Determination of Methomyl by Using 1-Fluoro-2,4-dinitrobenzene Reaction and Gas–Liquid Chromatography

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A method using 1-fluoro-2,4-dinitrobenzene (DNFB) for determination of methomyl (syn) isomer or Lannate (*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate) is reported. It involves hydrolysis of methomyl by sodium hydroxide immediately before addition of DNFB. The methylamine derived from methomyl is then allowed to react with DNFB at 80–82° in a water bath equipped with a shaker. The reaction product 2,4-dinitrophenylmethylamine (DNPMA) is extracted with benzene and analyzed by gas–liquid chromatography. Optimum conditions affecting DNFB and methylamine are also reported. Recoveries of methomyl added to rapeseed oil at 0.01–1 ppm range from 80 to 105%, with an average of about 97%. The technique gives quantitative recoveries of DNPMA from methomyl (anti) isomer and carbaryl standards. Dinitrophenyldimethylamine was also obtained from Zectran.

1-Fluoro-2,4-dinitrobenzene (DNFB) was used in the determination of amines by gas–liquid chromatography (glc) by Day *et al.* (1966). The reaction conditions and the glc resolutions and responses were satisfactory, thus encouraging Holden *et al.* (1969) and Sumida *et al.* (1970a,b) to adapt the method for determination of carbamate standards and residues in plant crops. The method reported by Holden *et al.* was found unsatisfactory by Sumida *et al.* (1970a) because it was time consuming and tended to give low yields. Sumida *et al.* (1970a) suggested that the loss may be due to volatilization of methylamine prior to DNFB reaction. These workers (1970a) modified the method to avoid the loss of volatile methylamine derived from a carbamate pesticide. DNFB was dissolved in dioxane and was added to 5% borax solution containing the carbamate pesticide. The carbamate was hydrolyzed in a boiling water bath for 30 min. Recoveries for the seven carbamates tested were 91–106%.

We found that the method described by Sumida *et al.* (1970a) gave variable and generally low recoveries for methomyl (*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate). Heating at 100° for 30 min did not completely hydrolyze methomyl. Because of these inconsistencies, optimum reaction conditions were studied. Factors also studied were (1) the rate of methomyl hydrolysis by NaOH, (2) DNFB concentrations, (3) temperature and duration of reaction, (4) concentrations of methomyl in the reaction media, and (5) optimum conditions for detection of the reaction product 2,4-dinitrophenylmethylamine (DNPMA). The modified method was evaluated for the determination of methomyl added to rapeseed oils.

Methomyl has been used in Canada to control armyworms attacking rapeseed plants in Saskatchewan. It is registered in the Food and Drug Act and Regulations (National Health and Welfare, Queen's Printers, 1972) for cabbage under 5-ppm tolerance. No method is available for quantitative determination of methomyl residues in rapeseed oils.

MATERIALS AND METHODS

Reaction and Extraction Procedures. Five milliliters of 5% borax and 0.5 ml of 1 *N* NaOH were pipeted into a 15-ml graduated centrifuge tube containing methomyl (syn isomer) standard, methomyl residue, or methylamine hydrochloride. (Methomyl was a gift from DuPont de Nemours Co., Wilmington, Del.) The solution was mixed thoroughly and heated for 10 min in an 82° water bath equipped with a shaker. DNFB (10 μl) (Eastman Kodak Co., Rochester, N. Y.) dissolved in 1 ml of dioxane was added to the solution and allowed to react with methylamine. After 10 min of heating, 1 ml of saturated glycine solution was added. After another 10 min, the tube was cooled in an ice bath. The reaction product DNPMA was extracted with 4 ml of benzene and then with two 2-ml portions of benzene. The aqueous layers were discarded. The benzene fractions were combined in a 15-ml centrifuged tube, which was then filled with 0.1 *N* Na_2CO_3 to the mark and shaken vigorously. The benzene layer was transferred into another tube. The Na_2CO_3 layer was washed with 4 ml of fresh benzene. The benzene fractions were combined and concentrated to approximately 0.5 ml under a gentle flow of nitrogen.

