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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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to determine the initial insecticide concentration.

Water Treatments. To treat nutrient solutions with insecticides, 1 mL of an insecticide–acetone solution was first pipetted into empty 75-mL test tubes. The solvent was then evaporated under a stream of nitrogen, and 40 mL of nutrient solution was added, followed by mixing for 10 s on a vortex mixer. The homogeneous distribution of all radiocarbon derived from ^{14}C-labeled insecticide was demonstrated by analyses of aliquots of the nutrient solutions by liquid scintillation counting.

Extraction. Soils were extracted twice with acetone–methanol (1:1) followed by a third extraction with acetone–methanol–benzene (1:1:1) as described by Lichtenstein et al. (1973). Water and nutrient solutions were extracted 3 times with benzene. Macerated *Elodea* plants were extracted as described for soils. After these extractions, the remaining plant pulp was combusted to $^{14}CO_2$ and analyzed by LSC as described below.

Analyses. Liquid scintillation analyses (LSC) of all benzene and water extraction phases were performed as described by Lichtenstein et al. (1972). Unextractable (bound) ^{14}C-labeled products in soils or plants were determined by their oxidation to $^{14}CO_2$ and subsequent LSC as described by Flashinski and Lichtenstein (1974). Gas–liquid chromatography (GLC) of benzene extracts was performed as described by Walter-Echols and Lichtenstein (1977).

EXPERIMENTAL SECTION

Effects of *Elodea* Plants on the Fate and Metabolism of Soil-Applied [^{14}C]Phorate in a Flooded Soil System. The fate of soil-applied [^{14}C]phorate as affected by the presence of *Elodea* plants in water was studied in triplicate tests with soil–water–plant systems as described by Walter-Echols and Lichtenstein (1978a). Soil (10 g dry weight), previously treated with [^{14}C]phorate at 4 ppm (0.19 μCi), was added to 200 mL of tap water in each of six 18 × 6 cm glass cylinders. After 1 h, when the soil particles had settled and the water appeared clear, four 8 cm long apical pieces of *Elodea*, loosely tied together with a string, were added to the water in each of three containers. Roots started to develop after approximately 7 days of incubation. To avoid pickup of radiocarbon via the root system, plants were periodically lifted out of the water and the roots were cut off before they could touch the insecticide-treated soil. The three remaining containers without plants served as controls. Each of the six containers was closed with Saran Wrap to reduce the evaporation of water, and all were incubated at 20–24 °C under a bank of Gro-Lux lamps on a 16-h photoperiod. After the 14-day incubation period, soils, water, and plants were separated and extracted with organic solvents for analyses by LSC and GLC as described.

Uptake of Radiocarbon by *Elodea* Plants from [^{14}C]Phorate-Treated Water. To study the potential uptake of radiocarbon by *Elodea* plants from insecticide-treated water, experiments were conducted in nine 75-mL test tubes (20 × 2.2 cm), each filled with 40 mL of nutrient solution, previously treated at 1 ppm with [^{14}C]phorate (0.23 μCi). After the addition of one 8 cm long apical piece of an *Elodea* plant to each tube, the nine tubes were closed with Saran Wrap and incubated for 14 days at 20–24 °C and at a 16-h photoperiod. After 3, 7, or 14 days of incubation, three plants each were removed, rinsed with water, dried, combusted, and analyzed by LSC. Aliquots of water (100 μL) were also analyzed by LSC after 0, 1, 2, 3, 5, 7, 11, and 14 days.

Release of Radiocarbon from *Elodea* Plants into Water. Experiments described above showed that *Elodea* takes up foreign compounds from contaminated water. To study the potential release of [^{14}C]phorate-derived radiocarbon by *Elodea* into water, six plants each were grown for 2 weeks as described above in 40 mL of nutrient solution treated at 2 ppm with [^{14}C]phorate (0.50 μCi). After that three plants were analyzed to determine the base radiocarbon content. The remaining three plants were each rinsed and grown for an additional 2 weeks in an insecticide-free nutrient solution. The radiocarbon released from these plants into the originally uncontaminated nutrient solutions was then determined by direct LSC after 0, 1, 2, 3, 5, 7, 10, and 14 days. At the end of the experiments nutrient solutions were extracted with benzene for analyses by LSC.

