Gas Chromatographic and Colorimetric Measurement of Dimethoate Residues

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Dimethoate residues were analyzed on field-treated snap bean extracts, and the dinitrochlorobenzene colorimetric procedure was compared with detection by gas chromatography. Residue data determined on common extracts by electron affinity detection averaged 97% of those data obtained by the conventional colorimetric procedure. Disappearance curves indicated dimethoate residues on snap beans were below 2.0 p.p.m. 1 week after 0.5 pound actual per acre treatment. Dimethoate oxygen analog could not be satisfactorily separated from the parent material or quantitatively detected under the gas chromatographic conditions tested.

DIMETHOATE [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorodithioate] exhibits contact and systemic activity against certain insect pests attacking plants and animals. It has an acute toxicity to rats of about one fiftieth of parathion, but, unlike many organophosphates, dimethoate readily passes into the aqueous tissues of the plant where it undergoes oxidation and hydrolysis. The selective toxicity of dimethoate according to Dauterman *et al.* (*4*) may be dependent on the ability of the mammal to attack the C—N bond more vigorously than can the insect. Alessandrini (*1*) reports that dimethoate residues in heavily-treated olives were down to 2 or 3 p.p.m., 10 days after application. Most of the dimethoate and derivatives were found in the aqueous tissues, while practically none was detected in the olive oil, according to Santi and Giacomelli (*8*), indicating the lack of liposolubility of the compound or its metabolites.

As a result of oxidation and hydrolysis, various metabolites are formed. From a toxicological standpoint, however, only the oxygenated analog need be considered. Plant and animal metabolism of dimethoate show marked differences as is illustrated in Figure 1. Dauterman *et al.* (*5*) report that the important pathways for dimethoate in plant foliage are either to the oxygen carboxy or to the desmethyl derivatives. In animal systems, these metabolites are relatively minor, with the primary product reported to be the carboxy derivative. This metabolite is apparently not produced by foliar applications of dimethoate to plants. Labeled dimethoate metabolic studies with sheep by Chamberlain *et al.* (*2*) indicate three possible degradation pathways in animals: rupture of the C—N or amide bond to yield the thiocarboxy derivative, replacement of the sulfur atom by an oxygen atom to produce the oxygen analog, and loss of a methyl group to produce the desmethyl derivative. The desmethyl derivative

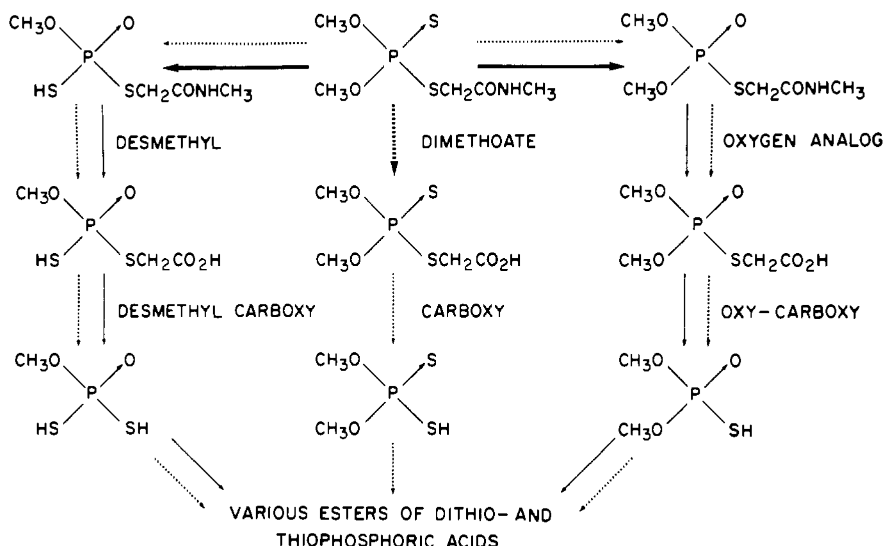

Figure 1. Pathways of dimethoate metabolism in plants (⟶) and animals (- - ->)