The procedure was also applied to the following insecticide standards: (a) methomyl anti isomer, (b) carbaryl (1-naphthyl methylcarbamate), and Zectran (4-dimethylamino-*m*-tolyl methylcarbamate).

Microcolumn Cleanup Procedure. The extract, which was concentrated to ~0.5 ml, was transferred to a microcolumn (pasteur pipet) containing 5 cm of silica gel G-HR. The centrifuge tube was rinsed with 0.5 ml of benzene; the rinse was combined with the extract in the column. The extract was eluted under vacuum at a rate of 60–70 drops/min. The tube was rinsed again with three 1.5-ml portions of benzene. Each 1.5-ml benzene fraction was added as the solvent meniscus reached the top of the gel. The eluate was diluted to obtain a concentration that was appropriate for glc analysis. Details of the microcolumn cleanup procedure will be described elsewhere.

Glc Analysis. The reaction product of DNFB and methylamine was analyzed by glc before and after microcolumn cleanup. The chromatograph (Aerograph HyFi Model 500-C) was equipped with an electron capture detector. Column temperatures tested were 195–200 and 210–215°. The nitrogen flow was adjusted to give 9–10 min retention time for DNPMA. The glass column, 4 ft × 0.25 in., was packed with 80–100 mesh Chromosorb W-HP coated with 4% SE-30 and 6% QF-1. Columns made of 10% OV-225, 2.5, 5, and 10% SP-525, and 4% SE-30–2.5% SP-525 were also tested.

Preparation of DNPMA Standard. Methylamine hydrochloride salt was allowed to react in an alkaline medium with DNFB to obtain DNPMA (Day *et al.*, 1966). The solids obtained were washed several times with 0.1 *N* Na_2CO_3 and recrystallized six times either in ethanol–water followed by petroleum ether–cyclohexane (1:1, v/v)

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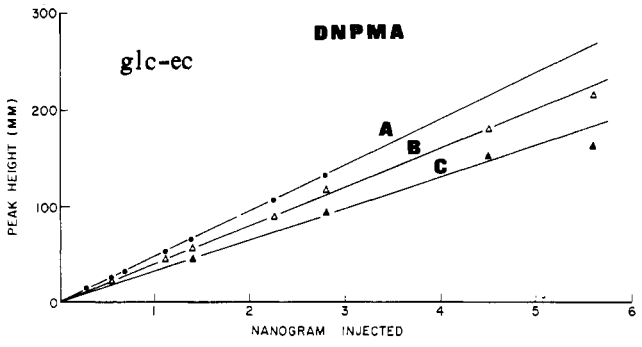

Figure 1. Standard curves for DNPMA with a 4% SE-30-6% QF-1 column at 212° (A,C) and at 197–198° (B). Each injection of the standard solution was in 5 μl; glc-ec means gas-liquid chromatography-electron capture detection.