Effects of Light on the Uptake of [^{14}C]Phorate and Some of Its Metabolites by *Elodea* Plants. To study the effects of light, two series of experiments were conducted. In the first one, plants were grown for 72 h in [^{14}C]phorate-treated water as described above but under conditions of constant light, darkness, or a 12-h photoperiod. Three tubes for each light condition were prepared, and the radiocarbon present in the water was monitored after 0, 12, 24, 36, 48, 60, and 72 h by LSC. The total radiocarbon taken up by the plants during the 72-h incubation period was determined by combustion of dried plants and subsequent analyses by LSC.

In the second series of experiments, the uptake of radiocarbon derived from some phorate metabolites was investigated. In these tests, all plants were grown on a 16-h photoperiod for 72 h in triplicate 40 mL of nutrient solution treated at 1 ppm with either [^{14}C]phorate sulfoxide (0.21 μCi), [^{14}C]phorate sulfone (0.21 μCi), ^{14}C-labeled hydrolysis products of [^{14}C]phorate (0.21 μCi), or [^{14}C]-phorate (0.23 μCi). The radiocarbon in the water was monitored after 0, 12, 24, 36, 48, 60, and 72 h and that taken up by *Elodea* was determined at the end of the experiment as described above.

Significance of Live Plant Tissues Relative to the Uptake and Metabolism of [^{14}C]Phorate from Water. Studies conducted by different authors have shown that insecticides are accumulated at the same rate by both live and dead organisms (Derr and Zabik, 1974; Paris and Lewis, 1976). To test if dead *Elodea* plants also adsorb [^{14}C]phorate residues and whether the uptake of these residues from the water by *Elodea* is of biological or physical nature or both, the following experiments were conducted. Three 8 cm long apical pieces of *Elodea* plants were air-dried for 24 h. This was sufficient to kill the plants since they started to disintegrate when again being placed into water. Nine 20 × 2.2 cm test tubes were each filled with 40 mL of Hoagland nutrient solution, treated previously at 1 ppm with [^{14}C]-phorate (0.23 μCi). One dead plant was placed into each of three test tubes and one live plant into each of three additional tubes while the remaining test tubes remained without plants. All tubes were closed with Saran Wrap and incubated at 20–24 °C under a bank of Gro-Lux lamps on a 16-h photoperiod. Initially, and at intervals over a 2-week period, the water in each tube was thoroughly mixed and 100-μL aliquots were analyzed by LSC. At the end of the 14-day incubation period both live and dead plant materials were combusted to $^{14}CO_2$ and analyzed by LSC.

RESULTS AND DISCUSSION

Effects of *Elodea* Plants on the Fate and Metabolism of Soil-Applied [^{14}C]Phorate in a Flooded Soil System. As shown in Table I, the total amounts of radiocarbon recovered from soil–water systems without plants were 81.1% of the applied dose but amounted to

Table I. Distribution and Metabolism of [^{14}C]Phorate in a Flooded Soil System after 14 Days of Incubation without and with *E. nuttallii* Plants[a]

	recovered from extraction phases in % of soil-applied [^{14}C]phorate[b]							
	radiocarbon				benzene phase			
substrates	benzene	water	bound[c]	total	phorate	phorate sulfoxide	phorate sulfone	total
				Without Plants				
soil	24.6 ± 1.0	0.3 ± 0.1	8.3 ± 0.7	33.2 ± 1.4	18.8 ± 0.6	3.7 ± 0.4	0.3 ± 0.1	22.7 ± 0.3
water	29.2 ± 2.7	18.7 ± 0.7		47.9 ± 3.1	3.4 ± 1.2	25.5 ± 4.0	1.6 ± 0.3	30.5 ± 3.6
total	53.8 ± 2.9	19.0 ± 0.6	8.3 ± 0.7	81.1 ± 4.1	22.3 ± 1.7	29.2 ± 4.4	1.8 ± 0.4	53.2 ± 3.3
				With Plants				
soil	21.4 ± 1.1	0.5 ± 0.1	11.4 ± 0.7	33.3 ± 1.3	15.2 ± 1.4	3.0 ± 0.2	0.7 ± 0.4	19.0 ± 1.0
water	16.5 ± 2.7	18.3 ± 0.5		34.8 ± 2.3	0.6 ± 0.3	12.3 ± 1.8	3.6 ± 1.8	16.5 ± 3.1
plants	0.2 ± 0.0	2.8 ± 0.6	26.3 ± 2.1	29.4 ± 2.0	NA[d]	NA	NA	
total	38.1 ± 2.2	21.6 ± 1.2	37.7 ± 1.6	97.5 ± 1.0	15.8 ± 1.7	15.4 ± 2.0	4.3 ± 2.2	35.4 ± 2.4