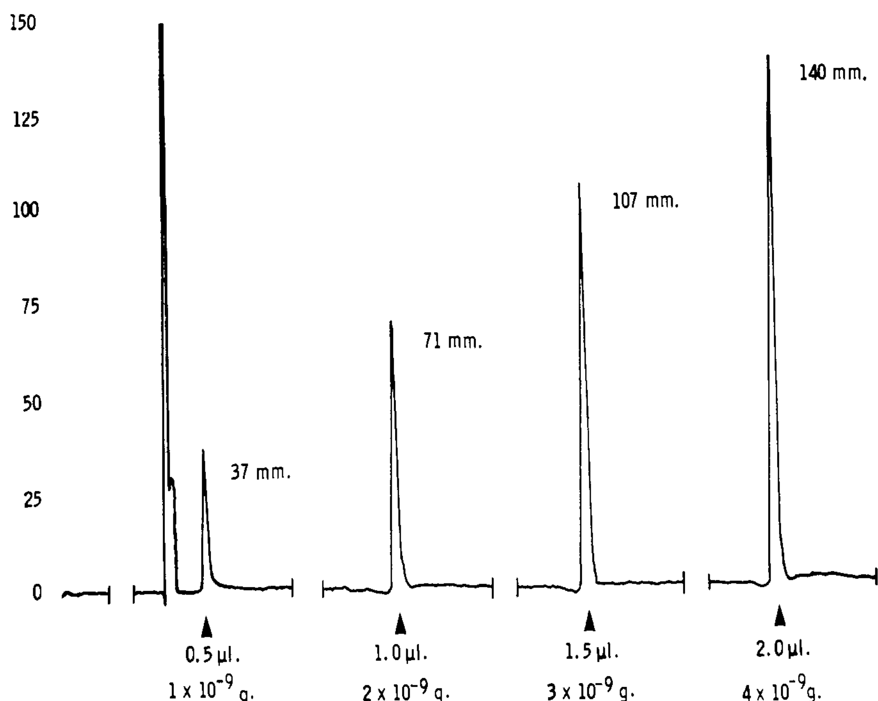

Figure 2. Dimethoate standard curve by electron capture detection

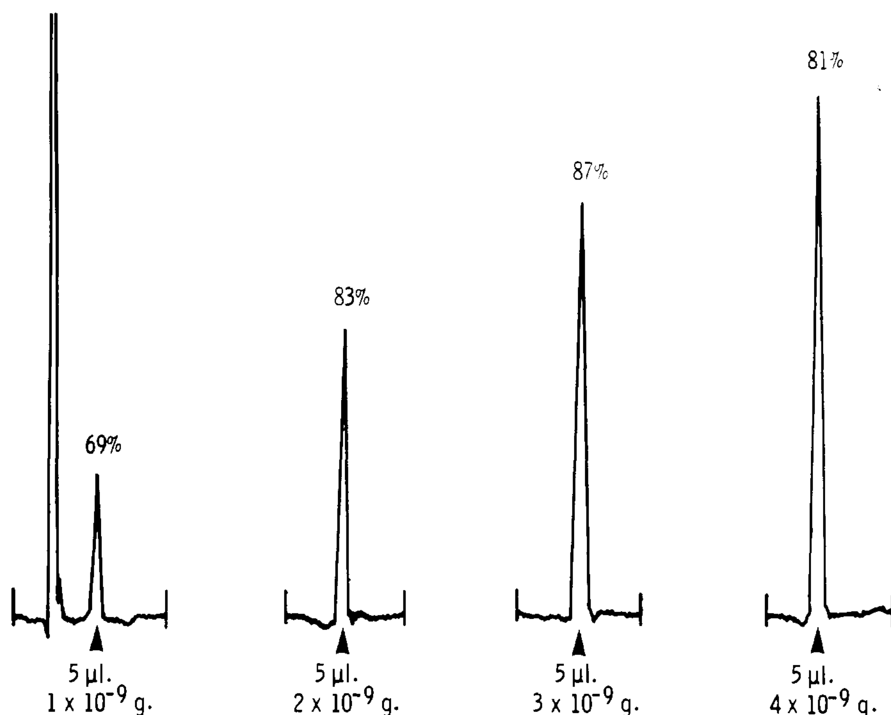

Figure 3. Dimethoate recoveries from snap bean extracts

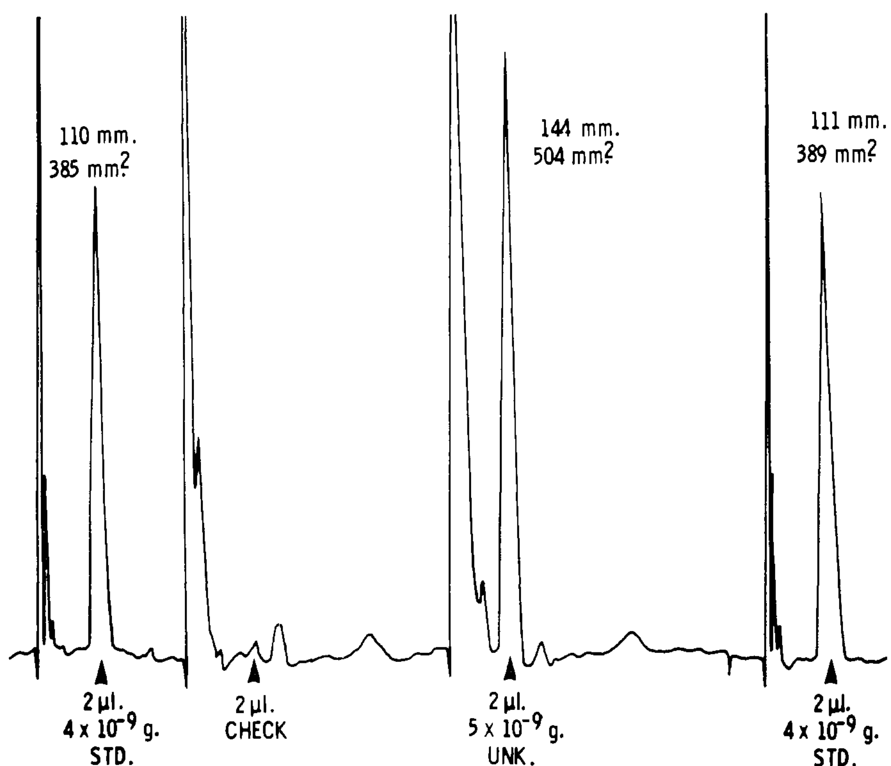

Figure 4. Dimethoate standards compared to check and treated unknown

does not appear to have anticholinesterase properties, and its mammalian toxicity is comparatively low. The oxygen analog, according to Dauterman *et al.* (*4*), exhibits an oral LD_{50} value of 55 mg. per kg. on white rats as compared to 600 mg. per kg. for dimethoate on the same test animal.

In green vegetable crops, Chilwell *et al.* (*3*) report that more dimethoate residues were found in the outer leaves than in the edible parts of the crops tested. For instance, brussels sprouts analyzed 8 days after spraying contained 4.9 p.p.m. dimethoate in the leaves and only 0.7 p.p.m. in the sprouts. Cabbages contained 2.4 p.p.m. in the outer leaves, 1.4 p.p.m. in the wrapper leaves, and 0.4 p.p.m. in the edible heart 7 days after spraying. Following a 9.6-ounce active per 100-gallon spray, broad beans contained 0.5 p.p.m. 1 week after treatment. Chilwell and coworkers concluded from their animal feeding studies that residual dimethoate and its metabolites in lettuce leaves are no more toxic to mammals than is pure dimethoate. Dauterman *et al.* (*5*) found that dimethoate was rapidly absorbed and decomposed by oxidation and hydrolysis both on the surface and inside the foliage of corn, cotton, pea, and potato plants. Only trace amounts of dimethoate or its oxygen analog were present in these plants 32 days after treatment.

Systemic property studies by Santi and de Pietri-Tonelli (*9*) indicate that both dimethoate and its oxygen analog, when applied to roots, stems, or leaves of beans, are absorbed and translocated to other parts of the plant in amounts lethal to the test mites. These authors state that the oxygen analog, present only in trace amounts in plants, is more acaricidal than is the parent compound.

