# Metabolic Degradation of *O*-Ethyl *S*-Phenyl Ethylphosphonodithioate (Dyfonate) in Potato Plants

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The metabolites of the soil insecticide Dyfonate (*O*-ethyl *S*-phenyl ethylphosphonodithioate) in potato plants grown in treated soil were identified by chromatographic and autoradiographic methods, combined with degradative and derivatization procedures. Metabolites arising from cleavage of Dyfonate *in vivo* were examined in separate but identical experiments using preparations labeled with $^{14}C$ in the α-ethoxy carbon of the phosphonodithioate moiety and in the ring of the thiophenyl moiety. *O*-Ethylethanephosphonic acid is the major phosphonate metabolite, while the thiophenyl moiety is liberated, but thiophenol is not detected because it undergoes rapid methylation, sulfoxidation, and conjugation.

The insecticide chemical Dyfonate (*O*-ethyl *S*-phenyl ethylphosphonodithioate) controls wireworms attacking potatoes (Baranowski, 1964; Burrage *et al.*, 1967; Onsager *et al.*, 1966), and has shown utility in managing populations of many other soil-inhabiting pest organisms (van den Brink *et al.*, 1967). Accordingly, it is necessary to understand the chemical nature of Dyfonate-derived products, if any, which might be present in plants growing in Dyfonate-treated soil.

This study deals with the absorption, distribution, and metabolic degradation of Dyfonate in Irish potato plants grown in soil containing either a ring-$^{14}C$ (R-$^{14}C$) or an ethoxy-α-$^{14}C$ (E-$^{14}C$) preparation of this insecticide chemical. These labeling positions are useful in characterizing and identifying metabolites arising from both the thiophenyl and thiophosphonic acid moieties of Dyfonate.

## METHODS AND MATERIALS

**Chemicals.** The compounds, their chemical names, and sources are listed in Table I. Dyfonate-R-$^{14}C$ (4.65 mc per mmole) had a radiochemical purity of 99.2%, and Dyfonate-E-$^{14}C$ (5.75 mc per mmole) was 99.0% radiochemically pure. These purity determinations were made with the C:H thin-layer chromatography (tlc) system indicated in Table II. The general procedures of the synthesis of Dyfonate-E-$^{14}C$ were described by Kalbfeld *et al.* (1968).

**Soil Treatment and Potato Planting.** Three-gallon plastic buckets were filled with Felton sandy loam to an approximate depth of 6 in.; then, a 3 in. layer of similar soil, treated at the rate of 2.64 ppm with either Dyfonate-R-$^{14}C$ or -E-$^{14}C$, was placed on top. This treatment with Dyfonate is within the range of recommended field application. Sprouting Irish potato seed pieces were treated with Captan fungicide, held overnight at 40° F to induce suberization, and planted, one per container, in the treated zone. The containers were held in an outdoor lathhouse on location for the duration of the experiment, which was conducted during the months of August, September, and October.

**Extraction of Metabolites.** Foliage samples were harvested from 27-, 60-, and 83-day-old plants, and tubers from 67- and 83-day-old plants. The foliage was weighed and homogenized with acetone and methanol in a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, N.Y.). The insoluble portion was washed on a sintered-glass filter with several portions of acetone followed by methanol until completely decolorized, leaving the bound or unextractable fraction. The extracts were combined, concentrated in a rotary vacuum evaporator to near dryness, and the $^{14}C$-components separated into benzene-soluble and water-soluble fractions by adding water and extracting three times with equal volumes of benzene. Potato tubers were thoroughly washed with a soft brush under running cold water, peeled, and the separated pulp and peel were extracted in a similar manner to the foliage. Subsequently, radioactive residues from each of foliage, tuber pulp, and tuber peel were partitioned into three fractions: benzene-soluble, water-soluble, and bound or unextractable.

The radiocarbon in all samples was quantified by liquid scintillation counting (LSC) using a Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3214, equipped with external standardization. The counting solution used was described by Ford *et al.* (1966).

Insoluble plant solids were finely pulverized in each of three 50 mg aliquots, and each was suspended in LSC fluid for radioassay. Duplicate aliquots of plant extracts were radioassayed directly.

Benzene extracts were reduced to small volumes with a rotary vacuum evaporator for subsequent chromatography. The aqueous extracts were concentrated by lyophilization without significant loss of radiocarbon.

**Autoradiography of Plant Material.** Sixty-eight-day-old plants were cut at the soil line, immediately placed on a thin plastic sheet covering an X-ray film in film holders, and held in a freezer; the plastic served to minimize chemical reaction between plant and film. Potato tubers from 83-day-old plants were scrubbed to remove adhering soil particles, and thin slices were removed near the center of each potato and exposed to X-ray film in the same manner as the foliar portions of the plant.

