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Received for review December 27, 1976. Accepted June 10, 1977. Contribution No. 120 from the Division of Agricultural Chemicals, I.A.R.I., New Delhi-110012, India.

Mechanisms of Production of Soil-Bound Residues of [^{14}C]Parathion by Microorganisms

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Unextractable (bound) radiolabeled residues are formed when [^{14}C]parathion is incubated in soil. The role of microorganisms in producing these bound residues was investigated by incubating [*ring*-^{14}C]-parathion in soil-free culture media that had been inoculated with soil microorganisms. The amounts of compounds in culture supernatants, that upon addition to soil became unextractable, increased up to 12 h of microbial culture incubation, when 43% of the added radiocarbon was bound after a 2-h soil incubation period. The increase in soil bound residues was correlated with a decrease in the amount of parathion in the microbial culture and a concomitant increase in the appearance of the major degradation product, aminoparathion. Microbial cells contained only a minor proportion of ^{14}C-bound residues. Addition of natural microbial growth metabolites to the soil did not affect binding of either parathion or aminoparathion. This latter compound was highly bound to both sterile and nonsterile soils. When [^{14}C]parathion, [^{14}C]paraoxon, *p*-[^{14}C]nitrophenol, and their respective ^{14}C-amino compounds were added to soil, the amino compounds were bound to a much greater extent than were the nitro compounds. It appears that the process of parathion degradation has to be separated from that of binding and that the role of the microorganisms in soil binding phenomena consisted in degrading [^{14}C]parathion to compounds which are more tightly bound to soil than the parent insecticide. Once these compounds were formed, their binding to soil was found to be high.

The fate of pesticides in the environment has been the subject of numerous studies. Because of potential hazards to living organisms, some of the more persistent insecticides were replaced by less persistent ones which rapidly "disappear" from environmental components, such as soil. However, the "disappearance" of a pesticide from soil may not only reflect its degradability, but can also reflect our inability to detect its residues by conventional procedures. One reason a chemical is not detected is its potential conversion to compounds which cannot be extracted, thus forming "bound" residues. The formation of these residues in soils can be followed by using ^{14}C-labeled compounds and combusting the previously extracted soil to $^{14}CO_2$. Formation of bound residues was shown with the herbicide, propanil (Bartha, 1971), and with the fungicide, 2,6-dichloro-4-nitroaniline (DCNA) (Van Alfen and Kosuge, 1976). The production of bound residues in soil was recently demonstrated with several insecticides which usually are considered to be nonpersistent (Katan et al., 1976; Lichtenstein et al., 1977). It was shown in this study that the binding of [^{14}C]parathion residues to soil was the result of the activity of soil microorganisms, and it was also suggested that microorganisms degraded [^{14}C]parathion to amino compounds which are rapidly and tightly bound to soil. However, other possibilities, such as binding to microbial cells and binding by means of microbial growth metabolites, should not be excluded.

The study of the mechanisms of binding of pesticides is important since this may assist us in obtaining information pertaining to the problem of the potential release of bound residues. In the present investigations degradation products of [^{14}C]parathion were produced by soil microorganisms in soil-free microbial cultures, followed by a separate study of the binding potential of these compounds to soils. The possible role of microorganisms in binding insecticide residues directly to microbial cells was also studied.

MATERIAL AND METHODS

Chemicals. [*ring*-^{14}C]Parathion (sp act. 2 μCi/mg) was purchased from Amersham-Searle Corporation, Arlington, Ill. Its radio purity was at least 99% after cleanup and isolation by thin-layer chromatography (TLC). In addition, five potential metabolites were prepared from [*ring*-^{14}C]parathion: [^{14}C]paraoxon was prepared as described by Fuhremann and Lichtenstein (1972), [^{14}C]-aminoparathion and [^{14}C]aminoparaoxon were obtained by reducing [^{14}C]parathion or [^{14}C]paraoxon, respectively,

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with nascent hydrogen produced from HCl and zinc, and *p*-[^{14}C]nitrophenol and *p*-[^{14}C]aminophenol were prepared from [^{14}C]parathion and [^{14}C]aminoparathion, respectively, by hydrolysis in 1 N NaOH at 90 °C for 30 min, subsequent acidification and extraction with diethyl ether. Isolation and purification by TLC was conducted as described by Fuhremann and Lichtenstein (1972).

