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Comparison of Solvent Systems for the Extraction of Diclofop Acid, Picloram, Simazine, and Triallate from Weathered Field Soils

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The extraction of diclofop acid [2-[4-(2,4-dichlorophenoxy)phenoxy]propionic acid], picloram (4-amino-3,5,6-trichloropicolinic acid), simazine (2-chloro-4,6-diethylamino-1,3,5-triazine), and triallate (*S*-2,3,3-trichloroallyl diisopropylthiocarbamate) from three field soils that had received treatments of the individual herbicides 12 months previously was compared by using different solvent systems. The highest recoveries for dicolofop acid and triallate were achieved with 30% aqueous acetonitrile containing 2.5% of glacial acetic acid. The same extraction solvent was also the most effective for recovering weathered residues of picloram and simazine from a clay soil. For the extraction of picloram and simazine from treated clay loam and sandy loam soils, acetonitrile containing 18% of water and 12% of ammonium hydroxide proved to be the most effective solvent system. In all cases for maximum recoveries, the soils were initially extracted for 0.5 h on a wrist-action shaker and then allowed to stand for 18 h before being shaken for a further 0.5-h period.

When a pesticide residue remains in contact with field soils for prolonged periods of a phenomenon kown as aging, or weathering, can occur which renders the chemical residue more resistant to solvent extraction (Hamaker et al., 1966; Chiba and Morley, 1968; Chiba, 1969; Saha et al., 1969; Mattson et al., 1970). This resistance to solvent extraction has been considered to result from an increased adsorption of the pesticide to soil colloids and a diffusion into the interior of humic colloids (Hamaker et al., 1966; Chiba, 1969; Adams, 1973; Khan, 1973).

Inadequate extraction procedures present problems to analysts monitoring persistent pesticide residues in the soil. In addition, unextracted residues could be considered to be bound to the soil [cf. Kearney (1976)] when, in fact, they are merely being inefficiently extracted. A practical solution to this problem has been to take samples of field soils that have received previous applications of pesticides and compare several extraction systems, selecting for general analytical use that procedure which recovers the greatest amounts of the particular residue (Mattson et al., 1970; Johnsen and Starr, 1970, 1972; Khan et al., 1975; Smith, 1978, 1981; Cotterill, 1980). The results from such studies provide more reliable information on the solvent extractability of residues than do those which simply rely on the recovery of pesticide residues from recently fortified soils. In the latter case the test chemicals are allowed to equilibrate with the soil for a few hours, or a few days, before extraction, and although a particular procedure may indicate that over 90% of the applied pesticides is being recovered, there are no means of knowing whether the recovery efficiency of the same pesticide from field soils treated several months previously is the same (Hamaker et al., 1966; Saha et al., 1969).

In the studies to be reported, field plots were separately treated with the commonly encountered herbicides diclofop-methyl (Figure 1, 1, R = CH_3), picloram (Figure 1, 2), simazine (Figure 1, 3), and triallate (Figure 1, 4), all of which can persist for over a year in Canadian field soils (Smith, 1982). Following natural weathering in the field for over 12 months, the soils were sampled and various solvent systems compared to determine which extractant resulted in the highest amounts of herbicide recovered. Since diclofop-methyl (Figure 1, 1, R = CH_3) undergoes rapid hydrolysis in soils (Smith, 1977) to diclofop acid (Figure 1, 1, R = H), the extraction of the acid, rather than the ester, was investigated. Triallate was included in this study since its extraction from weathered field soils was previously reported (Smith, 1978) from a single soil.

MATERIALS AND METHODS

Soils. The composition and physical characteristics of the clay (C), clay loam (CL), and sandy loam (SL) have already been described (Smith, 1981).