Experimental Procedure

Dimethoate (Cygon 2E, 25%) was applied to snap beans in four-row field plots with a John Bean Hi-Lo sprayer at a pressure of 100 to 125 pounds per square inch to control aphids, thrips, and leaf miners. Dosage rates applied to three field replications were 0.25-, 0.50-, and 1.00-pound active ingredient per 100 gallons per acre. Two applications were applied 7 days apart, and samples were taken 1, 3, 7, and 14 days following the second application.

Approximately 1000-gram samples of snap beans were picked at random from the center rows of each field plot. Untreated (check) samples were selected on each sampling date. A representative 500-gram subsample was finely ground on a Hobart food cutter and frozen until time for analysis.

Dinitrochlorobenzene (Colorimetric) Determination. EXTRACTION. On the day of analysis, and depending on the suspected amount of insecticide in the field sample, from 10 to 75 grams of finely chopped snap beans were removed from the frozen gross sample. The beans were macerated on a Lourdes Multi-mix blender at medium speed in 150 ml. of methylene chloride for 3 minutes. The macerate was then filtered through four layers of cheesecloth and subsequently squeezed to remove as much of the solvent from the pulp as possible.

CLEANUP. The resulting extract was divided into equal portions and centrifuged for 20 minutes at 3000 r.p.m. to separate the water and emulsion layer from the methylene chloride phase. Transfers were made to separatory funnels, and the bottom methylene chloride layer was drawn off and saved. Approximately 5 grams of unwashed

Table I. Recovery of Dimethoate (Chlorodinitrobenzene)[a]

Added, μg.	Recovered, %	Added, μg.	Recovered, %
20	62	60	70
40	70	80	71

[a] Sensitivity = 0.05 p.p.m.

C-190-N nuchar was thoroughly mixed with the extract and filtered through porcelain Büchner funnels containing anhydrous sodium sulfate and a glass wool plug. The recovered volume was noted and added to Rinco evaporators containing 50 ml. of distilled water heated at 40° C. The evaporation was carried out until all methylene chloride was removed. The remaining water phase was then passed through a 20- × 300-mm. column containing 10 grams of polyethylene-coated alumina at a rate of approximately 60 drops per minute.

PROCEDURE. The remainder of the dinitrochlorobenzene procedure was carried out as outlined in the procedure adapted by the American Cyanamid Co. from the original method by George (*6*). Per cent recovery studies conducted with this procedure are outlined in Table I. The recovery studies were carried out as follows: 50 grams of check crop + 150 ml. of methylene chloride + standard were added prior to maceration and compared with a methylene chloride only standard curve.

Gas Chromatographic Measurement by Electron Affinity Detection. EXTRACTION. On the day of analysis, 25 grams of finely chopped snap beans were removed from the frozen gross sample. The beans were macerated in a Lourdes Multi-mix blender for 4 minutes at medium speed in the presence of 150 ml. of purified methylene chloride.

CLEANUP. The macerate was filtered through cheesecloth followed by the addition of 1 gram of unwashed C-190-N nuchar and 30 grams of anhydrous sodium sulfate to the recovered extract. After blending for 1 minute, the extract was filtered through sharkskin filter paper. The cake was thoroughly rinsed twice with 25-ml. portions of methylene chloride. After the addition of 50 ml. of reagent grade benzene, the solution was evaporated to approximately 2 to 5 ml. with gentle heat and air flow. The sample was made to final volume in 10- or 25-ml. volumetric flasks with reagent grade benzene.

PROCEDURE. Usually 5 or 10 μl. of sample from volumetric flask were injected into the instrument. Results obtained in this study were conducted under the following conditions by means of an electron capture detector in a Jarrell-Ash Universal Chromatograph Model 700. The 4-foot, U-shaped, 1/4-inch stainless steel column was packed with 2% SE-30 on 80- to 90-mesh Anakrom A.