Soil. The soil type used was a Plano silt loam (organic matter 4.7%, sand 5.0%, silt 71%, clay 24%, and pH 6.0). Soil sterilization was carried out by γ irradiation at 45 000 rad for 70 h. The sterility of the soils was confirmed by incubating samples in yeast extract–dextrose medium. The extraction solvents used were methanol and redistilled acetone and benzene.

Microbial Culture and Insecticide Treatment. Microorganisms were grown in liquid cultures in cotton plugged 25-mL Erlenmeyer flasks, each containing 7.5 mL of growth medium. The medium contained per 1000 mL of distilled water 2.0 g of $NaNO_3$, 1.0 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of KCl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 10.0 g of peptone, 10.0 g of yeast extract, and 10.0 g of dextrose. The pH of the medium was 6.7.

Prior to the inoculation of the growth media, 0.22 mL of acetone solutions containing 80 μg (0.16 μCi) of [^{14}C]parathion were added to each 7.5 mL of growth medium to give a final insecticide concentration of 10 ppm (final volume with inoculum = 8 mL). To remove the acetone as much as possible, culture flasks were placed into a transfer chamber, unplugged, and left for 2 min while sterilized air was allowed to flush through the chamber. This procedure did not contaminate the media. Preliminary experiments had shown that growth of microorganisms in acetone-treated media was similar to that in the nontreated controls.

For the utilization of soil-free, but soil-derived, microbial cultures, special inocula were prepared. To that effect 200 mg of loam soil in 50 mL of the growth medium was shaken 3 days at room temperature. After settling of the soil, 5 mL of the culture suspension was used to inoculate 50 mL of fresh medium which was then incubated for 2 days. Microscopy indicated that this culture was composed mainly of bacteria. It was finally used for inoculation of the growth media in the Erlenmeyer flasks. In this way a microbial inoculum was obtained that originated from soil but did not contain soil particles, which subsequently could have interfered with binding tests. Finally, 0.5 mL of this inoculum suspension served to inoculate the 7.5 mL of growth media in each of the 25-mL Erlenmeyer flasks. Cultures were incubated in the dark and at room temperature (20–22 °C) on an Eberbach shaker over various time periods at 140 strokes/min. All experiments were carried out in triplicate and were repeated once or twice.

Microbial Counts. At the end of the incubation period the density of microbial cells in [^{14}C]parathion-treated cultures was determined at a 1:20 dilution ratio with a Beckman Model 1401 DB spectrophotometer at a wavelength of 500 nm.

Extraction and Analyses. Soils utilized in experiments described below, and which had been treated with ^{14}C-labeled compounds, were extracted three times with 60 mL of a mixture of benzene, methanol, and acetone (1:1:1) (Katan et al., 1976). These soils were then tested for unextracted radioactivity. Preliminary studies with [^{14}C]parathion had shown that six additional extractions yielded a total of only 2.4% of the originally applied radiocarbon. Unextractable or bound residues were, therefore, defined as ^{14}C residues remaining in soils which had been extracted three times as described. To measure the amounts of these bound residues, three 1.5-g aliquots of the extracted soil from each replicate were combusted to $^{14}CO_2$ in a Packard Model 305 Tri-Carb Sample Oxidizer. Subsequent liquid scintillation analyses (LSC) were performed as described by Flashinski and Lichtenstein (1974). Results were finally expressed as bound ^{14}C in percent of radiocarbon applied to soil.

Supernatants of microbial cultures were extracted with benzene and partitioned into benzene and water extraction phases. The radioactivity in each extraction phase was then determined, followed by analyses of the benzene extracts for parathion and aminoparathion by GLC as described (Lichtenstein and Fuhremann, 1971) except that a 1.8 m × 4 mm column containing 10% DC-200 in 80/100 Gas-Chrom Q maintained at 180 °C was used. The amounts of parathion and of aminoparathion were finally expressed in percent of the applied parathion, with a correction being made for the difference in molecular weight of these compounds. Analyses by TLC were conducted as described by Lichtenstein and Fuhremann (1971) and autoradiography as described by Flashinski and Lichtenstein (1974).