Field Treatments. Commercial formulations of diclofop-methyl, picloram, and simazine were applied as unicorporated treatments of 1.25 kg/ha to the surface of fallow plots at three locations in Saskatchewan. In the case of diclofop-methyl, treatments were made only on the clay. All applications were made during the second week of May 1981. At the same time, and at the same locations,

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Table I. Comparison of Extraction Procedures for Recovery of Diclofop Acid from Weathered Clay Field Soil

solvent	conditions	diclofop acid recovered, μg/g[a]
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 0.5 h; stand 18 h; shake 0.5 h	0.37 a
acetonitrile + water + acetic acid (70 + 30 + 2.5)	wet soil with water 18 h; shake with acidic acetonitrile 1 h	0.32 b
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 1 h	0.27 bc
acetonitrile + water + ammonium hydroxide (70 + 18 + 12)	shake 0.5 h; stand 18 h; shake 0.5 h	0.30 b
water + methanol + ethyl acetate + acetic acid (40 + 40 + 20 + 1)	shake 1 h	0.23 cd
acetonitrile + water (70 + 30)	shake 1 h	0.22 d
methanol + water (40 + 10)	shake 0.5 h; stand 18 h; shake 0.5 h	0.13 e
methanol + water (40 + 10)	shake 1 h	0.09 e

[a] Average from four replicate extractions; means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table II. Comparison of Extraction Procedures for Recovery of Picloram Residues from Weathered Field Soils

solvent	conditions	picloram recovered, μg/g[a] C	CL	SL
acetonitrile + water + ammonium hydroxide (70 + 18 + 12)	shake 0.5 h; stand 18 h; shake 0.5 h	0.056 b	0.934 a	0.143 a
acetonitrile + water + ammonium hydroxide (70 + 18 + 12)	shake 1 h	0.036 c	0.707 b	0.134 a
methanol + water + ammonium hydroxide (70 + 18 + 12)	shake 0.5 h; stand 18 h; shake 0.5 h	0.031 cd	0.469 c	0.088 bc
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 0.5 h; stand 18 h; shake 0.5 h	0.067 a	0.291 d	0.057 de
methanol + water (40 + 10)	shake 1 h	0.024 de	0.178 de	0.047 e
methanol + water + acetic acid (70 + 30 + 2.5)	shake 1 h	0.024 de	0.218 d	0.095 b
water + calcium hydroxide (50 mL + 1 g)	shake 1 h	0.022 def	0.178 de	0.068 cd
acetone + 0.5% phosphoric acid (50 + 10)	shake 1 h	0.020 ef	0.227 d	0.087 bc
0.1 N KOH containing 10% KCl	shake 1 h	0.018 ef	0.065 ef	0.097 b
methanol containing 0.2% of 12 N HCl	shake 1 h	0.011 f	0.050 f	0.048 de
acetone containing 0.2% of 12 N HCl	shake 1 h	0.000 g	0.011 f	0.004 f

[a] Average from triplicate extractions; means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

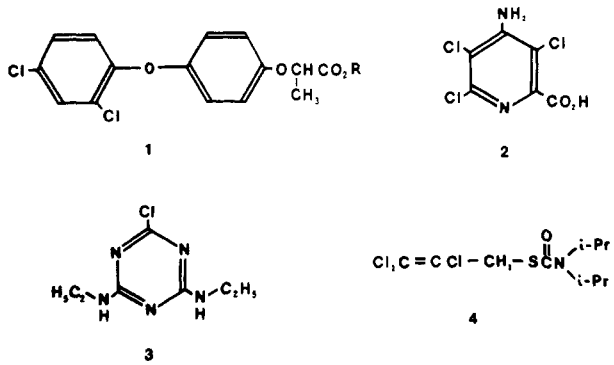

Figure 1. Structures of herbicides.

treatments of 1.25 kg/ha of an emulsifiable concentrate formulation of triallate were incorporated to a depth of 5 cm into fallow plots.

Representative soil samples were removed from the top 5 cm of all treated plots during the second week of May 1982. The soils were air-dried at laboratory temperature, ground, and thoroughly mixed in a laboratory mixer for 20 min to ensure even distribution of the chemicals throughout the soils.

Extraction Procedures. *Diclofop Acid.* The solvent systems and procedures compared are displayed in Table I. In all cases, four replicate soil samples (20 g) were weighed into 150-mL glass-stoppered flasks and shaken with the respective solvent system (50 mL) on a wrist-action shaker as required. In one case the aqueous component of the extractant was added to the soil 18 h prior to addition of the acidic acetonitrile and the commencement of shaking. After centrifugation at 3500 rpm for 5 min, supernatant (25 mL, equivalent to 10 g of soil) was added to 5% (w/v) aqueous sodium carbonate (100 mL) and shaken with *n*-hexane (25 mL). The organic layer was discarded.