**Chromatographic Systems and Detection of Resolved Metabolites.** The tlc systems used are given in Table II. Analytical separations were performed on Silica Gel H precoated (0.25 mm) tlc plates (Analtech Inc., Wilmington, Del.). Preparative separations were carried out with Silica Gel H with a gel thickness of 1.0 mm. Various products were detected on the tlc plates, as follows: radioactive materials by using autoradiography; Dyfonate, oxygen analog of Dyfonate designated oxon (*O*-ethyl *S*-phenyl ethylphosphonothiolate), and ETP with a spray solution of 0.5% (w/v) 2,6-dibromo-*N*-

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Table I. Names and Sources of Compounds Used

| Compound | Chemical Name | Source |
|---|---|---|
| Dyfonate | *O*-Ethyl *S*-phenyl ethylphosphonodithioate | Stauffer Chemical Co., Richmond, Calif. |
| Oxon | *O*-Ethyl *S*-phenyl ethylphosphonothiolate | Stauffer Chemical Co. |
| ETP | *O*-Ethylethanephosphonothioic acid | Stauffer Chemical Co. |
| EOP | *O*-Ethylethanephosphonic acid | Stauffer Chemical Co. |
| PSH | Thiophenol | Pitt-Consol Chemical Co., Newark, N.J. |
| MPSO | Methyl phenyl sulfoxide | Stauffer Chemical Co. |
| $MPSO_2$ | Methyl phenyl sulfone | Aldrich Chemical Co., Milwaukee, Wis. |
| 4-OH-$MPSO_2$ | 4-(Hydroxyphenyl) methyl sulfone | Crown Zellerbach, Camas, Wash. |
| 3-OH-$MPSO_2$ | 3-(Hydroxyphenyl) methyl sulfone | Stauffer Chemical Co. |
| 2-OH-$MPSO_2$ | 2-(Hydroxyphenyl) methyl sulfone | Stauffer Chemical Co. |
| Dyfonate-R-$^{14}C$ | *O*-Ethyl *S*-phenyl-$^{14}C$-ethylphosphonodithioate | Stauffer Chemical Co. |
| Dyfonate-E-$^{14}C$ | *O*-Ethyl-$\alpha$-$^{14}C$ S-phenyl ethylphosphonodithioate | Stauffer Chemical Co. |

Table II. $R_f$ Values of Compounds Identified as Dyfonate-$^{14}C$ Metabolites as Separated by Tlc in Four Solvent Systems

| Metabolite | B:A:W[a] | B:E[b] | C:EA[c] | C:H[d] |
|---|---|---|---|---|
| Dyfonate | 0.96 | 0.95 | 0.83 | 0.4 |
| Oxon | 0.87 | 0.16 | 0.60 | 0.0 |
| EOP | 0.59 | ... | 0.00 | ... |
| EOP-$CH_3$ | 0.87 | ... | 0.18 | ... |
| ETP | 0.81 | ... | 0.00 | ... |
| ETP-$CH_3$ | 0.95 | ... | 0.35 | ... |
| MPSO | ... | 0.04 | 0.28 | ... |
| $MPSO_2$ | ... | 0.42 | 0.69 | ... |

[a] B:A:W *n*-butanol:glacial acetic acid:water (2:1:1). [b] B:E benzene-ethyl ether (7:3). [c] C:EA chloroform-ethyl acetate (1:1). [d] C:H chloroform-hexane (2:3).

chloro-*p*-benzoquinoneimine (DCQ) in cyclohexane (Menn *et al.*, 1957) followed by exposure of the tlc plates to HCl fumes to enhance the color development; EOP with the ammonium molybdate-ammonium chloride reagent (Block *et al.*, 1958), and/or DCQ; MPSO with a saturated aqueous $KMnO_4$ solution; $MPSO_2$ with iodine vapors. To quantify the radioactive metabolites, radioactive gel regions were scraped free of the glass support, then placed in vials for radioassay by LSC.

When individual or composited foliage, tuber pulp, or tuber peel extracts were examined for metabolites, reference standards were chromatographed alone and in combination with the plant metabolites for comparison.

The possible presence of $MPSO_2$ and EOP was tested by radio-gas-liquid chromatography (rglc). $MPSO_2$ was analyzed directly, while ETP, EOP, and EOP-$^{14}C$ from the foliage were methylated by the procedure of Fieser and Fieser (1968) to give *O*-ethyl *S*-methyl ethylphosphonothioate (ETP-$CH_3$), and *O*-ethyl *O*-methyl ethylphosphonate (EOP-$CH_3$). The sample of ETP-$^{14}C$ from foliage was insufficient for rglc analysis. A Barber-Coleman (Rockford, Ill.) rglc Selecta-System, Series 5000, was used under the following conditions:

| | $MPSO_2$ | EOP-$CH_3$ |
|---|---|---|
| Oven temp. (Isothermal) | 160° C | 158° C |
| Injector temp. | 220° C | 228° C |
| Flame Ionization Detector temp. | 210° C | 242° C |
| Combustion temp. | 700° C | 725° C |
| Carrier gas (He) flow | 60 ml/min | 45 ml/min |
| Column | 10% DC-200 on Gas Chrom Q 80/100 mesh 4 ft × 1/8 in. stainless steel (Applied Sciences, Los Angeles, Calif.) | 3% OV-17 on Gas Chrom Q 100/120 mesh 6 ft × 1/8 in. stainless steel (Varian Aerograph, Walnut Creek, Calif.) |