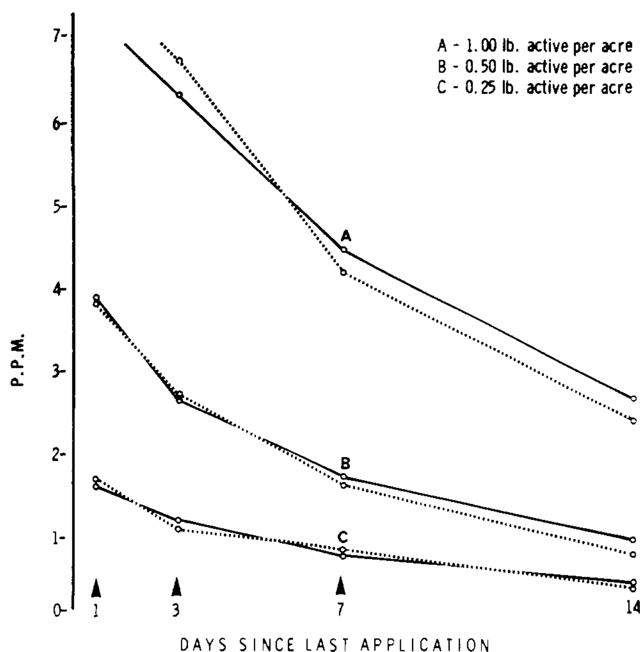

Figure 5. Dimethoate disappearance curves by two methods of detection

Electron affinity (........) and colorimetric (———)

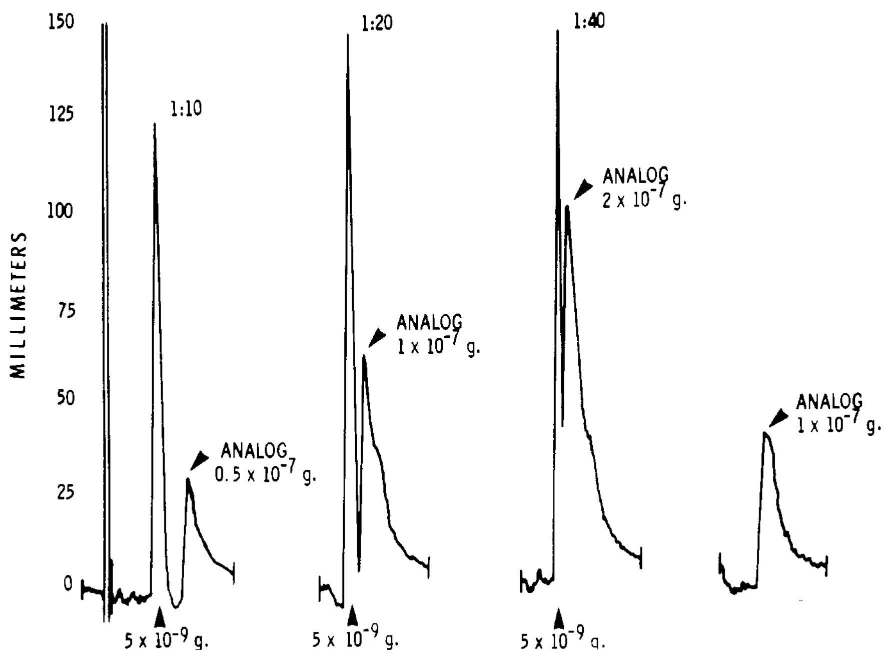

Figure 6. Response of dimethoate and oxygen analog benzene mixtures (5 μl.) with increasing ratios of dimethoate:oxygen analog

The carrier gas controller was set at 30 pounds per square inch to deliver 280 to 300 ml. per minute nitrogen gas flow rate through the column. The instrument was operated with a detector voltage of 10 volts, amplifier range of 2×10^{-9} amp f.s.d., and a chart speed of 15 inches per hour. The optimum operating temperatures for the dimethoate chromatographs were found to be as follows: injector, 200° C.; column, 175° C.; splitter collector, 200° C.; and detector, 200° C.