[a] Results are means ± SD of triplicate tests. Ten grams (dry weight) of insecticide-treated loam soil was added to 200 mL of tap water. After 1 h, four *Elodea* plants were placed into the water and incubated for 14 days. [b] [^{14}C]Phorate was applied to loam soil at 4 ppm (0.19 μCi). [c] Bound = unextractable residues determined by combustion to $^{14}CO_2$. [d] NA = not analyzed because benzene extraction phases of plants contained only traces of ^{14}C-labeled residues.

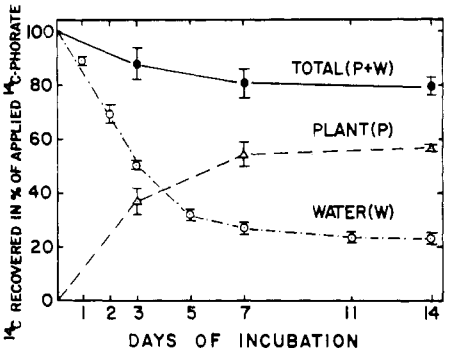

Figure 1. Uptake of radiocarbon by *E. nuttallii* from water treated at 1 ppm with [^{14}C]phorate. Results are means ● SD of triplicate tests.

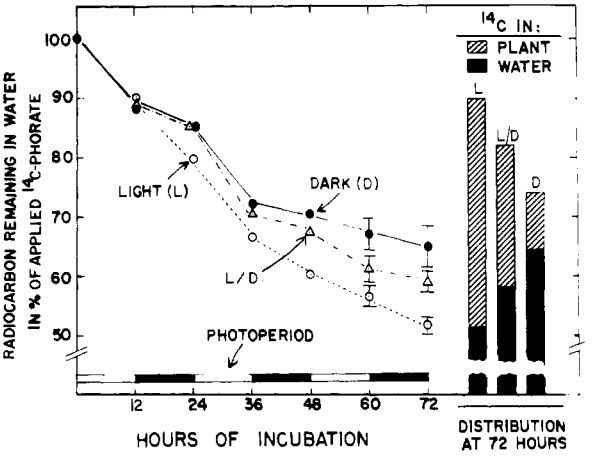

Figure 2. Effect of photoconditions on the loss of radiocarbon from [^{14}C]phorate-treated water and on the uptake of radiocarbon from water by *E. nuttallii* plants. The samples were incubated under continuous light (L), under continuous darkness (D), or on a 12-h photoperiod (L/D). Results are the means or means ± SD of three replicates. Bar graphs depict the distribution of ^{14}C between plants and water, expressed in percent of originally applied radiocarbon to water.

97.5% when *Elodea* plants had been added. Since plants contained 29.4% of the applied ^{14}C—most of it in the form of bound ^{14}C-labeled residues—only 34.8% of soil-applied ^{14}C were left in the water as opposed to 47.9% in water without plants. The amounts of benzene-soluble ^{14}C were smaller in soils when plants were present, while the amounts of water-soluble radiocarbon in soil and water were identical in both the absence and presence of plants. As shown in the right-hand portion of Table I, the major insecticide residue in soil was associated with the originally applied phorate. In water, however, phorate sulfoxide was the major compound recovered. Of the total (53.2% of soil-applied ^{14}C) recovered from the benzene phase of the systems without plants, 41.8% (=22.3% of soil-applied ^{14}C) were associated with phorate, 54.8% with phorate sulfoxide, and 3.5% with phorate sulfone. In systems with plants these figures amounted to 44.6%, 43.5% and 12.1%, respectively.