**Hydrolysis of Plant Metabolites.** Acid hydrolysis of selected unknown metabolites, isolated by preparative tlc from the benzene and water-soluble fractions, involved refluxing in 3*N* HCl for 24 hr. Little or no radioactivity was lost during hydrolysis, as determined by comparative radioassay of pre- and posthydrolyzed samples. Hydrolyzates were concentrated to near dryness, under vacuum, reconstituted with water, and evaporated to near dryness again to remove any residual HCl. The hydrolyzed samples were chromatographically analyzed, along with their nonhydrolyzed counterparts and appropriate standards.

For enzymatic hydrolysis of water-soluble metabolites, aliquots from each extract at different time intervals were combined to obtain representative samples of metabolites from each of foliage, tuber pulp, and tuber peel. These combined samples were evaporated to near dryness, then reconstituted in 1.0 ml deionized water and 4.5 ml 0.1*M* citric acid: 0.2*M* disodium phosphate (at pH 4.4). The buffered sample was divided into four equal portions and the following enzyme preparations were added:

Sample I: 3 mg $\beta$-glucosidase (1500 units) (Calbiochem, Los Angeles, Calif.)
Sample II: 10 $\mu$l glusulase (1600 units aryl sulfatase + 1500 units $\beta$-glucuronidase) (Calbiochem)
Sample III: 4.2 mg $\beta$-glucuronidase (1500 units) (Calbiochem)
Sample IV: Buffer only—control

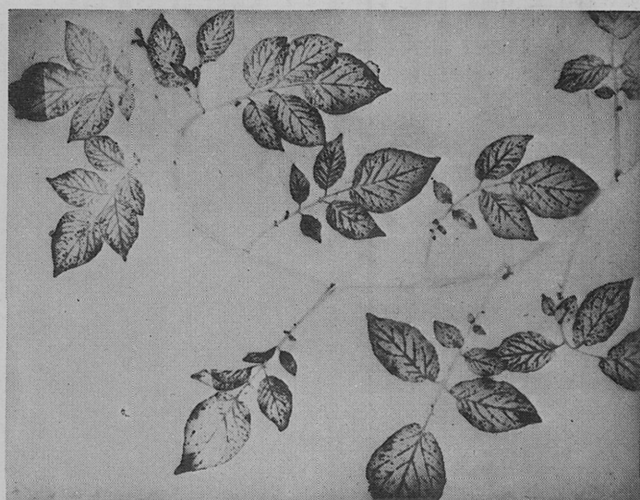
The samples were incubated with shaking for 20 hr at 37° C, after which time an additional amount (in the same proportions) of enzyme was added to half of each sample, which was then incubated again under the same conditions for an additional 20 hr.

The 20 hr and 40 hr incubation mixtures were analyzed by tlc in *n*-propanol:*n*-butanol:0.17*N* $NH_4OH$ (2:1:1) mixture (P:B:A).

**Toxicity Determinations of Plant Metabolites.** Male white rats of the Sprague-Dawley strain weighing 200–250 g were fasted overnight, and then treated with the test compound dissolved or suspended in carrier vehicles which are described below. Female houseflies of the S-Stauffer susceptible strain were treated, 2 days after emergence, with the test compounds in acetone (0.5–2.0 $\mu$l) using an Isco, model M, Microapplicator (Instrumentation Specialties Co., Lincoln, Neb.). $LD_{50}$ values were determined from mortality data at 14 days for the rats and 24 hr for houseflies.

## RESULTS

**Distribution of Radiocarbon in Potato Foliage and Tubers.** Dyfonate and/or its breakdown products are absorbed from the soil and transported within the growing potato plant.