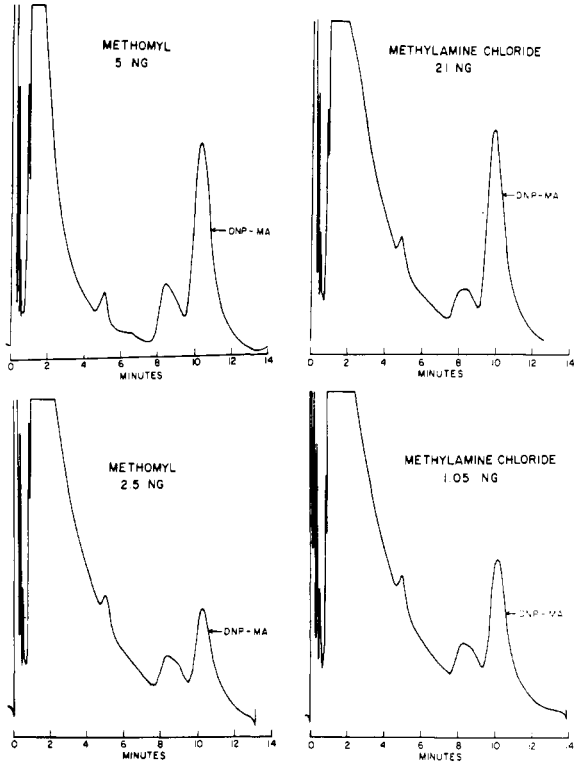

Figure 2. Typical chromatograms of solutions extracted from the reaction media in which DNFB was incompletely dissolved. The DNPMA peaks at about 10.5 min are pointed by arrows. Note the huge peak extending from 1 min to about 7 min of the resolution period.

or in ethanol-water (1:1, v/v) alone. The standards gave a single DNPMA peak under the glc conditions described in the preceding paragraph. The melting point (uncorrected) of the crystals was 177° and agreed well with the previous reports (Hollingsworth, 1959; Asatoor, 1960; Day *et al.*, 1966). The elemental composition (C, 42.66; H, 3.64; N, 21.20) agreed with the theoretical values (C, 42.79; H, 3.55; N, 21.37).

Sampling. Duplicate tests, each test replicated at least twice, were done. Each sample was analyzed at least twice by glc.

RESULTS AND DISCUSSIONS

Under the conditions used, the 4% SE-30-6% QF-1 column was found superior over the other types of column tested. Figure 1 shows DNPMA standard curves obtained by glc-ec (electron capture) detection at column temperatures of 212° (A and C) and 197–198° (B). Linear curves were obtained from 0.2 to 5 ng of DNPMA regardless of

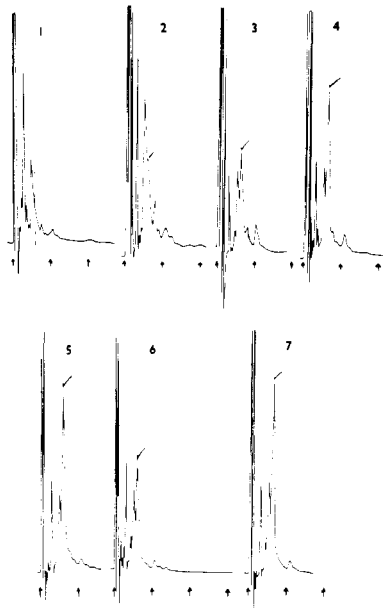

Figure 3. Typical glc chromatograms after reaction of methylamine derived from methomyl with DNFB. Unless specified each final extract was in 10 ml of benzene. Methomyl added was in parts per million: (1) blank, without methomyl, 50 g of rapeseed oil; (2) 0.01 ppm in 50 g of oil; final extract in 5 ml of benzene; (3) 0.05 ppm in 50 g of oil; (4) 0.1 ppm in 50 g of oil; (5) 0.5 ppm in 10 g of oil; (6) 0.5 ppm in 5 g of oil; (7) 1.0 ppm in 5 g of oil. The check marks indicate peaks corresponding to DNPMA. Arrows at the bottom of each chromatogram indicate the time interval: 9 min between arrows.

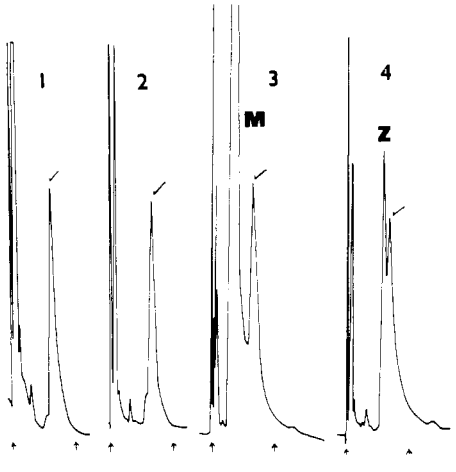

Figure 4. Glc chromatograms of DNPMA obtained from the reaction of DNFB and methylamine derived from methomyl (anti) isomer, carbaryl, and Zectran standards: (1) methomyl isomer in acetone; (2) carbaryl in acetone; (3) carbaryl in methanol; M indicates the peak for possibly 1-methoxydinitrobenzene; (4) Zectran in acetone; Z corresponds to the dinitrophenyldimethylamine product.

the detection sensitivity and the column temperature. It must be noted that A and B curves were done immediately one after the other and the C curve on a later date. Because of fluctuations in the detection sensitivity (see A and C) the glc should be monitored by injecting standard solutions at intervals.