Uptake of Radiocarbon by *Elodea* Plants from [^{14}C]Phorate-Treated Water. Analyses of plants and [^{14}C]phorate-treated water during the 2-week incubation period indicated (Figure 1) that plants picked up increasing amounts of radiocarbon from the [^{14}C]phorate-treated water, especially during the first few days of incubation: 37.6 ± 5.4% of the radiocarbon applied to the water were found in plants after 3 days, 54.2 ± 5.4% after 7 days, and 56.1 ± 1.2% after 14 days of incubation. Conversely, the radiocarbon in the water declined during the 2-week incubation period to 22.9 ± 2.1% of the applied [^{14}C]phorate. Since up to 20% of the applied radiocarbon could not be accounted for after the 2-week incubation period ("total" in Figure 1), it was presumably lost through volatilization.

The reduced rate of ^{14}C uptake by the plants with increasing incubation time could have been the result of the formation of more water-soluble [^{14}C]phorate metabolites (Bowman and Sans, 1979) which were taken up at a slower rate by the plants. This latter hypothesis was tested with some of the oxidation and hydrolysis derivatives of [^{14}C]phorate in experiments described below relative to the effects of light on their uptake by plants from water.

Release of Radiocarbon from *Elodea* Plants into Water. Plants grown in [^{14}C]phorate-contaminated water contained after 2 weeks 0.22 ± 0.01 μCi of radiocarbon. When placed for an additional 2 weeks into insecticide-free water, only 6.9 ± 0.7% of the base amount of radiocarbon determined within the plants 2 weeks earlier had been released into the originally uncontaminated nutrient solution. Of the radiocarbon released, 95% were in water-soluble and 5% in benzene-soluble form.

Effects of Light on the Uptake of [^{14}C]Phorate and Some of Its Metabolites by *Elodea* Plants. Exposure of plants to [^{14}C]phorate-treated water under conditions of constant light, darkness, or a 12-h photoperiod did affect the uptake of the insecticide into plants as well as the distribution of radiocarbon within the water-plant system. Results are presented in Figure 2 where the left side shows

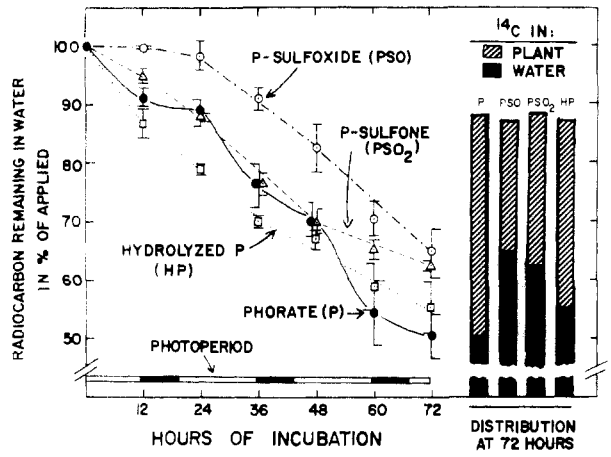

Figure 3. Loss of radiocarbon from water treated with [^{14}C]-phorate [^{14}C]phorate sulfoxide, [^{14}C]phorate sulfone, or hydrolyzed [^{14}C]phorate in the presence of *E. nuttallii* plants. Results are the means or means ± SD of triplicate tests. Bar graphs depict distribution of ^{14}C between plants and water, expressed in percent of originally applied radiocarbon to water.

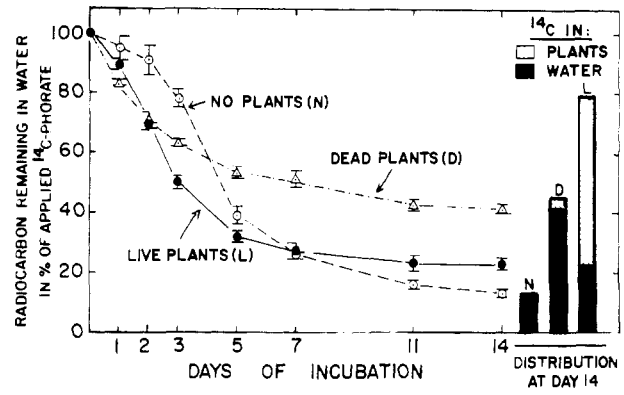

Figure 4. Loss of radiocarbon from [^{14}C]phorate-treated water incubated with none (N), dead (D), or live (L) *E. nuttallii* plants. Bar graphs to the right show distribution of radiocarbon between water and plants at day 14, expressed in percent of [^{14}C]phorate applied originally to the water. Results are the means or means ± SD of triplicate tests.