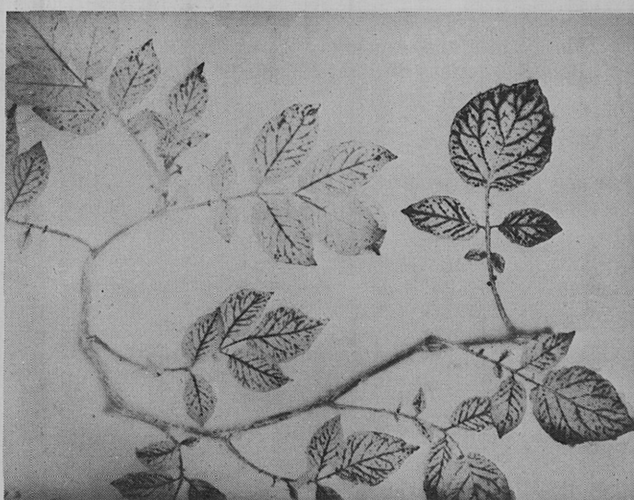


**Figure 1. Autoradiograms showing distribution of radiocarbon in the foliage of potato plants grown for 68 days in soil treated with Dyfonate-R-$^{14}C$ (left) or -E-$^{14}C$ (right)**

The autoradiographs in Figures 1 and 2 show the uptake and distribution of radiocarbon in potato foliage and tuber slices, respectively. Quantitative data on the radioactive residues are given in Table III as ppm distributed in the benzene-soluble, water-soluble, and bound fractions, and changes in these residues occurring as a function of time. The radiocarbon from both labeled preparations appears uniformly distributed in the foliage. In the tuber, the concentration of radiocarbon from both Dyfonate-$^{14}C$ labels appears to be higher in the tuber peel than in the tuber pulp, and in the tuber pulp residues from Dyfonate-E-$^{14}C$ are generally higher than those from Dyfonate-R-$^{14}C$ (Table III). The concentrating effect of the tuber peel for Dyfonate-derived products is more evident in the autoradiograms (Figure 2) than in the quantitative data (Table III), possibly because, in the quantitative studies, the peel contained significant amounts of adhering pulp which had a diluting effect on the peel residues. Lichtenstein (1965) reports that in several carrot varieties, residues of other soil applied insecticides were concentrated in the peel.

The preponderance of radiocarbon as water-soluble metabolites in all plant parts and at all time intervals indicates that extensive degradation of Dyfonate occurs in the plant since Dyfonate and its oxon partition into benzene from aqueous solutions (Batchelder, 1966). This process appears to be relatively rapid, possibly as rapid as the uptake of labeled products, because there is little change in the proportion of benzene-soluble radiocarbon after 27 days for the foliage or 67 days for the tuber.

Significantly more bound radiocarbon is found in potato plants treated with Dyfonate-E-$^{14}C$ than with Dyfonate-R-$^{14}C$. A relationship appears to exist between the water-soluble and bound components of the residues of Dyfonate-E-$^{14}C$. With the progression of time, the water-soluble radiocarbon in tuber pulp and tuber peel (Table III) generally decreases in magnitude, while the bound radiocarbon increases.

**Extraction and Chromatography of Dyfonate-$^{14}C$ Metabolites.** Table II presents the identified metabolites and their $R_f$ values in several solvent systems. Of the products listed, only EOP and ETP appear in the water-soluble fraction on extraction with benzene.

**Characterization of Benzene-Soluble Metabolites.** The R-$^{14}C$ benzene-soluble extract contains Dyfonate, and the oxon, MPSO, and several minor unknown metabolites (Table IV), as determined by tlc, and $MPSO_2$ as determined by both rglc (retention time 2.5 min, Figure 3) and tlc. As MPSO decreases in the foliage, more $MPSO_2$ is found. In addition to Dyfonate and the oxon, the C:EA system resolves to minor

**Table III. Distribution of Radiocarbon Derived from Dyfonate-R-$^{14}C$ and -E-$^{14}C$ in Foliage, Tuber Peel, and Tuber Pulp at Indicated Intervals, Days**

| Dyfonate | Fraction | Foliage 0–27 ppm | Foliage 0–60 ppm | Foliage 0–83 ppm | Tuber Peel 0–67 ppm | Tuber Peel 0–83 ppm | Tuber Pulp 0–67 ppm | Tuber Pulp 0–83 ppm |
|---|---|---|---|---|---|---|---|---|
| R-$^{14}C$ | Benzene soluble | 0.30 | 1.06 | 0.84 | 0.12 | 0.26 | 0.03 | 0.05 |
| | Water soluble | 2.31 | 4.19 | 4.26 | 1.13 | 1.90 | 0.85 | 0.98 |
| | Bound | 0.21 | 0.18 | 0.35 | 0.38 | 0.57 | 0.08 | 0.10 |
| | Total | 2.82 | 5.43 | 5.45 | 1.63 | 2.73 | 0.96 | 1.13 |
| E-$^{14}C$ | Benzene soluble | 0.44 | 0.60 | [a] | 0.26 | 0.41 | 0.11 | 0.18 |
| | Water soluble | 2.51 | 2.03 | [a] | 0.70 | 0.74 | 1.11 | 0.84 |
| | Bound | 1.10 | 0.97 | [a] | 0.79 | 1.38 | 0.54 | 0.78 |
| | Total | 4.05 | 3.60 | | 1.75 | 2.53 | 1.76 | 1.80 |

[a] Leaf samples were depleted at this interval.

