from 12.1 g by refiltering and concentrating the acetone portion. The remaining oil, together with 150 ml of tetrahydrofuran, was filtered and 150 ml of hexane was added. An oil again separated which, after decanting the solvent layer, amounted to 2.6 g. A 0.466-mg portion was purified on a 20 × 20 cm 2-mm thick layer of silica gel F-254 (E. Merck) using 85:15 acetone–ethanol for developing. The major band at *ca.* R_f 0.63 was scraped from the plate and 0.291 mg of colorless oil was recovered: ir 1530, 1660, and 3220 (secondary amide), 1240 cm^{-1} (P=O); nmr ($CDCl_3$) δ 8.4 (br, *ca.* 1 H, NH), 4.76 (d, J = 6 Hz, 2 H, NCH_2O), 3.82 (d, J = 13 Hz, 8 or 9 H with δ 3.59 d and possible OH), 3.59 (d, $J \simeq 14$ Hz, $PSCH_2$). A small companion doublet at δ 3.65 suggested the presence of an impurity. *Anal.* Calcd for $C_5H_{12}NO_5PS$: C, 26.20; H, 5.28; N, 6.11; P, 13.51; S, 13.99. Found: C, 26.24; H, 5.23; N, 6.11; P, 13.50; S, 14.20.

Synthesis of N-[β-D-Glucopyranosyloxy)methyl-2-mercaptoacetamide, Tetraacetate, S-Ester with O,O-Dimethyl Phosphorodithioate (VII). To a solution of 4.9 g (0.02

mol) of *O,O*-dimethyl *S*-[(hydroxymethyl)carbamoyl]-methyl phosphorodithioate in 250 ml of methylene chloride was added 5.2 g (0.021 mol) of mercuric cyanide (previously dried at 2–3 mm over P_2O_5) plus 8.0 g of finely powdered Drierite (crushed and dried overnight at 150° (1 mm)). The reaction mixture was stirred at room temperature for 45 min, after which 8.2 g (0.02 mol) of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (Gallard-Schlesinger) was added. The reaction mixture was then stirred for 6 days, as its progress was followed by tlc (1:1 CH_2Cl_2–MEK on silica gel; product R_f 0.5, starting materials R_f 0.19 and 0.53). The reaction mixture was then filtered and the solvent evaporated, leaving 12.82 g of tar. This was purified by dry column chromatography (3:1 methylene chloride–methyl ethyl ketone on silica gel), and the product recovered between R_f values of 0.46 and 0.80. This was used for the deacetylation reaction without further purification.

A small amount of the above product was streaked onto a 2.0-mm silica gel plate (prewashed with acetone), and the plate was developed with 1:1 methylene chloride–methyl ethyl ketone. The band at *ca.* R_f 0.34–0.47 was scraped off the plate and extracted with redistilled acetone. The acetone extract was concentrated, and the residue taken up in methanol and filtered through a cotton plug. The methanol solution was evaporated at reduced pressure, and the residue dried overnight *in vacuo* at 56°. Mass spectroscopy of the resulting product showed a molecular ion at *m/e* 576, P − 60 ion (*m/e* 516, loss of acetic acid *via* a McLafferty rearrangement), and major ions at *m/e* 331, 289, 228, and 199, the latter two being also the major fragments from *N*-hydroxymethyl dimethoate (VI). This appears to be completely consistent with the assigned structure: ir (neat) 1740 (acetyl), 1980 (amide I), and 1520 cm^{-1} (amide II); nmr ($CDCl_3$) δ 1.9–2.3 (m, 12 H), 3.63 (d, J = 19 Hz, $PSCH_2$), 3.83 (d, J = 15 Hz, CH_3OP), 4.0–5.7 (m, together with 3.63 and 3.83, 16 H), 6.37 (d, J = 3.5 Hz, <1 H, OCHO), *ca.* 7.6 (br, *ca.* 1 H, NH). *Anal.* Calcd for $C_{19}H_{30}NS_2O_{13}P$: C, 39.65; H, 5.25; N, 2.43; S, 11.14; P, 5.38. Found: C, 43.97; H, 6.00; N, 2.33; S, 9.68; P, 4.15.