Table I shows that satisfactory recoveries were obtained when 10–40 μl of DNFB was allowed to react with methylamine. The recoveries with 2.1 μg of methylamine were generally lower than those with 4.2 μg. The glc chromatograms obtained from the reaction using 80 μl of DNFB had huge background peaks and could not be evaluated.

Table I. Effects of Varying Amounts of DNFB on DNPMA Recovery

Chemical	Amount[a] reacted, μg	% DNPMA recovery[b] A	B	C	D
Methylamine	2.1	94	87	80	NE[c]
	4.2	98	99	91	NE
Methomyl	5	50	*d*	*d*	*d*
	10	63	*d*	*d*	*d*

[a] Methomyl in 10 μl of acetone and 0.5 ml of 1 *N* NaOH were added to each 5-ml reaction solution. Each experiment was in duplicate, with 10- and 20-μl tests replicated twice. Standard errors vary from 0 to 8%. [b] Volumes of DNFB used in each reaction solution were A = 10, B = 20, C = 40, and D = 80 μl. [c] NE indicates that no evaluation of results was carried out because of glc interferences. [d] The experiment was not carried out.

Table II. Effect of Temperature on the Recovery of Methomyl after Reaction with 10 μl of DNFB at 60–100° for 30 min[a]

Test no.	Amt used, μg	Recovery, %, at 60°	75°	82°	100°
1	5	78	51	91	74
2	5	42	58	84	65
1	10	7	39	89	77
2	10	71	71	82	77

[a] Per cent recovery = (peak height for methomyl)/(peak height for methylamine) × 100.

Table III. Effects of NaOH, Which Was Added at Different Stages in the Procedure, on the Recovery of Methomyl after Reaction with DNFB at 82°

Chemical	% recovery[a] I	II	III	IV	V	VI
Methomyl	66	94	64	95	60	60
	64	100	59	63	59	62
Methylamine (control)	94	*b*	*b*	*b*	*b*	*b*
	94	*b*	*b*	*b*	*b*	*b*

[a] I, control, without NaOH; II, NaOH added to methomyl in borate solution and then heated for 30 min before adding DNFB; III, NaOH, borate, and dioxane–DNFB solutions were mixed before addition of methomyl; IV, dioxane and borate were mixed before DNFB and NaOH were added; V, NaOH added to the reaction solution immediately after 60-min heating; VI, NaOH was added 10 min after heating the solution with glycine. All solutions were heated for 60 min with DNFB and another 10 min with glycine. [b] Not tested.

Table IV. Effects of the Length of Heating on Hydrolysis of Methomyl by NaOH

Temp, °C	% recovery[a] 5 min	10 min	20 min	30 min	45 min	60 min
27	n.t.	64 ± 0	60 ± 2	75 ± 1	60 ± 2	69 ± 1
82	92 ± 2	89 ± 1	93 ± 2	92 ± 2	96 ± 0	98 ± 0

[a] Glc analyses were carried out at 195–198° and were in duplicates except that values obtained after 5, 10, and 20 min at 82° were in quadruplicates; n.t. means not tested.

The data in Table II show that maximum interaction between methylamine and DNFB was at 82°. Poor recoveries of DNPMA were obtained at 60, 75, or 100°. Since the reaction at 100° was done in a water bath without a shaker, low DNPMA yields could be expected. Percent recoveries were inconsistent when the reaction was done at 60 and 70°. The discrepancies between duplicates may also be due to inadequate dissolution of DNFB. To avoid this problem DNFB should be dissolved in an adequate amount of dioxane before addition to the borate solution.