The aqueous phase containing the diclofop acid was acidified with concentrated hydrochloric acid (15 mL) and ether extracted (2 × 50 mL), and the evaporated extracts were methylated with ethereal diazomethane. After evaporation of excess reagent and ether, the residue was dissolved in *n*-hexane (25 mL), and aliquots were examined by using gas chromatography. Full details for the workup and derivatization have been published (Smith, 1976).

Picloram. The solvent systems compared are displayed in Table II. Three replicate soil samples (20 g) were placed in 150-mL glass-stoppered flasks and shaken with extraction solvent (50 mL) on a wrist-action shaker for the necessary period. After centrifugation at 3500 rpm for 5 min, supernatants (25 mL) from all the extractants containing ammonium hydroxide were evaporated to approximately 5 mL with a rotary evaporator at 40 °C. To the evaporation flasks was added 5% (w/v) aqueous sodium carbonate (100 mL), and the well-shaken mixtures were transferred to a separatory funnel and extracted with *n*-hexane (25 mL). The hexane layer was discarded, and the aqueous phase, containing the picloram, was extracted with ether and worked up exactly as described above for diclofop acid, except that boron trifluoride–methanol (14% by weight) was the methylating reagent. Thus, the evaporated extracts containing picloram were heated at 65 °C

Table III. Comparison of Extraction Procedures for Recovery of Simazine from Weathered Field Soils

solvent	conditions	simazine recovered, μg/g[a] C	CL	SL
acetonitrile + water + ammonium hydroxide (70 + 18 + 12)	shake 0.5 h; stand 18 h; shake 0.5 h	0.228 b	0.160 a	0.378 a
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 0.5 h; stand 18 h; shake 0.5 h	0.305 a	0.092 c	0.378 a
acetonitrile + water (90 + 10)	shake 0.5 h; stand 18 h; shake 0.5 h	0.185 bc	0.121 b	0.335 ab
methanol + (40 + 10)	shake 0.5 h; stand 18 h; shake 0.5 h	0.155 c	0.077 c	0.307 b

[a] Average from triplicate extractions; means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

Table IV. Comparison of Extraction Procedures for Recovery of Triallate Residues from Weathered Field Soils

solvent	conditions	triallate recovered, μg/g[a] C	CL	SL
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 0.5 h; stand 18 h; shake 0.5 h	1.42 a	1.35 a	0.55 ab
methanol + water (40 + 10)	wet soil with water 18 h; shake with methanol 1 h	1.36 b	1.23 b	0.56 a
acetonitrile + water + acetic acid (70 + 30 + 2.5)	wet soil with water 18 h; shake with acidic acetonitrile 1 h	1.32 b	1.36 a	0.52 bc
acetonitrile + water + acetic acid (70 + 30 + 2.5)	shake 1 h	1.32 b	1.19 b	0.52 abc
acetonitrile + water + ammonium hydroxide (70 + 18 + 12)	shake 0.5 h; stand 18 h; shake 0.5 h	1.14 d	1.21 b	0.49 c
methanol + water (40 + 10)	shake 1 h	1.23 c	1.05 c	0.45 d

[a] Average from triplicate extractions; means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test.

for 1 h with the reagent (5 mL). After being cooled, excess reagent was decomposed by using a saturated aqueous solution of sodium chloride (10 mL) and the methyl ester of picloram was extracted into benzene (25 mL).

Following centrifugation, supernatants (25 mL) derived from all other soil extractants were treated as described for diclofop acid above, with the esterification being achieved by using boron trifluoride–methanol reagent.

All methylated solutions containing the methyl ester of picloram were quantified gas chromatographically.

Simazine. The solvent systems used are shown in Table III. Three replicate soil samples (20 g) were weighed into 150-ml glass-stoppered flasks and shaken with extractant (50 mL). Following centrifugation at 3500 rpm for 5 min, supernatant (25 mL) was added to a separatory funnel containing 10% (w/v) sodium chloride (100 mL) and ammonium hydroxide (2 mL) and shaken with methylene chloride (50 mL and then two 25-mL portions). The combined organic extracts were evaporated to dryness by using a rotary evaporator at 40 °C, and the residue was dissolved in chloroform (10 mL). The chloroform solution was examined for simazine by using a gas chromatograph equipped with a nitrogen-specific detector.