EXPERIMENTAL SECTION

Formation of Soil-Bound Compounds by Soil Microorganisms in Culture. To test whether the binding of [^{14}C]parathion to soil was a particular function of microorganisms which first degraded the insecticide to bindable compounds, [^{14}C]parathion metabolites were produced in the absence of soil with cultures of microorganisms. These metabolites were then added to insecticide-free soil. To that effect, soil-free microbial cultures were prepared as described above, treated with [^{14}C]parathion and incubated for 0, 3, 6, 12, 24, 48, and 96 h. After each incubation period, the radiocarbon in 0.5 mL of whole cultures or in 0.5 mL of their respective supernatants was determined by LSC as described (Lichtenstein et al., 1972). These supernatants were obtained by centrifuging each culture in Corex glass tubes at 10 000 rpm (12 000*g*) for 10 min in a refrigerated Sorvall centrifuge. Contrary to tests with cellulose nitrate centrifuge tubes, no ^{14}C residues remained on the Corex glass tube walls. After that, 1.5-mL aliquots of the whole cultures or of the supernatants were thoroughly mixed with 10 g of air-dried loam soil and then incubated for the relatively short period of 2 h. The soil samples were then extracted and analyzed for ^{14}C-bound residues as described above. For control purposes the same procedure was employed, except that sterile (15 min at 121 °C and 1 atm), noninoculated media were used. Data were finally expressed as radiocarbon recovered in percentages of that added with the whole culture or its supernatant to the soil.

Binding of Potential Parathion Metabolites to Soil. To test the capacity of potential [^{14}C]parathion metabolites to be bound to soils, 10-g samples of moist loam soil were mixed with 0.4 mL of acetone solutions of [^{14}C]parathion, [^{14}C]paraoxon, *p*-[^{14}C]nitrophenol or their respective amino derivatives to give final concentrations of 1 ppm of each compound. After 2 h of incubation, these soils were extracted and the amount of ^{14}C-bound residues was determined as described.

Distribution of ^{14}C Residues in Microbial Cultures after Incubation with [^{14}C]Parathion. To determine if cells of microorganisms themselves could bind [^{14}C]-parathion residues thus making them unextractable, insecticide-treated microbial cultures were incubated for 24, 48, or 216 h, and then separated into supernatants and the microbial pellets. In order to remove ^{14}C compounds adhering to the microbial cells, pellets were resuspended

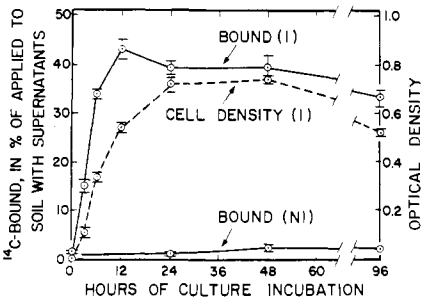

Figure 1. Binding of ^{14}C compounds to soil within 2 h after the addition of supernatants obtained from 0- to 96-day old microbial cultures, treated with [^{14}C]parathion and inoculated (I) with soil microorganisms; NI = noninoculated controls.

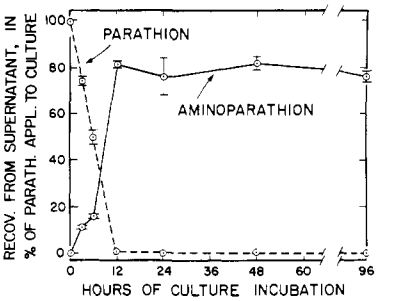

Figure 2. Amounts of parathion and aminoparathion recovered by gas–liquid chromatography from benzene extracts of supernatants of microbial cultures after treatment with [*ring*-^{14}C]-parathion at 10 ppm and incubation for 0 to 96 h.

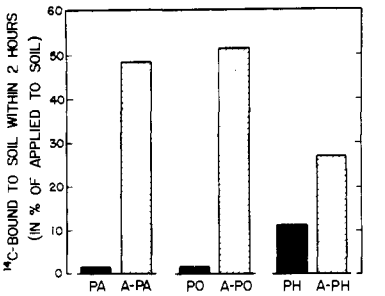

Figure 3. Amounts of ^{14}C-bound residues in soils, 2 h after soil treatment at 1 ppm with [^{14}C]parathion (PA), [^{14}C]aminoparathion (A-PA), [^{14}C]paraoxon (PO), [^{14}C]aminoparaoxon (A-PO), *p*-[^{14}C]nitrophenol (PH), or *p*-[^{14}C]aminophenol (A-PH).

with 40 mL of distilled water, shaken for 1 min on a Vortex Genie mixer, and centrifuged at 3000 rpm for 15 min. The resulting pellet was again resuspended and centrifuged as described. The combined water fractions are referred to as the water wash. The pellet was then suspended in a 40-mL 1:1:1 mixture of benzene–methanol–acetone, mixed for 1 min on a Vortex Genie mixer, and centrifuged, thus yielding an organic solvent extract of the cells referred to as a solvent wash. The amounts of radiocarbon in the water and solvent washes were then determined by LSC. The extracted pellets were transferred with cotton onto Whatman No. 1 filters which were then combusted to $^{14}CO_2$ for the determination of bound or unextractable ^{14}C residues in or on microbial cells.