Figure 2 shows typical chromatograms for the reaction solutions in which DNFB was incompletely dissolved (see Table III, test IV). Each chromatogram showed a huge peak extending from 1 to 7 min of the resolution period. In addition, incompletely dissolved DNFB also gave variable and low DNPMA yields. Holden *et al.* (1969) also reported a DNFB peak near the solvent front. This may be due to DNFB that was not dissolved in the reaction medium and was extractable by benzene.

The mean per cent recoveries of duplicate samples tested under seven conditions are shown in Table III. Recoveries of DNPMA from test II indicate that methomyl was completely hydrolyzed before reaction with DNFB (*cf.* Table II). Tests III, IV, V, and VI were expected to give unsatisfactory results as test I because NaOH hydrolyzed DNFB before it could hydrolyze methomyl.

The data in Table IV show that methomyl was adequately hydrolyzed in 5–60 min at 82°. DNPMA recoveries were 89–98% when methomyl was heated with NaOH at 82° for 5–60 min. At 27°, only 60–75% recoveries were obtained.

Table V shows that values obtained from the same solutions analyzed by glc at 195–200 and 210–215° were comparable. However, the peak heights at 210–215° were generally higher than those at 195–200°. The table further shows that maximum interaction between DNFB and methylamine was obtained from 5- to 30-min incubation and agitation at 82°.

Figure 3 shows typical gas–liquid chromatograms of extracts of rapeseed oils fortified with methomyl at 0-, 0.01-, 0.5-, and 1.0-ppm levels. Chromatogram 2 shows that 0.01-ppm methomyl cannot be quantitated because of the interfering peak before the DNPMA peak (see check mark). For accurate quantitation, this unknown peak must not be higher than that of DNPMA.

Table VI shows the mean per cent recoveries of methomyl added to rapeseed oils. Recovery values based on peak heights for methomyl at 0.05, 0.5, and 1 ppm were from 95 to 105% (Table VI, A and A_1). Those obtained from a 0.1-ppm level were lower, *i.e.* 80–84%, but were within the acceptable recovery values. The data also indicate a loss of residue when the extracts were chromatographed again using another fresh cleanup column (Table VI, A_3). Recovery values obtained by integration of peak areas (Hewlett-Packard 3370B integrator) were inconsistent (Table VI, A_2) and did not agree with those obtained by means of peak heights. The inconsistency was due to occasional appearance of a tiny peak on the "tailing" side that terminated integration. The resulting values were smaller than those obtained in the absence of this peak. Therefore, to obtain reliable results the integrator programmer and adjustment must be fully controlled.

The procedure developed was also applied to other carbamate insecticide standards. Figure 4 shows gas–liquid chromatograms of DNPMA obtained from the reaction of DNFB and methylamine derived from methomyl (anti) isomer, carbaryl, and Zectran. As expected, chromatograms obtained from the anti isomer and carbaryl were similar to that from the syn isomer. The figure shows also that DNFB was very reactive with methanol (see chromatograph 3, peak M) or ethanol. Chromatogram 4 shows two DNFB reaction products obtained from Zectran. The second product was found to correspond to dinitrophenyldimethylamine. Under similar conditions, Frei and Lawrence (1972) found that dimethylamine was readily hydrolyzed from the phenolic moiety of Zectran.

CONCLUSIONS

The DNFB method as described in this report was found satisfactory for analysis of methomyl standards

Table V. Mean Per Cent Recoveries of Methylamine and Methomyl Incubated for 10 min with NaOH and Then with DNFB for 5, 10, 20, and 30 min at 82°