Synthesis of N-[β-D-Glucopyranosyloxy)methyl-2-mercaptoacetamide, S-Ester with O,O-Dimethyl Phosphorodithioate (VIII). To a solution of 3 g of the tetraacetyl glucoside VII (0.005 mol) in 60 ml of methanol at 0° was added 120 ml of an ice-cooled, saturated solution of methanolic ammonia. The reaction mixture was stirred at 0° for 3.5 hr. It was then concentrated at room temperature and reduced pressure and put on a 3 cm i.d. × 30.6 cm silica gel column with methylene chloride. The column was then eluted with 2 l. of ethyl acetate after which the solvent was changed to acetone (using a 100-ml ethyl acetate–acetone mixture to change solvents), and elution was continued. After 700 ml of acetone had been collected, the following 200 ml of acetone eluate was concentrated and the residue stirred with 2-propanol. The white solid was collected by filtration and washed with ether, giving 156 mg of product. This was taken up in methanol and filtered and the solvent evaporated giving 127 mg of product: mp 123.5–125°; nmr (CD_3OD) δ 3.43–4.15 (m, 10 H, including δ 3.52, d, J = 17 Hz, $PSCH_2CO$, and δ 3.81, d, J = 15 Hz, CH_3OP), and 4.15–5.7 (m, 12 H); ir (KBr) 3260 (amide I), 1550 (amide II), 1010 (CO(P) stretch), 830 (PO(C) stretch) and 665 cm^{-1} (P=S). *Anal.* Calcd for $C_{11}H_{22}NS_2O_9P$: C, 32.43; H, 5.44; N, 3.44; S, 15.74; P, 7.60. Found: C, 32.47; H, 5.51; N, 3.87; S, 15.08; P, 7.21.

Field Treatment of Grapes. The treatment schedules for the grape samples analyzed and the residue values obtained are presented in Tables II and III. These grape samples were analyzed for residues of dimethoate and dimethoxon using the procedure of Steller and Pasarela (1972).

Gas Chromatography. An F&M Model 402 gas chromatograph equipped with a thermionic detector as previously described by Steller and Pasarela (1972) with the following glc column and conditions was used: column, 120 cm, 3-mm i.d. Pyrex glass U-tube packed with 1% EGSS-X (Catalog No. 10401, Applied Science Labs., Inc.) on Gas Chrom Q 60–80 mesh (Catalog No. 02001, Applied Science Labs., Inc.); column temperature, 180°; injection port temperature, 225°; detector temperature, 210°; carrier (helium) flow, 100 cm^3/min; air flow, 220 cm^3/min; hydrogen flow, 30 cm^3/min. The retention times for de-*N*-methyl and *N*-hydroxymethyl dimethoxon were 2.3 min and for de-*N*-methyl and *N*-hydroxymethyl dimethoate they were 3.2 min. The retention times for dimethoxon and dimethoate were 1.2 and 1.5 min, respectively.

The analysis was conducted as follows. Condition the glc column by heating for 2 hr at 190° with the exit end disconnected from the detector. When the oven temperature reaches approximately 150° inject 10 μl of Silyl 8 (Pierce Chemical Co.). With the exit end of the column connected to the detector, alternately inject aliquots of de-*N*-methyl dimethoate containing 200 and 10 ng until response for the latter quantity is reproducible. Subsequent injection of grape extracts will further condition the column and result in enhancement of the response, particularly for de-*N*-methyl dimethoxon and *N*-hydroxymethyl dimethoxon.

Table II. Summary of Dimethoate and Dimethoxon Residues in Grapes[a]

Sample no.	Rate	Interval after last application (days)	ppm of dimethoate	ppm of dimethoxon
A-1	A	0	7.12	0.19
A-2	B	0	13.0	0.28
A-3	Check	0	0.02	<0.04
A-4	A	21	0.89	0.26
A-5	B	21	1.91	0.43
A-6	Check	21	0.02	<0.04
A-7	A	28	0.40	0.22
A-8	B	28	0.73	0.27
A-9	Check	28	<0.01	<0.03
A-10	A	35	0.21	0.10
A-11	B	35	0.43	0.21
A-12	Check	35	<0.01	<0.01

[a] Location, Reedley, Calif.; product, Cygon systemic 25 insecticide; treatment: A, three applications at 1.92 lb of A/a; B, two applications at 1.92 lb of A/a and final application at 3.84 lb of A/a; gallons/acre, 180; treatment dates: July 24, July 31, and Aug 7, 1971; A/a, actual/acre.