the rate of disappearance of radiocarbon from the water over the 72-h incubation period. During the first 12 h radiocarbon content in the water decreased equally under all three conditions. After that, however, it decreased fastest in samples kept under continuous light (L) and slowest in those that were kept in darkness (D). Results obtained with plants incubated on a 12-h photoperiod (L/D) were in between and exhibited some cycling. Bar graphs on the right side of Figure 2 show the amounts of radiocarbon that remained in water or were picked up by plants under the three conditions. Data are expressed in percent of the water-applied [^{14}C]phorate. Total recoveries of [^{14}C]phorate-derived radiocarbon from water plus plants were largest under conditions of constant light (90% of applied) and smallest under conditions of darkness (74% of applied). Also, under conditions of constant light plants contained 38.4 ± 1.2% of the applied dose but only 9.0 ± 0.9% when kept in the dark. Results, therefore, indicate that [^{14}C]phorate residues are primarily taken up by the plants from water during photoperiods and to a much lesser extent in darkness.

Results obtained in experiments with phorate and some of its metabolites are summarized in Figure 3. After plants had grown for 72 h under a 12-h photoperiod in nutrient solutions containing [^{14}C]phorate, [^{14}C]phorate sulfoxide, [^{14}C]phorate sulfone, or ^{14}C-labeled hydrolysis products of [^{14}C]phorate, amounts of radiocarbon remaining in the water and taken up by the plants were different for each treatment. Thus, the amounts of radiocarbon derived from hydrolyzed phorate (HP) decreased most rapidly while those derived from [^{14}C]phorate sulfoxide (PSO) decreased at the slowest rate. However, these differences were no longer apparent by the end of the 72-h incubation period. Amounts of ^{14}C recovered from the water treated with [^{14}C]phorate (P) were affected by the light–dark periods as exhibited by some cycling during incubation, thus confirming results described above (Figure 2). Bar graphs to the right of Figure 3 show the amounts of ^{14}C-labeled compounds recovered after 72 h of incubation from plants and water. They indicate that the total ^{14}C recovered from both plants and water was quite similar (87–81% of applied) for all four compounds used. However, the distribution of the radiocarbon between water and plants was not similar: plants exposed to water treated with [^{14}C]-phorate, hydrolyzed [^{14}C]phorate, [^{14}C]phorate sulfone, or [^{14}C]phorate sulfoxide contained after 72 h of incubation 37.6 ± 5.4%, 31.4 ± 4.1%, 26.1 ± 1.6%, or 21.8 ± 1.3% of the applied radiocarbon, respectively, while the amounts remaining in the water at the end of the incubation period were 50.5 ± 3.7%, 55.8 ± 5.1%, 62.5 ± 1.2%, and 65.2 ± 4.1% of the applied dose, respectively.

Significance of Live Plant Tissues Relative to the Uptake and Metabolism of [^{14}C]Phorate from Water. Results obtained after incubation of [^{14}C]phorate-treated water with none, dead, or live *Elodea* plants are summarized in Figure 4. Radiocarbon was rapidly lost from water under all three conditions, leaving in the water 41.1 ± 1.9%, 22.9 ± 2.1%, or 13.2 ± 0.9% of the radiocarbon applied to water which was incubated with dead, live, or no plants, respectively. Figure 4 also shows that in the presence of both live and dead plants, amounts of radiocarbon in the water decreased during the first 2 days at a similar rate. The larger amounts of radiocarbon remaining in the water with dead plants during the second week of incubation could possibly be attributed to a slow release of ^{14}C from the decomposing plants (average fresh weight 35 mg) which contained after 14 days 2.3 ± 0.3% of the applied radiocarbon while live plants (average fresh weight 170 mg) contained 56.5 ± 1.2%. But even on an equal weight basis, live plants contained 5 times more radiocarbon residues. Total recoveries of radiocarbon were highest in the presence of live plants (Figure 4, bar graph L) amounting to 79.4% of the applied [^{14}C]phorate. Of this amount, 56.5% were associated with the living plant tissue. In the presence of dead plants, the total recovery of ^{14}C amounted to 43.4% of applied, and only a minor fraction (2.3% of applied) was recovered from dead plant tissue. A possible explanation for these findings may be that organic matter of any kind, absent in the systems without plants, retained radiocarbon.