Table IV. Nature and Amounts (%) of Dyfonate-R-$^{14}C$ and -E-$^{14}C$ Metabolites in the Benzene-Soluble, Water-Soluble, and Bound Fractions of Potato Plants at Indicated Intervals (Days) as Determined by the B:A:W, B:E, C:EA, C:H, and P:B:A Tlc Systems

| | | Foliage | | | | | Tuber Peel | | Tuber Pulp | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0–27 | | 0–60 | | 0–83[a] | 0–83 | | 0–83 | |
| Fraction | Metabolites | R-$^{14}C$ | E-$^{14}C$ | R-$^{14}C$ | E-$^{14}C$ | R-$^{14}C$ | R-$^{14}C$ | E-$^{14}C$ | R-$^{14}C$ | E-$^{14}C$ |
| | Dyfonate | 0.48 | 0.51 | 0.06 | 0.40 | 0.12 | 6.01 | 5.57[b] | 0.10 | 0.11[b] |
| | Oxon | 2.31 | 3.36 | 0.83 | 1.27 | 1.25 | 1.01 | ... | 0.12 | ... |
| | EOP-$CH_3$ | ... | ... | ... | 1.29 | ... | ... | ... | ... | ... |
| Benzene | ETP-$CH_3$ | ... | ... | ... | 1.74 | ... | ... | ... | ... | ... |
| | MPSO | 1.11 | ... | 0.51 | ... | 0.18 | 0.20 | ... | 0.17 | ... |
| | $MPSO_2$ | 5.74 | ... | 16.19 | ... | 12.33 | 1.78 | ... | 3.76 | ... |
| | Unknowns | 1.26 | 7.53 | 1.91 | 12.00 | 1.52 | 0.50 | ... | 0.29 | ... |
| | Total | 10.9 | 11.4 | 19.5 | 16.7 | 15.4 | 9.5 | 16.2 | 4.4 | 10.0 |
| | EOP | ... | 51.61 | ... | 30.68 | ... | ... | 17.08 | ... | 31.90 |
| Water | ETP | ... | 2.86 | ... | ... | ... | ... | ... | ... | ... |
| | $MPSO_2$ | ... | ... | ... | ... | 18.06 | ... | ... | ... | ... |
| | Unknowns | 81.8 | 7.63 | 77.2 | 25.72 | 60.14 | 69.6 | 12.22 | 86.8 | 14.80 |
| | Total | 81.8 | 62.1 | 77.2 | 56.4 | 78.2 | 69.6 | 29.3 | 86.8 | 46.7 |
| Bound | Total | 7.3 | 26.5 | 3.3 | 26.9 | 6.4 | 20.9 | 54.5 | 8.8 | 43.3 |

[a] E-$^{14}C$ leaf samples were depleted at this interval. [b] Tuber peel and tuber pulp E-$^{14}C$ residues streaked badly, making it impossible to quantify metabolites other than Dyfonate E-$^{14}C$.

metabolites derived from Dyfonate-E-$^{14}C$ which behave chromatographically as EOP-$CH_3$ and ETP-$CH_3$ (Table IV). Due to their small amounts in foliage, no other evidence is available in support of these observations. Acid hydrolysis of the E-$^{14}C$ labeled "unknowns" (Table IV) did not yield ETP or EOP, and so they remain unidentified.

Dyfonate and the oxon originating from both labeled preparations are detected in the foliage in progressively decreasing amounts with time. At 83 days, the young potatoes contained 0.002 ppm Dyfonate and oxon in the pulp, and 0.15 ppm Dyfonate and 0.03 ppm oxon in the peel.

**Characterization of the Water-Soluble Metabolites.** The major ethoxy-labeled plant metabolite, representing 54–83% of the radiocarbon in the water-soluble fraction of foliage, tuber pulp, and tuber peel is identified by tlc as EOP (Table IV). The methyl derivative of this metabolite cochromatographs by rglc (retention time 0.5 min) with methylated authentic standard EOP. On acid hydrolysis (pH 1.0), 49% of the radiocarbon of the ethoxy-labeled composited "unknowns" converts to EOP. A closely-related metabolite ETP (2.9%) was identified in 0–27-day foliage by tlc. The unknown, ring-labeled, water-soluble plant metabolites of Dyfonate (Table IV) resolved into two radioactive bands (A and B; $R_f$ 0.37 and 0.47, respectively) when chromatographed in the P:B:A tlc system. Enzymatic cleavage with β-glucosidase or glusulase but less so with β-glucuronidase resulted in partial conversion of metabolites A and B to cleavage product C ($R_f$ 0.58, P:B:A tlc system) (Table V). Deconjugation increased with an increase in incubation time from 20 to 40 hr. Metabolite A is cleaved most rapidly by glusulase, while metabolite B cleaves equally well with β-glucosidase or glusulase. These results suggest that metabolites A and B are present in the plant largely as sulfate and glycoside conjugates.