Triallate. The solvent systems compared are shown in Table IV. Triplicate soil samples were weighed (20 g) into flasks and shaken with solvent systems (50 mL). In two instances, the water component was added to the soils 18 h before addition of the remaining components and shaking was started. After shaking and centrifugation at 3500 rpm for 5 min, supernatant (25 mL) was extracted with 5% (w/v) aqueous sodium carbonate (100 mL) and *n*-hexane (50 mL). The aqueous phase was discarded and the organic layer run into a stoppered flask and dried over anhydrous sodium sulfate (5 g). Aliquots were analyzed gas chromatographically for triallate.

Gas Chromatographic Analysis. Diclofop acid and picloram (as their methyl esters) together with triallate were analyzed by using a Hewlett-Packard Model 5700A gas chromatograph equipped with a radioactive nickel detector operated at 350 °C. All columns were of glass (1.5 m × 4 mm i.d.), with argon containing 5% of methane at a rate of 40 mL/min as the carrier gas. All samples were injected directly onto the column packings. For diclofop and picloram methyl esters the column packing was 2% Apiezon L on 80–100-mesh Gas-Chrom Q. At a column temperature of 205 °C picloram methyl ester had a retention time of 6.85 min, while at 240 °C the diclofop-methyl had a retention time of 6.25 min. For triallate the column was 5% Dexsil 300 on 80–100-mesh Chromosorb W HP at 200 °C, on which the herbicide had a retention time of 3.16 min.

Simazine samples were analyzed by using a Hewlett-Packard 5730A gas chromatograph equipped with a nitrogen-phosphorus flame ionization detector operated in the nitrogen mode. The glass column (1.5 m × 4 mm i.d.) was packed with 5% Dexsil 300 on 80–100-mesh Chromosorb W, HP. The column carrier gas was helium at a flow rate of 40 mL/min. Flow rates of hydrogen and air through the detector was maintained at 3 and 50 mL/min, respectively. The detector voltage was operated at 18 V. All samples were injected directly onto the column packing. With a column temperature of 210 °C, the retention time for simazine was 4.25 min.

For all chromatographic analyses, standards were prepared in the same solvents as those used for the samples, and the concentrations of the various herbicides recovered from the soils were calculated by comparing the sample peak heights with those of the standards.

RESULTS AND DISCUSSION

The mean amounts of the various compounds recovered from the aged field soils by using the various extraction systems are summarized in Tables I–IV. The data are expressed as micrograms of herbicide recovered per gram of air-dried soil. Since at least three replicate soil samples were analyzed for each solvent system, it was possible to statistically determine (by using Duncan's multiple range test) significant differences between the various amounts

recovered using the different extraction procedures.

Acidic acetonitrile has been reported (Smith, 1976, 1979) to be a suitable solvent system for the extraction of diclofop acid from field soils. Other extractants compared (Table I) were based on aqueous aceonitrile and aqueous methanol, solvent systems frequently employed by analysts for the extraction of herbicide residues from treated soils.

Amounts of diclofop acid recovered from aged clay (Table I) indicated that solvent systems containing acetic acid were, in general, more efficient than were alkaline or neutral mixtures. It can also be noted (Table I) that an overnight extraction involving a 0.5-h shake followed by an 18-h equilibration period prior to a second 0.5-h shaking resulted in greater recoveries than did a simple 1-h shake. Moistening of the soil with water for 18 h before extraction did not result in a significantly greater recovery of diclofop acid than was achieved by an overnight extraction. The greatest recoveries of the diclofop acid were obtained by using aqueous acidic acetonitrile in conjunction with the overnight extraction procedure.

For the extraction of picloram from the weathered field soils, solvent mixtures included both acidic and alkaline solutions derived from acetonitrile and methanol (Table I), since such solvents have proved satisfactory for the extraction of various acidic herbicides from treated soils (Smith, 1976, 1979, 1981). Other extractants reported for the recovery of picloram residues from soils and included in this study were acetone containing hydrochloric acid (Merkle et al., 1966), acetone containing phosphoric acid (Saha and Gadallah, 1967), aqueous potassium chloride containing potassium hydroxide (Leahy and Taylor, 1967; Cheng, 1969; McKone and Cotterill, 1974; Grover and Bowes, 1981), and water containing solid calcium hydroxide (McKone and Cotterill, 1974). Since aqueous methanol has been reported (Cotterill, 1980) to be an efficient solvent for the extraction of a number of herbicides from weathered British soils, aqueous methanol was included in the study.