Table III. Summary of Dimethoate and Dimethoxon Residues in Grapes[a]

Sample no.	Rate	Interval after last application (days)	ppm of dimethoate	ppm of dimethoxon
B-1	A	0	10.1	0.21
B-2	B	0	18.1	0.26
B-3	Check	0	0.03	<0.04
B-4	A	28	0.50	0.18
B-5	B	28	0.65	0.24
B-6	Check	28	<0.01	<0.01
B-7	A	35	0.24	0.13
B-8	B	35	0.50	0.20
B-9	Check	35	<0.01	<0.01

[a] Location, Reedley, Calif.; product, Cygon systemic 25 insecticide; treatment: A, three applications at 1.82 lb of A/a; B, two applications at 1.82 lb of A/a and final application at 3.64 lb of A/a; gallons/acre, 27.5; treatment dates, July 24, July 31, and Aug 7, 1971.

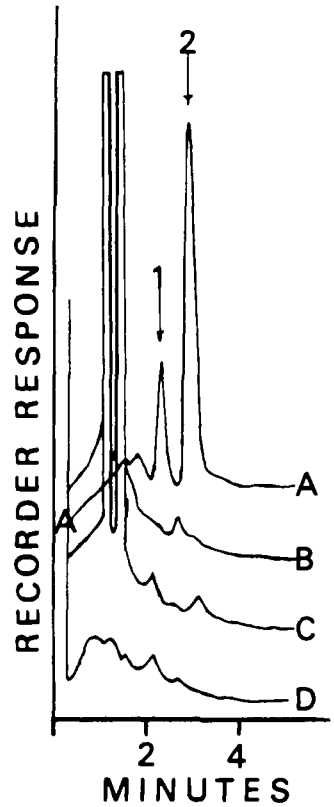


Figure 1. Typical chromatograms of response for *N*-hydroxymethyl dimethoate and *N*-hydroxymethyl dimethoxon in grapes using a thermionic phosphorus detector: (A) from control grape fortified with 0.05 ppm each of *N*-hydroxymethyl dimethoate and *N*-hydroxymethyl dimethoxon; arrow 1 represents the retention time of de-*N*-methyl dimethoxon and *N*-hydroxymethyl dimethoxon while arrow 2 represents the retention time of de-*N*-methyl dimethoate and *N*-hydroxymethyl dimethoate; (B) from grape sample (No. B-5) field-treated with dimethoate and analyzed for residues of de-*N*-methyl dimethoate, *N*-hydroxymethyl dimethoate, de-*N*-methyl dimethoxon, and *N*-hydroxymethyl dimethoxon; (C) from grape sample (No. B-5) field-treated with dimethoate and analyzed for residues of the *O*-glucoside of *N*-hydroxymethyl dimethoate; (D) from control grapes.

Sample Preparation Procedures. Use a food chopper to pulverize the prefrozen field-treated grape samples in the presence of sufficient Dry Ice to maintain the sample in the frozen state. To analyze the grapes for potential residues of the four unconjugated metabolites (de-*N*-methyl dimethoate, *N*-hydroxymethyl dimethoate, de-*N*-methyl dimethoxon, or *N*-hydroxymethyl dimethoxon), use a representative 40-g grape sample and follow the extraction and cleanup procedure previously described by Steller and Pasarela (1972) for the determination of residues of dimethoate and dimethoxon in grapes. Strip the solvent (methylene chloride) on a rotary evaporator and dissolve the residue in a 1-ml measured volume of acetone for glc analysis.

To analyze the grapes for residues of *N*-hydroxymethyl dimethoate *O*-glucoside (VIII) blend a representative 20-g grape sample with 70 ml of distilled water for 1 min. Transfer the macerate to a 250-ml erlenmeyer flask and adjust the pH to 0.9 with 1 *N* hydrochloric acid. Place the flasks into the holders of a reciprocating water-bath shaker with the temperature of the bath previously equilibrated at 70°. Allow the flasks to shake gently for 1 hr. Filter the extract with the aid of vacuum through a fritted Buchner funnel covered with a piece of Whatman No. 40 filter paper. Add 40 g of sodium sulfate ($Na_2SO_4 \cdot 10H_2O$) to the filtrate and heat to dissolve the salt. Cool to room temperature and transfer to a 500-ml separatory funnel. Extract the aqueous phase with four 100-ml portions of chloroform by shaking *gently* with each portion for 45 sec.