Metabolites A and B are not converted to product C in the absence of enzyme at pH 4.4; however, when subjected to acid hydrolysis (pH 1.0, reflux), metabolite A, and to a lesser

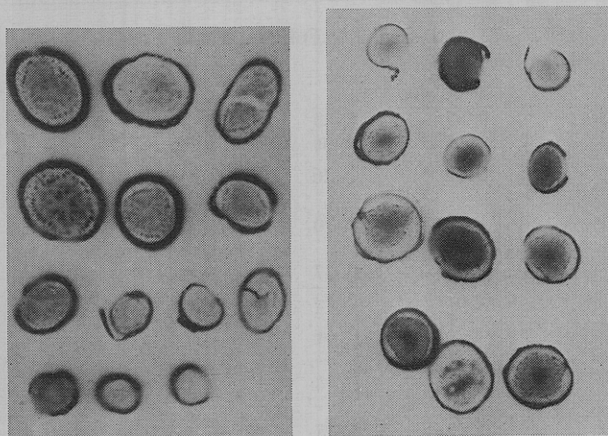


Figure 2. Autoradiogram showing distribution of radiocarbon within the tubers of potato plants grown for 80 days in soil treated with Dyfonate-R-$^{14}C$ (left) or -E-$^{14}C$ (right)

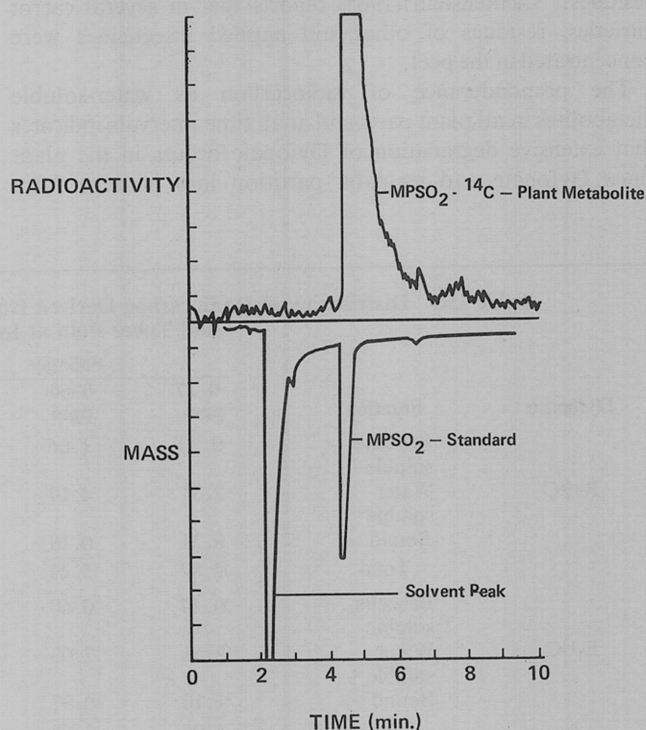



Figure 3. Radio-gas-liquid chromatogram of $MPSO_2$ standard with $MPSO_2$-$^{14}C$ plant metabolite