The extraction data (Table II) indicated that for the clay loam, which contains 12% organic matter, aqueous alkaline acetonitrile recovered more picloram than any other solvent system tested. Also, an overnight extraction appeared to be better than a 1-h shaking. For the sandy loam (4% organic matter) the aqueous alkaline acetonitrile also proved the most effective extractant, and shaking for 1 h recovered the same amounts of picloram as the overnight procedure. For the clay (4% organic matter), aqueous acidic acetonitrile was the extractant of choice. Aqueous methanol, alkaline potassium chloride, water containing calcium hydroxide, and acetone and methanol containing small amounts of mineral acids were inadequate for recovering weathered residues of picloram from field soils (Table II).

The soil extractions containing ammonium hydroxide were evaporated to dryness before further workup, since simple acidification of the unevaporated solutions in the aqueous sodium carbonate resulted in a slight precipitate of humic colloids with a resultant lowering of herbicide recovery. It was presumed that adsorption of the picloram to the precipitated humic material had occurred, since experiments by Grover (1968) has shown that an increased adsorption of picloram to soils occurs with a lowering of pH. Evaporation of the alkaline extracts prior to treatment with the sodium carbonate solution overcame this problem.

The extraction of simazine from aged field soils is compared in Table III. Aqueous acetonitrile containing ammonia with the overnight extraction procedure was used since an earlier study (Smith, 1981) had shown that this method yielded the greatest recoveries of the structurally similar atrazine from weathered Saskatchewan field soils. Aqueous acetonitrile and aqueous methanol were also compared since these are excellent solvents for the recovery of simazine from weathered Brittish soils (Cotterill, 1980). Aqueous acidic acetonitrile was included because of proven usefulness as an extractant of herbicides from prairie soils (Smith, 1976, 1978, 1981).

The results (Table III) indicate that the amounts of simazine recovered by using a particular extractant were dependent upon soil type. Thus, for the sandy loam, all extractants, except for the aqueous methanol, were equally effective in recovering the simazine residues. For the clay loam, alkaline aqueous acetonitrile was superior, while for the weathered clay, aqueous acidic acetonitrile was the most efficient extractant for simazine (Table III).

The recovery of triallate from weathered clay field soils using different systems has been compared and discussed (Smith, 1978); aqueous acidic acetonitrile was observed to be the most effective extractive solvent. The recoveries of triallate from the three aged field soils using the reported solvent systems are compared in Table IV. Aqueous acidic acetonitrile with an overnight extraction was again the most effective solvent system. Wetting of the soils for 18 h before extraction for 1 h did not significantly increase the recoveries in the case of aqueous acidic acetonitrile but did so when methanol was used as the extractant (Table IV). In the latter instance, the recovery of triallate from the sandy loam was the same as for the acidic acetonirile extractant.

Most of the solvent systems compared (Table I–IV) have been reported to give almost quantitative recoveries of the various herbicides from fortified soils. However, as with the earlier studies (Smith, 1978, 1981), the present investigations indicate that the recovery of herbicide residues from field soils treated 12 months previously is very much dependent upon the extraction procedure adopted by the analyst and that short-term fortification experiments may not give accurate information regarding herbicide extraction from weathered soils.

Additional efficiency of herbicide recovery must be attributed to the 18-h period for which the soil was in contact with the water-containing extraction solvents. During this interval more desorption of the herbicides is likely to occur than during a 1- or 2-h extraction period.

The study also indicates that the same solvent system may not be ideal for the recovery of a particular herbicide from all soil types (cf. picloram and simazine, Tables II and III). Thus, different soil types may require different extraction systems. This may partially explain why aqueous methanol was not such an effective extractant of herbicides weathered in Saskatchewan field soils as for British soils (Cotterill, 1980), though the latter study (Cotterill, 1980) the herbicides were weathered in the field for only 3 months and not for 12 months as in the present study. In Saskatchewan, the extra weathering under hot dry summer conditions and the long cold winter temperatures may have resulted in a greater recalcitrance to extraction.

Registry No. 1 (R = H), 40843-25-2; picloram, 1918-02-1; simazine, 122-34-9; triallate, 2303-17-5.