Potential Effects of Microbial Growth Metabolites on the Binding of [^{14}C]Parathion Residues to Soil. Microorganisms usually produce extracellular growth metabolites, which potentially could affect the binding of [^{14}C]parathion residues to soil. Since some microbial metabolites could potentially be produced and secreted into the cultures only upon induction by parathion, microbial cultures were treated with nonradioactive parathion at 10 ppm and incubated for 3 days. These cultures were then centrifuged at 10 000 rpm for 10 min as described. Acetone solutions (0.1 mL) containing 10 μg (0.02 μCi) of either [^{14}C]parathion or [^{14}C]aminoparathion were added to 1.5-mL aliquots of suspensions of the original culture or of the supernatant and immediately utilized to treat 10 g of air-dried soil. This resulted in a final concentration of the ^{14}C compound of 1 ppm in the soil. After soil incubation for 2 h, analyses for ^{14}C-bound residues were performed as described. Controls (treatments without microbial metabolites or without addition of ^{14}C compounds) were prepared in a similar manner.

RESULTS AND DISCUSSION

Formation of Soil-Bindable Compounds by Soil Microorganisms in Culture. Results, summarized in Figure 1, indicate that primarily within the first 12 h of culture incubation, ^{14}C compounds had been produced, which could rapidly be bound to the loam soil. Thus, after 12 h of incubation of microbial cultures, 43% of the radiocarbon added with the supernatants to the soil, became bound within 2 h of soil incubation. The rate of binding to soil within 2 h decreased somewhat with supernatants from [^{14}C]parathion-treated microbial cultures that had been incubated for 24 to 96 h. After the addition of [^{14}C]parathion to sterile, noninoculated culture media (controls), insignificant amounts (1.4 to 2.7%) of applied radiocarbon derived from these sterile culture media were bound when added to soil (NI in Figure 1). The increase in the production of bindable compounds by inoculated cultures with time was accompanied by an increase in the density of microorganism cells as determined by measuring the optical density of the cultures (Figure 1).

Supernatants of inoculated cultures which had been incubated for 0 to 96 h, were analyzed by GLC. Results (Figure 2) showed that the production of bindable compounds was accompanied by a rapid increase in the appearance of aminoparathion and a concomitant decrease in parathion, which had practically disappeared from the supernatants 12 h after culture inoculation. These findings were also confirmed by TLC and autoradiography. The presence of aminoparathion in the supernatant of the inoculated cultures was further confirmed by a color reaction specific for amino compounds (Lichtenstein and Fuhremann, 1971). The autoradiograms showed that cultures incubated for 6 h or more also contained trace amounts of compounds having R_f values similar to those of *p*-nitrophenol or aminoparaoxon. With controls (noninoculated cultures), however, analyses by GLC and TLC of benzene fractions of supernatants revealed that only parathion was present throughout the entire experimental period, thus indicating that no insecticide breakdown had occurred in the absence of microorganisms.

Microbial degradation of parathion by soil microorganisms in culture was also evident by the production of water-soluble degradation products which increased with time. Thus, 12 h after incubation of [^{14}C]parathion in a microbial culture, 35% of the applied radiocarbon was associated with the water extraction phase of the supernatant. This figure increased to 42% after 96 h of incubation. In controls (noninoculated cultures), however, practically no water-soluble degradation products had been produced (0.9–3.6% of the applied radiocarbon).

Binding of Potential Metabolites of [^{14}C]Parathion to Soil. After incubation of soils for 2 h with [^{14}C]-parathion, [^{14}C]paraoxon, *p*-[^{14}C]nitrophenol and their respective amino derivatives, the amounts of unextractable, bound radiocarbon in the soil were determined as described. As shown in Figure 3 the reduction of the nitro group to an amino group resulted in a pronounced increase

in binding. Binding of the amino compounds was 14–26 times higher than that of parathion. Since binding was assessed after a relatively short (2 h) period of incubation in soil, the mechanism of binding of the amino compounds apparently does not involve a significant soil microbial activity.