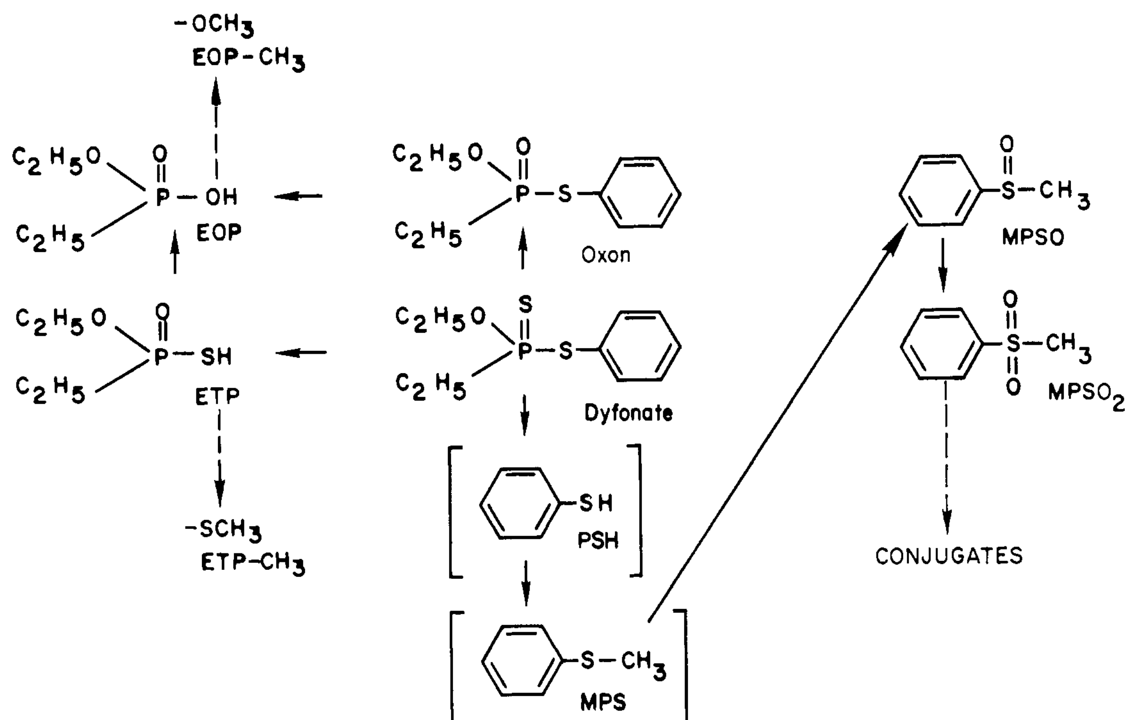



Figure 4. Proposed metabolic pathway of Dyfonate in the potato plant

extent B, convert to cleavage product C and $MPSO_2$ (Table V). The identity of product C remains unknown, as it fails to cochromatograph with 2-, 3-, or 4-OH-$MPSO_2$. However, it converts to a less polar product on reaction with $CH_3N_2$, and so product C probably is a phenolic compound and possibly is a hydroxylated $MPSO_2$ derivative.

**Mammalian and Insect Toxicity of Dyfonate Plant Metabolites.** The comparative mammalian and insect toxicity of Dyfonate and its metabolites is given in Table VI. Of the metabolites found in the plant, only Dyfonate and its oxon were toxic to both animal species. PSH, which is toxic to rats, was not detected in the potato plant.

## DISCUSSION

Although Dyfonate is a nonsystemic insecticide, autoradiography shows that radiocarbon is actively transported throughout all plant parts. The same metabolites were present in the plant at early and late intervals. It is not possible to state whether some metabolites represent biotransformation products formed in the plant after uptake of Dyfonate from the soil or whether, to some extent, they were formed in the soil and subsequently adsorbed into the plant. Preliminary work, now under way, indicates that Dyfonate in the soil undergoes similar biotransformation steps to those in the plant.

The proposed metabolic pathway of Dyfonate in the plant, based on metabolites which were identified by tlc and rglc, is presented in Figure 4. Dyfonate undergoes extensive biotransformation involving cleavage to yield metabolites of the phosphonic acid and thiophenyl moieties. The major terminal metabolite of the phosphonic acid moiety in foliage, tuber pulp, and tuber peel is EOP, with minor involvement of ETP and the methyl esters of both acids. It is not known whether the small amounts of the two methyl esters represent *in vivo* formed metabolites or whether they arise from nonmetabolic products. *O*- or *S*-Methylation of exogenous

**Table V. Cleavage of Dyfonate-R-$^{14}$C Water-Soluble Metabolites Recovered from Combined Potato Foliage, Tuber Pulp, and Tuber Peel by Enzyme and Hydrochloric Acid**

| Cleavage Conditions | Incubation Time hr | Cleavage, %[a] Metabolite Designation A | B | C | Remainder of Chromatogram |
|---|---|---|---|---|---|
| Buffer only (Control) | 20 | 21.4 | 76.8 | 0 | 1.8 |
| β-Glucosidase and | 20 | 17.8 | 58.5 | 20.0 | 3.6 |
| buffer | 40 | 18.6 | 24.8 | 56.7 | 0 |
| Glusulase and | 20 | 14.3 | 45.0 | 36.7 | 4.0 |
| buffer | 40 | 8.4 | 25.2 | 66.5 | 0 |
| β-Glucuronidase and | 20 | 20.4 | 74.0 | 5.6 | 0 |
| buffer | 40 | 21.3 | 73.1 | 3.7 | 1.9 |
| Hydrochloric acid (pH 1.0) | 24 | 0 | 36.4 | 34.8 | 28.8[b] |

[a] Percentages are based on radioassay of radioactive gel regions from pre- and post-cleavage of metabolites A and B in mixture (P:B:A tlc system). [b] 23.1% represents a metabolite that cochromatographs two-dimensionally with authentic $MPSO_2$.