In summary, results obtained from the five experiments described above, indicate that *Elodea* plants in water accumulate radiocarbon derived from [^{14}C]phorate contained in bottom soil deposits or after a direct application of the insecticide to water. Insecticidal compounds thus taken up are primarily bound to plant tissues, which might explain the small amounts of ^{14}C-labeled compounds released from these plants into noncontaminated 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 to [^{14}C]phorate-contaminated water for 72 h. Moreover, most of the insecticide and some of its metabolites are primarily being picked up by the plants when exposed to light, a time when

photosynthetic processes take place. Plants kept in the dark contained less than $^1/_4$ of the amounts of radiocarbon taken up by plants exposed to light.

Registry No. Phorate, 298-02-2.

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In Vitro Release of Bound (Nonextractable) Atrazine Residues from Corn Plants by Chicken Liver Homogenate and Bovine Rumen Liquor

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The release of bound (nonextractable) residues from corn plants treated with [^{14}C]atrazine was investigated by in vitro incubation of the extracted plant tissue with chicken liver homogenate and bovine rumen liquor. Liver homogenate released bound ^{14}C residues from the plant tissues. However, no such release was observed with rumen liquor in the in vitro incubation system. The ^{14}C-bound residues in plant shoots or roots, mainly present as 2-chloro mono-N-dealkylated compounds, were released into the incubation mixture and subsequently metabolized to 2-hydroxy analogues.

Many studies have revealed that a considerable portion of pesticide residues may become bound (nonextractable) in plants (Fuhremann and Lichtenstein, 1978; Helling and Krivonak, 1978; Rouchaud et al., 1978, 1979; Fuhr and Mittelstaedt, 1980; Khan, 1980; Still et al., 1981). Oral administration of plant material containing ^{14}C-bound pesticide residues to animals has shown a rapid elimination of these residues via feces (Bakke et al., 1972; Paulson et al., 1975; Sutherland, 1976; Dorough, 1976; Marshall and Dorough, 1977). Thus, bound residues are considered to be of limited toxicological concern because of their limited bioavailability to animals.

In vitro studies have been undertaken to investigate the metabolism of pesticides by mammalian liver enzymes homogenates. Similarly, in vitro techniques in studies concerning metabolism of pesticides by rumen fluid have often been found useful. These methods applied to plant materials containing bound residues may provide useful information on the metabolic fate of these residues. Previously, we reported that the soluble fraction from chicken liver homogenates contained a heat-labile, glutathione-dependent enzyme that metabolized the herbicide atrazine in in vitro incubation (Foster et al., 1979). This paper reports the results of our investigation concerning the in vitro release of bound ^{14}C residues from corn plants by the chicken liver homogenates and rumen liquor from a lactating cow.

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EXPERIMENTAL SECTION

Plant Material. Ten corn plants (*Zea maise*), 19 days old, were exposed to 5 ppm of aqueous solution of ^{14}C-ring-labeled atrazine (0.16 Ci/jar). The plants were grown in Hoagland nutrient solution (300 mL/jar) and were maintained in a growth chamber for 8 days after herbicide treatment. Nutrient solution was added intermittently to the jar to replace loss due to transpiration over the growing period. At the end of the treatment period, each plant was harvested and the shoots and roots were separated. The latter was washed with cold water and the shoots and roots were stored at −20 °C until analyzed. Aliquots of the dried (24 h at 30 °C) plant tissues were combusted to $^{14}CO_2$ for determination of the total ^{14}C residues.

Chemicals. All solvents were of pesticide grade and used as received. Uniformly ^{14}C-ring-labeled atrazine, reference standards of atrazine, and metabolites were gifts from Ciba-Geigy, Ltd., Switzerland.

Determination of Radioactivity. Combustion of dried plant tissues was done in a Packard sample oxidizer, Model 306, to produce $^{14}CO_2$. Aliquots of various extracts (de-

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