Table IV. Recovery of *N*-Hydroxymethyl Dimethoate, De-*N*-methyl Dimethoate, *N*-Hydroxymethyl Dimethoxon, and De-*N*-methyl Dimethoxon from Grapes

Compound	Fortification, ppm	Apparent residues, ppm	Recovery, %
N-Hydroxymethyl dimethoate	0.00	<0.003	
	0.05	0.055	110
	0.25	0.260	104
De-*N*-methyl dimethoate	0.00	<0.003	
	0.05	0.047	93
	0.25	0.230	92
N-Hydroxymethyl dimethoxon	0.00	<0.01	
	0.10	0.096	96
	0.25	0.184	74
De-*N*-methyl dimethoxon	0.00	<0.007	
	0.05	0.047	93
	0.25	0.280	112

Table V. Recovery of *N*-Hydroxymethyl Dimethoate *O*-Glucoside from Grapes

Fortification, ppm	Equiv of *N*-hydroxymethyl dimethoate, ppm	Apparent *N*-hydroxymethyl dimethoate, ppm	Apparent glucoside, ppm	Recovery, %
0.00	0.00	<0.001	<0.002	
0.08	0.05	0.034	0.056	70.0
0.41	0.25	0.176	0.294	70.8
0.80	0.50	0.363	0.605	72.9
1.66	1.00	0.760	1.268	76.4

Transfer the chloroform phases to a 1-l. round-bottomed flask with a ₹ 24/40 joint. Strip the combined chloroform phases on a rotary evaporator. Dissolve the residue in a small volume of acetone and transfer to a 100-ml pear-shaped flask. Strip the solvent on a rotary evaporator and dissolve the residue in 1 ml of acetone for glc analysis.

Gas Chromatographic Analysis. Using a microliter syringe, inject appropriate aliquots of the prepared residue samples into the gas chromatograph. After each two sample injections, inject an appropriate aliquot of the appropriate standard. For those samples analyzed for residues of *N*-hydroxymethyl dimethoate *O*-glucoside use a standard consisting of 1 μg/ml of *N*-hydroxymethyl dimethoate since the method is based upon hydrolysis of the glucoside to that compound. Convert apparent parts per million of *N*-hydroxymethyl dimethoate to parts per million of its *O*-glucoside by multiplying by 407/245.

RESULTS AND DISCUSSION

The glc column (11% DC 200 on Gas Chrom Q) previously used to determine residues of dimethoate and dimethoxon as reported by Steller and Pasarela (1972) was not applicable for the chromatographic separation of de-*N*-methyl dimethoate, de-*N*-methyl dimethoxon, *N*-hydroxymethyl dimethoate, and *N*-hydroxymethyl dimethoxon. De-*N*-methyl dimethoate was not completely resolved from dimethoate while de-*N*-methyl dimethoxon and *N*-hydroxymethyl dimethoxon did not elute as discrete peaks. A column packed with 1% EGSS-X on Gas Chrom Q was found to be satisfactory for the chromatography of the four compounds of interest while saparating them from any dimethoate and/or dimethoxon present in the field-treated samples. The elution pattern of the four compounds of interest indicated apparent conversion of the *N*-hydroxymethyl derivatives of dimethoate and dimethoxon to their corresponding de-*N*-methyl derivatives by heat on the glc column. (This phenomenon

was previously reported for the corresponding derivatives of Bidrin and Azodrin by Giang and Beckman (1968).) Hence, *N*-hydroxymethyl dimethoate and de-*N*-methyl dimethoate eluted at the same retention time (3.2 min) while *N*-hydroxymethyl dimethoxon and de-*N*-methyl dimethoxon also eluted together (2.3 min).

Conventional recovery studies were run by dripping dilute acetone solutions of the four unconjugated metabolites (or dilute methanol solutions of *N*-hydroxymethyl dimethoate *O*-glucoside) onto the surface of the grapes. After evaporation of the solvent, the grapes were extracted as previously described. Recovery values are reported in Table IV. Figure 1 shows typical chromatograms obtained using the thermionic detector.