**Table VI. Comparative Acute Toxicity Values for Dyfonate and Metabolites in the Rat and Housefly**

| Compound[a] | Vehicle Used for Administration to Rats | LD$_{50}$, mg/kg Rat, Oral | Housefly, Topical |
|---|---|---|---|
| Dyfonate | corn oil | 14.7 | 9 |
| Oxon | corn oil | 2.75 | 15 |
| ETP | distilled water | 600.0[b] | 500 |
| EOP | undiluted | 2112.0[b] | >1000[c] |
| PSH | polyethylene glycol | 65.0 | 1500 |
| MPS | corn oil | 891.0 | >1000[c] |
| MPSO | ... | ... | >1500[c] |
| $MPSO_2$ | corn oil | 1470.0 | >1000[c] |

[a] Chemical names are given in Table I. [b] LD$_{50}$ value for female rat. [c] No mortality at indicated dosages.

phosphonic or thiophosphonic acids have not previously been found in plants. The oxon occurs in very small amounts only; so cleavage of Dyfonate to ETP and subsequent transformation to EOP is probably the predominant pathway for EOP formation.

The biotransformation of the thiophenyl moiety (PSH) involves *S*-methylation, sulfoxidation, and possibly glycoside and sulfate conjugation.

*S*-Methylation and subsequent sulfoxidation of PSH in the plant parallels this detoxication pathway found in the animal (McBain and Menn, 1969). The methylation of PSH is apparently nonreversible based on the present study and that of Bull (1969), who reported the sulfoxidation of intact dimethyl *p*-(methylthio-$^{14}C$)-phenyl phosphate (GC-6506) in the growing cotton plant and subsequent cleavage to derivatives of MPSO and $MPSO_2$.

The mechanism of PSH methylation of plants was not studied, but it is known that homocysteine is methylated by folic acid coenzyme in the presence of ATP to form methionine (Fairley, 1966), and that cysteine to methylcysteine conversion in plants involves *S*-adenosylmethionine as the immediate methyl donor (Thompson, 1967). Various higher plants are also known to sulfoxidize methylcysteine to methylcysteine sulfoxide (Thompson, 1967). Similar biochemical transformations may be involved in the metabolism of PSH in the plant.

Although the polar metabolites, derived from methylated and sulfoxidized PSH, are not identified, the enzyme cleavage and acid hydrolysis studies indicate that MPSO, or more likely $MPSO_2$, are further metabolized via glycoside and sulfate conjugation. Based on the currently available data, it is not known whether these conjugates arise from $MPSO_2$ or MPSO; $MPSO_2$ may be the conjugate precursor based on its abundance in the plant, or the findings of only small amounts of MPSO could be due to rapid conversion to metabolites that are conjugated.

Each of the biotransformation steps involved in the metabolism of Dyfonate in the plant are associated with detoxication processes, with the exception of oxon formation, as shown by the acute toxicity studies with mammals and insects (Table VI).

This study leads to the conclusion that Dyfonate represents, by virtue of its extensive metabolism to relatively nontoxic metabolites, a biodegradable insecticide which is not likely to leave persistent, toxic residues in food crops.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LITERATURE CITED


Baranowski, R. M., *Proc. Fla. State Hort. Soc.* **77,** 219 (1964).
Batchelder, G. H., Stauffer Chemical Company, Western Research Center, Richmond, Calif., personal communication (1966).
Block, R. J., Durrum, E. L., Zweig, G., "A Manual of Paper Chromatography and Paper Electrophoresis," 710 pp., Academic Press, New York (1958).
Bull, D. L., *Entomol. Soc. Amer. Nat. Meeting,* Paper No. 82, Chicago, Ill., Dec. 1–4, 1969.
Burrage, R. H., Menzies, J. A., Zirk, E., *J. Econ. Entomol.* **60,** 1489 (1967).
Fairley, J. L., Kilgour, G. L., "Essentials of Biological Chemistry," p. 231–232, Reinhold Publications, New York (1966).
Fieser, L. F., Fieser, M., "Reagents for Organic Synthesis," p. 191, Wiley, New York (1968).
Ford, I. M., Menn, J. J., Meyding, G. D., J. AGR. FOOD CHEM. **14,** 83 (1966).
Kalbfeld, J., Gutman, A. D., Herman, D. A., *J. Label. Compounds* **IV,** 367 (1968).
Lichtenstein, E. P., "Problems Associated with Insecticidal Residues in Soils," In *Research in Pesticides,* Chichester, C. O., Ed., p. 202, Academic Press, New York (1965).
McBain, J. B., Menn, J. J., *Biochem. Pharmacol.* **18,** 2282 (1969).
Menn, J. J., Erwin, W. R., Gordon, H. T., J. AGR. FOOD CHEM. **5,** 701 (1957).
Onsager, J. A., Landis, J. B., Rusk, H. W., *J. Econ. Entomol.* **59,** 441 (1966).
Thompson, J. F., *Ann. Rev. Plant Physiol.* **18,** 59, Machlis, L., Ed., Ann. Revs. Inc., Palo Alto, Calif. (1967).
van den Brink, B. J., Antognini, J., Menn, J. J., Proc. Fourth British Insecticide and Fungicide Conf., Brighton, England, **1,** 139 (1967).



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