Chemical	Glc column temp, °C	Evaluation method[a]	Mean % recovery ± SE at 5 min	10 min	20 min	30 min
Methylamine	195–200	A	85 ± 1[b]	98 ± 1[b]	90 ± 6	93 ± 3
		B	n.t.	n.t.	101 ± 3	107 ± 4
	210–215	A	92 ± 3	77 ± 1	85 ± 4	81 ± 4
		B	102 ± 2	90 ± 0[b]	90 ± 1	n.t.
Methomyl	195–200	A	96 ± 0[b]	98 ± 1[b]	92 ± 6	91 ± 5
		B	n.t.	n.t.	108 ± 5	114 ± 3
	210–215	A	96 ± 2[b]	94 ± 3[b]	91 ± 3	95 ± 2
		B	106 ± 2	106 ± 2	96 ± 3	94 ± 4[c]

[a] A, the peak height corresponding to methomyl or methylamine product was compared with that of standard DNPMA added to the reaction blanks; B, the peak height corresponding to methomyl product was compared with that of standard DNPMA alone. Each test was in duplicate and replicated twice. Unless specified, each mean was based on four injections; n.t. means not tested. [b] Based on two injections. [c] Based on three injections.

Table VI. Per Cent Recoveries of Methomyl Added to Rapeseed Oils

[Methomyl], ppm	Oil used, g	Equiv amt of oil injected, mg	Mean % recovery ± SE[a] A	A_1	A_2	A_3	B
1	5	3.75	98 ± 2 (4)	94 ± 0 (2)	78 ± 3 (2)	94 ± 4 (2)	96 ± 3 (2)
0.5	5	2.5	105 ± 5 (8)	104 ± 1 (4)	134 ± 8 (4)	94 ± 4 (2)	104 ± 0 (2)
0.5	10	5	95 ± 2 (7)	90 ± 2 (2)	92 ± 4 (2)	86 ± 0 (2)	97 ± 0 (2)
0.1	50	25	83 ± 1 (9)	80 ± 1 (2)	88 ± 27 (2)	78 ± 4 (2)	84 ± 2 (2)
0.05	50	25	100 ± 3 (6)	104 ± 4 (2)	84 ± 0 (2)	68 ± 1 (2)	99 ± 4 (2)
0.01	50	25	n.t.	n.t.	n.t.	n.t.	N[b]

[a] B is a replicate of A; both were based on peak heights. A_1, A_2, and A_3 values were obtained from extracts A. A_1 was based on peak heights obtained through an integrator. A_2 was based on integrated peak heights. A_3 was based on peak heights; the extracts were cleaned up twice by microcolumn chromatography using a silica gel column. Figures in parentheses indicate the number of glc determinations; n.t. means not tested. [b] N indicates negligible peak heights.

added to rapeseed oils. The recoveries were good and no glc interference was obtained from the oils.

To obtain reproducible results and maximum yields of DNPMA, DNFB must be dissolved properly in the aqueous reaction medium. Incomplete dissolution of DNFB may be one of the reasons why the method developed by Holden *et al.* (1969) was unsatisfactory (see also Sumida *et al.*, 1970a). Furthermore, the per cent recoveries reported by Holden *et al.* (1969) and Holden (1973) may not represent complete recoveries because they were based only on glc standard curves obtained from the reaction of DNFB and methylamine or the phenol moiety of the pesticide standard. This method of comparison would only be valid if (a) carbamate hydrolysis was complete (Holden *et al.*, 1969; Holden, 1973), (b) DNFB and methylamine moiety interaction was maximum (Holden *et al.*, 1969), and (c) there was no loss of volatile methylamine before DNFB reaction (Holden *et al.*, 1969). Their reports did not state that all the conditions were met. The DNPMA or dinitrophenyl ether derived from the reaction must be compared with its corresponding standard and not with the products obtained from a pesticide standard.

The method reported by Sumida *et al.* (1970a,b) was found unsatisfactory because of the interference that appeared just after the DNPMA peak. Glycine reduced this interference but the reduction was not sufficient for accurate quantitation. Column cleanup was necessary to remove this interference. The method of Sumida *et al.* (1970a) was also found unsatisfactory for methomyl because heating the reaction solution was not enough to completely hydrolyze this carbamate. This problem was solved by hydrolyzing the carbamate with NaOH immediately before reaction with DNFB.

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