In contrast to the conditions established by Giang and Beckman (1968) for the hydrolysis of the *O*-glucosides of *N*-hydroxymethyl Bidrin and *N*-hydroxymethyl Azodrin (pH 0.9, with stirring for 1 hr at room temperature), it was found necessary to apply heat to hydrolyze the glucoside of *N*-hydroxymethyl dimethoate. Hydrolysis at 70° for 1 hr resulted in essentially complete recovery of the equivalent amount of *N*-hydroxymethyl dimethoate upon subsequent extraction with chloroform and glc analysis. The recovery of *N*-hydroxymethyl dimethoate, when amounts of the *O*-glucoside varying from 10 to 110 μg were subjected to these hydrolysis conditions, averaged 80%. The values obtained for recovery of the *O*-glucoside from grapes are listed in Table V.

Grape samples field treated with Cygon systemic 25 insecticide and harvested 28 days after the final application (sample numbers A-7, A-8, B-4, and B-5 as described in Tables II and III) gave residue values of <0.05 ppm of apparent de-*N*-methyl dimethoate, *N*-hydroxymethyl dimethoate, de-*N*-methyl dimethoxon, or *N*-hydroxymethyl dimethoxon. No evidence for the presence of sugar adducts was found at the validated sensitivity limit of the hydrolysis–glc procedure (adduct equivalent, 0.05 ppm of *N*-hydroxymethyl dimethoate) in these field-treated grape samples.

The values obtained for residues of dimethoate, dimethoxon, and potential dimethoate metabolites in grapes field-treated with dimethoate render it highly unlikely that any *N*-hydroxymethyl dimethoxon *O*-glucoside could be formed in these grapes. These residue values demonstrate degradation of dimethoate residues from a maximum level of 18 ppm to approximately 0.5 ppm with no accumulation of dimethoxon (maximum of 0.3–0.4 ppm) and no indication of conversion to *N*-hydroxymethyl dimethoxon or even to *N*-hydroxymethyl dimethoate which might in turn be oxidized to *N*-hydroxymethyl dimethoxon. On the basis of these observations and the fact that any appreciable quantity (<0.05 ppm) of the *O*-glucoside of *N*-hydroxymethyl dimethoate was absent, the probability that the *O*-glucoside of *N*-hydroxymethyl dimethoxon might be present is considered unlikely and no attempt has been made to undertake the arduous task of synthesizing and testing this compound.

The work herein reported proving the absence of residues of unconjugated de-*N*-methyl dimethoate, *N*-hydroxymethyl dimethoate, de-*N*-methyl dimethoxon, or *N*-hydroxymethyl dimethoxon as well as conjugates of *N*-hydroxymethyl dimethoate adequately demonstrates that the presently recommended methods for residues of dimethoate and dimethoxon are a reliable measure of the total toxic dimethoate related residues in grapes treated with Cygon systemic 25 insecticide.

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Differential Pulse Polarographic Determination of Nicarbazin in Chicken Tissue

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An assay procedure for the quantitative determination of nicarbazin, which is an equimolar complex of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP), in chicken muscle, liver, kidney, and skin-fat is described. The polarographically active moiety DNC is extracted from the tissue by solvent extraction and is determined quantitatively by differential pulse polarography. The estimated sensitivity of the method is 2 ppm with a limit of detection of 0.2–0.3 ppm. Recovery of nicarbazin from tissues was 94 ± 5% with a range of 85–102%. Tissues from chickens which were fed nicarbazin in the diet were assayed. Average on-drug nicarbazin residues were 2.7 ppm in muscle, 3.5 ppm in skin-fat, 18.0 ppm in liver, and 10.1 ppm in kidney. All tissues had less than 2-ppm residue at 4 days off drug.

The coccidiostat nicarbazin is an equimolar complex of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethylpyrimidine (HDP). When chickens are given nicarbazin in the feed, the HDP moiety is absorbed and excreted more rapidly than the DNC moiety (Porter and Gilfillan, 1955). Thus, in the assay procedure described here, the more slowly eliminated DNC moiety is determined by differential pulse polarography after it is extracted from the tissue. Porter and Gilfillan (1955) developed a colorimetric assay procedure for nicarbazin which requires the use of nonmedicated tissues to correct for a tissue blank.

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