Per cent recoveries by electron capture detection are indicated in Table II. The recovery studies were carried out as follows. To each of five Mason jars was added 25 grams of finely chopped snap beans, 150 ml. of methylene chloride, and 0, 5, 10 and 20 μg. of dimethoate standard. After maceration, these samples were carried through the entire extraction cleanup and analyzed as previously outlined. A second dimethoate standard curve was carried through the analytical procedure with no crop maceration or cleanup. The peak heights obtained for the various injec-

Table II. Recovery of Dimethoate (Electron Affinity)[a]

Nanograms[b] Added	Recovered, %	Nanograms Added	Recovered, %
1	69	3	87
2	83	4	81

[a] Sensitivity = 0.08 p.p.m.
[b] 5-μl. injection.

tions of standard only and standard plus crop are illustrated, respectively, in Figures 2 and 3. An example of how a treated (unknown) sample was chromatographed and compared to a dimethoate standard is graphically illustrated in Figure 4.

Gas Chromatographic Measurement by Microcoulometric Titration. Dimethoate and its oxygen analog were subjected to gas chromatography and microcoulometric detection using a T-200-P sulfur-sensitive titration cell. Numerous injections were made with the instrument variables ranging as follows: column, 150° to 175° C.; block, 200° to 300° C.; combustion tube, 800° C.; and gas flow rates, 50 to 180 ml. per minute. The following types of columns were attempted: 1.5-foot × 1/4-inch aluminum, 10% DC-200 oil; 3-foot × 1/4-inch aluminum, 2.5% epon resin 1001; 6-foot × 1/4-inch aluminum, 20% DC silicone grease, 30/60 chromosorb P; 2-foot × 1/4-inch copper, 5% SE-30, 65/100 chromosorb W; 1.5-foot × 1/4-inch glass, 2% SE-30, 80/90 Anakrom A; 1.5-foot × 1/4-inch glass, 20% SE-30, Fluoropak; and 6-foot × 1/4-inch glass, 2% SE-30, 80/90 mesh Anakrom A.

The dimethoate peaks obtained under the above conditions were considered unsatisfactory because of excessive tailing. Efforts to eliminate tailing by raising the temperature and/or gas flow rate resulted in peaks that were not considered satisfactory because of excessive background interference.

Fairly satisfactory peaks were obtained using a 1.5-foot × 1/4-inch glass column packed with 2% SE-30 on 80- to 90-mesh Anakrom A support. The instrument at this time was operated at the following temperatures: column, 220° C.; block, 270° C.; combustion tube, 800° C., and flow rate, 120 ml. per minute.

Dimethoate injected into the Dohrmann microcoulometric gas chromatograph under the above conditions resulted in fair recoveries, but it was difficult to separate the solvent from the pesticide because of its very short retention time.

Because of excessive background and apparent degradation of dimethoate under the temperature limitations of this instrument, further dimethoate studies by microcoulometric gas chromatography were discontinued until additional solid and liquid phase research in various columns can be accomplished.