Distribution of ^{14}C Residues in Microbial Cultures after Incubation with [^{14}C]Parathion. Another possibility for explaining the role of microorganisms in the binding of [^{14}C]parathion in a nonsterile soil would be the binding of parathion or its degradation products to the cells of living microorganisms themselves. If this were the case, ^{14}C-bound residues should be detected in the microbial cells. To investigate this possibility microbial cells were analyzed for the presence of [^{14}C]parathion residues, after insecticide-treated microbial cultures had been incubated for 24, 48, or 216 h. Results indicated that approximately 12% of the applied radiocarbon was associated with the cells, but this was not tightly retained since it could be released by washing with water. Extraction of water-washed cells with organic solvents and subsequent combusting to $^{14}CO_2$, indicated that the cells did not bind the insecticide or its degradation product: only insignificant amounts of radiocarbon (0.10 to 0.91% of applied) were recovered from the solvent wash or the previously extracted cells.

An additional experiment was conducted to study the potential importance of microorganisms in affecting the binding of [^{14}C]parathion degradation products in soil. A supernatant derived from a [^{14}C]parathion-treated culture, incubated for 48 h, was mixed with either nonsterile soil or with a soil sterilized by γ irradiation. GLC analysis showed that the major compound present was aminoparathion. After 2 h of incubation, bound radiocarbon was determined. No significant difference in binding between the two soils was found: 39.6 and 42.0% of the applied radiocarbon were bound in nonsterile and sterile soils, respectively. This clearly indicated that rapid binding of ^{14}C compounds may occur in both sterile and nonsterile soils, provided that bindable ^{14}C compounds had previously been produced by soil microorganisms in culture.

Potential Effects of Microbial Growth Metabolites on Binding of [^{14}C]Parathion Residues in Soil. Previous experiments showed that the production of bindable degradation products of [^{14}C]parathion by microbial cultures increased with time and was correlated with microbial growth (Figure 1). Thus, it is expected that both the amounts of parathion degradation products and microbial growth metabolites would increase with time. The possibility that the growth metabolites of the microorganisms in the culture could affect the binding of parathion or its degradation products to soil was investigated as described. Results presented in Table I clearly show that addition of microbial growth metabolites with [^{14}C]parathion or with [^{14}C]aminoparathion did not affect the binding of either compound to the soil.

In summary, it appears that the process of parathion degradation has to be separated from that of binding. Once bindable compounds were formed, their binding to soil was found to be high, both in absence and in the presence of microorganisms and in both nonsterile and sterilized soils. This was not the case when parathion was directly added to soils, since in that system bindable degradation products had first to be produced. This occurred in nonsterile but not in sterilized soil (Katan et al., 1976). Binding to microbial cells was negligible, and microbial growth metabolites did not contribute to binding. Thus, the two-step process of binding, namely, a microbial degradation, followed by physicochemical binding is further supported. A similar binding mechanism through the liberation of easily bound products was suggested for propanil (Hsu and Bartha, 1974) and DCNA (Van Alfen and Kosuge, 1976).

Table I. Effect of Microbial Growth Metabolites on Binding of [*ring*-^{14}C]Parathion or of [*ring*-^{14}C]Aminoparathion to Soil within 2 h[a]

Soil treatment		^{14}C bound to soil
Compound	Combined with	in % of applied[b]
[^{14}C]Parathion	None	0.65
[^{14}C]Parathion	Whole culture[a]	0.77
[^{14}C]Parathion	Supernatant[a]	0.69
[^{14}C]Aminoparathion	None	39.7
[^{14}C]Aminoparathion	Whole culture	40.3
[^{14}C]Aminoparathion	Supernatant	38.3

[a] Aliquots of the cultures containing both microorganisms and their growth metabolites or cell-free supernatants of the cultures were mixed with the specified compound, incubated in soil for 2 h, followed by the determination of bound radiocarbon. [b] Determined by combusting the treated and extracted soil to $^{14}CO_2$. Differences observed between treatments with [^{14}C]parathion or with [^{14}C]-aminoparathion are nonsignificant (5%).