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Effects of Light and the Importance of Living Plant Tissues on the Fate of [^{14}C]Phorate in Water and *Elodea* Plants

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The importance of living plant tissues and light on the uptake and metabolism of [^{14}C]phorate-derived residues from water by *Elodea nuttallii* plants was investigated. Plants growing for 2 weeks in water with a bottom deposit of [^{14}C]phorate-treated soil accumulated in their tissues up to 30% of the originally soil-applied radiocarbon and 56% when the insecticide had been directly applied to the water. Most of the ^{14}C-labeled compounds thus taken up were bound to plant tissues, which might explain the small amounts of radiocarbon released later from these plants into insecticide-free water. The uptake of insecticides from water was a function of the living plant, since dead plant tissues contained only small amounts (2.6% of that found in living plants) of ^{14}C-labeled compounds after having been exposed for 72 h to [^{14}C]phorate-contaminated water. Moreover, most of the [^{14}C]phorate-derived compounds (38.4% of applied) were taken up by *Elodea* plants when exposed to light, while plants incubated in the dark contained only 9% of the radiocarbon originally applied to the water.

Lakes and rivers are often contaminated with pesticide chemicals, after their use for soil and plant pest control. This contamination is to some extent a result of soil runoff due to heavy rainfall, causing a transport of soil particles previously contaminated with pesticide chemicals. In the case of relatively water soluble chemicals, transport of pesticides with water through soils (leaching) is also possible, in particular with sandy soils. Once water has been contaminated, plant and animal life as well as microorganisms within the water are exposed to these chemicals.

Studies conducted previously in our laboratory dealt with the effects of lake bottom mud on the movement and metabolism of [^{14}C]phorate in a soil–lake mud–water system (Walter-Echols and Lichtenstein, 1977, 1978a). Phenomena related to the accumulation of the insecticide in *Elodea nuttallii*, a common macrophyte in North American lakes, have also been reported (Walter-Echols and Lichtenstein, 1978b) and were further investigated in this study relative to the effects of light and living plant tissues on the fate of [^{14}C]phorate in water.

MATERIALS AND METHODS

Chemicals. [*methylene*-^{14}C]Phorate (sp act. 9.7 mCi/mmol) was obtained through the courtesy of American Cyanamid Co. The insecticide was diluted with nonradioactive phorate before its addition to soils or water. Nonradioactive phorate, phorate sulfoxide, phorate sulfone, phoratoxon sulfoxide, and phoratoxon sulfone were also obtained from the American Cyanamid Co. These chemicals were determined to be at least 97% pure by thin-layer chromatography and autoradiography. [^{14}C]Phorate sulfoxide and [^{14}C]phorate sulfone were prepared from [^{14}C]phorate by oxidation with 30% H_2O_2 for 24 h or 0.1% $KMnO_4$ for 30 min, respectively (Schrader, 1963). The purity of [^{14}C]phorate metabolites, checked by GLC, TLC, and autoradiography, was at least 99.0%. Water-soluble hydrolysis products of [^{14}C]phorate were obtained after incubation of [^{14}C]phorate in 0.1 N NaOH for 3 days (Schrader, 1963). After that, the alkali was neutralized and extracted 3 times with benzene. The aqueous phase was adjusted to pH 7 and diluted with nutrient solution (Hoagland and Arnon, 1950) for use in experiments.

Solvents. Acetone and benzene were redistilled before use. Methanol was of analytical grade.

Soil. The agricultural soil was an insecticide-free Plano silt loam (4.7% organic matter, 5% sand, 71% silt, 24% clay; pH 6.0) which had been stored for 2 months in a moist condition at room temperature.

Plants. *E. nuttallii* (Plach.) St. John were grown under a bank of Gro-Lux lamps (Sylvania Electric Products) on a 16-h photoperiod in an aquarium containing tap water and a 2-cm bottom deposit of an insecticide-free agricultural loam soil.

Soil Treatment. In these studies, water was contaminated with [^{14}C]phorate-treated soil or by a direct application of the insecticide to water. Moist loam soil was screened through a 2-mm sieve and then treated with acetone solutions of [^{14}C]phorate to yield insecticide concentrations of 4 ppm on a dry weight basis. After removal of the acetone vapors and a thorough mixing of the insecticide-treated soil, portions were extracted for analyses

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