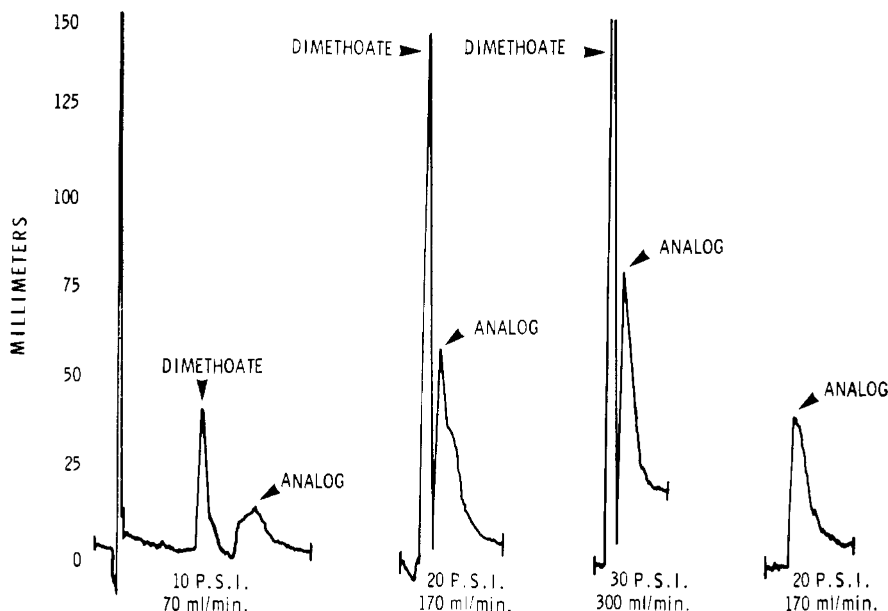

Figure 7. Effect of varying gas flow rate on oxygen analog recoveries of 5 μl. of 1:20 mixture of dimethoate (5×10^{-9} gram) and analog (1×10^{-7} gram)

Table III. Comparison of Dimethoate Residues[a] **(P.P.M.) in Snap Beans by GLC (Electron Affinity Detection) and Colorimetric (Chlorodinitrobenzene) Analyses**

Type of Analysis	Dosage, Lb. Act. per Acre	Time Lapse since Last Application, Days: 1	3	7	14
GLC	0.25	1.74	1.14	0.87	0.39
Color.	0.25	1.65	1.23	0.81	0.42
GLC	0.50	3.86	2.76	1.65	0.79
Color.	0.50	3.94	2.69	1.74	0.96
GLC	1.00	7.85	6.79	4.20	2.36
Color.	1.00	7.21	6.37	4.48	2.66

[a] Average of three field replications.

Results and Discussion

The variation in sensitivity of the two methods of detection, as indicated in Tables I and II, can be attributed to inherent variations in per cent recoveries. Dimethoate residue data presented in Table III were obtained from snap bean extracts analyzed by gas chromatography equipped with electron affinity detection and compared to colorimetric (chlorodinitrobenzene) analysis. The disappearance curves, as developed from the residue data obtained by the two analytical techniques, are graphically illustrated in Figure 5. Agreement between the two analytical procedures was excellent after an appropriate correction factor was applied, based on the respective per cent recoveries inherent to each technique. In fact, in averaging the 12 results shown in Table III, the residue data obtained by electron affinity detection are 97% of the average data obtained by the conventional colorimetric procedure.

Although to date no official tolerance has been established for the use of this pesticide on snap beans, Chilwell *et al.* (*3*) believe that a residue of 2.0 p.p.m. of dimethoate on a harvested crop would be safe for human consumption. This conclusion was based on results of dietary feeding trials on rats and other data on human ingestion.

Based on an unofficial tolerance of 2 p.p.m., data obtained by both procedures indicate that a 7-day interval would be sufficient on snap beans following the last 0.5-pound actual emulsifiable spray application. However, if the dosage rate was increased to 1.0-pound active per acre, more than 2 weeks of weathering would appear necessary to obtain harvested beans containing below the unofficial tolerance levels of dimethoate residues. The persistence of the residues might indicate that some of the systemic insecticide had penetrated the snap beans.

However, Dauterman and coworkers

(5) found that the breakdown rate of dimethoate was much slower on the corn leaf surface than inside the leaf. The rate of metabolism inside the corn leaf was found to be much faster than comparable areas of the potato or cotton leaves; but surface alteration on the corn leaf was the slowest. According to these workers, oxidation and not isomerization is the principal nonhydrolytic alteration of dimethoate on the leaf surface.

The oxygen analog, being a very polar compound, is infinitely soluble in water at room temperature, whereas dimethoate itself is only 2 to 3% water-soluble. The partition coefficient of dimethoate from water into chloroform is 20, whereas the value for the oxygen analog is only 0.7. Consequently, it is difficult to determine the oxygen analog by conventional means because of its poor distribution into organic solvents from plant macerates. Repeated extractions with fresh chloroform might have resulted in some partitioning out of the oxygen analog. This approach was not investigated. Attempts to modify the distribution of the oxygen analog by the addition of salts to the macerates results in the distribution of considerable interfering plant substances into the organic phase.

Maceration of plant extracts in the presence of anhydrous sodium sulfate followed by extraction resulted in considerable occlusion of the oxygen analog within the dry powder.

Other than chromatographic fractionation, there is apparently no efficient method at present for isolating quantitatively the oxygen analog formed on and in treated plant material. Moreover, the oxygen analog is such a weak cholinesterase inhibitor that it cannot be determined by conventional inhibition means.

Under the neutral plant macerate conditions employed with the dinitrochlorobenzene procedure, probably only dimethoate and its oxygen analog were removed to any degree. Because of distribution difficulties, undoubtedly very little of the oxygen analog was extracted and measured. Whatever was extracted would result in a color reaction since presumably only dimethoate and its oxygen analog give a positive reaction with the chlorodinitrobenzene solution. Using thin layer chromatography for separation and subsequent detection by micro infrared techniques, Sutherland (*10*) reports that, in general, only 10 to 15% of the originally applied dimethoate is metabolized to the oxygen analog in many crops even after 2 to 3 weeks of weathering.

Dauterman *et al.* (*5*) report that the quantity of oxygen analog found in the leaves of corn, potatoes, peas, and cotton plants was, in all instances, below 10% despite 12 days of weathering. These workers concluded that the formation of the oxygen analog on the leaf surface was apparently a nonenzymatic oxidation. However, within the plant, the oxygen analog is probably formed enzymatically.

Santi and Giacomelli (*8*) investigated the possible reasons for low residues of the P=O metabolite found in plant parts treated with dimethoate. Their studies indicated that the active ingredient penetrates and diffuses rapidly into the plant, simultaneously undergoing oxidation and hydrolysis. The P=O metabolite is detectable shortly after treatment, increasing to a maximum and then gradually decreasing. While residues of dimethoate and its oxygen analog are decreasing, products of hydrolysis are simultaneously increasing within the plant.

Once the proper conditions for satisfactory electron capture detection of dimethoate had been accomplished, it was thought that the oxygen analog would capture electrons as well as or even slightly better than the parent material. The only difference in the molecule is a substitution of a sulfur atom by an oxygen atom. According to Lovelock (*7*), oxygen and the halogens capture electrons readily to form stable negative ions. Hydrocarbons substituted with these elements, therefore, capture electrons, and the affinity of the molecule appears to be related to the ease of dissociation of the heteroelement from the compound. However, all compounds containing oxygen do not capture readily. For example, ethers, although containing oxygen, capture electrons weakly. On the other hand, anhydrides, peroxides, and diketones exhibit strong electron affinities.

The oxygen analog of dimethoate, under the conditions tested, exhibited weak electron capture properties. These may be partially explained by the fact that the substituted oxygen atom is not as readily removed from the molecule as was the original sulfur atom. This property may account for the transformation (during oxidation to the analog) from a compound considered a moderate capturer to one having low electron affinity.

Mixtures of purified dimethoate and its oxygen analog (90 plus % pure) were injected into the instrument. At a 1:1 ratio, the oxygen analog could not be detected. When the ratio was increased to 10 parts oxygen analog to one part dimethoate (10:1), the analog was detectable on the gas chromatograph. Figure 6 illustrates the response as the ratio was stepped up progressively to 20:1 and eventually to 40:1 but maintaining the dimethoate constant at 5 nanograms. The apparent dimethoate peak appears to increase along with the analog peak as the ratio of analog to dimethoate is extended. In an effort to increase the relative retention times between the two compounds, and thereby obtain a more pronounced separation, the gas flow rates were varied as indicated in Table IV.

Table IV. Relative Retention Times (Minutes) by Varying Flow Rates Using Electron Capture Detector

Compound Injected	10 P.S.I., 70 Ml. per Min.	20 P.S.I., 170 Ml. per Min.	30 P.S.I., 300 Ml. per Min.
Dimethoate	3.5	1.75	1.25
Oxygen analog	5.0	2.50	1.75

Figure 7 illustrates the effect of varying the gas flow rate on the oxygen analog recoveries. Fair separation from the dimethoate was achieved at the 170 ml. per minute flow rate (20 p.s.i.). Under the conditions of this study, the oxygen analog of dimethoate would have to be considerably in excess of the dimethoate present to be identified satisfactorily. Any oxygen analog that is not in excess will probably be chromatographed as dimethoate when electron capture detection is used.

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