Concomitant with an increase in microbial growth, aminoparathion was the major degradation compound produced in culture from parathion. Appearance of other degradation products with time, although present in minor quantities, may have contributed to the slight decrease in the amount of bindable compounds over a 96-h incubation period of [^{14}C]parathion in culture (Figure 1).

Aminoparathion has been reported to be a degradation product of parathion in flooded soils (Sethunathan and Yoshida, 1973; Katan et al., 1976), in lake sediments (Graetz et al., 1970), in a simulated cranberry bog (Miller et al., 1966), in a loam soil and inoculated soil water (Lichtenstein and Schulz, 1964), and in cultures of *Rhizobium* (Mick and Dahm, 1970), soil bacteria (Graetz et al., 1970), *Penicillium* (Rao and Sethunathan, 1974), and of *Chlorella* (Zuckerman et al., 1970). It was suggested that aminoparathion, formed in cells of *Chlorella*, does not accumulate and is rapidly released into the culture medium.

The mixed populations of soil-derived microorganisms used in this study may represent only a part of the microorganism populations existing in this soil. It is possible that under different conditions where other types of microorganisms are dominant, the rate and mechanisms of binding may be different. However, the role of microorganisms in binding phenomena of parathion derivatives was demonstrated in the present study both in soil and in culture.

Testing the relative binding capacity of parathion and five of its potential metabolites clearly showed that reduction of a nitro group to an amino group resulted in a significant increase in binding (Figure 3). The high binding of aminoparathion and *p*-aminophenol can explain the inability to recover these compounds from soil shortly after their application (Lichtenstein and Schulz, 1964). At that time the authors referred to these compounds as "nonpersistent" and also used the expression of "disappearance from soil". A high binding of another aromatic amino compound was shown with 4-chloroaniline (Hsu and Bartha, 1974). It is possible that with other types of molecules containing an amino group, the presence of additional substituents at certain positions may affect the high binding of the amino compound. Indeed, pentachloroaniline, a reduced degradation product formed under

anaerobic conditions can be easily extracted from soil (Ko and Farley, 1969; Wang and Broadbent, 1973). Another amino compound, DCNA, is not bound to nonflooded soil, but is highly bound when incubated in a flooded one, apparently due to transformation to unextractable compounds (Van Alfen and Kosuge, 1976). It is, therefore, not possible at this stage to predict which compound will be easily bound to soil. However, pesticides which contain amino groups or are degraded to such compounds, especially when under anaerobic conditions, should be looked upon as soil-bindable compounds.

The approach used in this study to elucidate the role of microorganisms in binding can probably be used with other pesticides as well. However, the microbial role in binding does not appear to be a general phenomena. Thus, the mechanism of binding of the insecticide [^{14}C]fonofos, whose rate of binding to soil is similar to that observed with [^{14}C]parathion, is not dependent on the presence of soil microorganisms (Lichtenstein et al., 1977).

ACKNOWLEDGMENT

Special thanks are expressed to B. N. Anderegg, T. W. Fuhremann, T. T. Liang, and K. R. Schulz for their assistance in performing this research. The degradation products of parathion were prepared by T. W. Fuhremann.

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Received for review January 10, 1977. Accepted July 18, 1977. This research was conducted during the senior author's sabbatical leave in the Department of Entomology of the University of Wisconsin. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, by the University of Wisconsin Graduate School, and by a grant from the National Science Foundation (BMS 72-02179). Contribution by Project 1387 from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project 96, entitled "Environmental Implications of Pesticide Usage".

Residues of Atrazine and Its Metabolites in an Orchard Soil and Their Uptake by Oat Plants

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Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and its metabolites persisted in a peach orchard soil for several years following nine consecutive annual applications of the herbicide at 4.5 kg/ha. Metabolites identified in soil samples taken 2 and 3.5 years after the last application of the herbicide were: deethylatrazine (2-chloro-4-amino-6-isopropylamino-*s*-triazine), hydroxyatrazine (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine), deethylhydroxyatrazine (2-hydroxy-4-amino-6-isopropylamino-*s*-triazine), and deisopropylhydroxyatrazine (2-hydroxy-4-ethylamino-6-amino-*s*-triazine). Partial N-dealkylation and hydrolysis reactions were involved in the breakdown of atrazine in soil. When oats were grown in the orchard soil, metabolites were absorbed as such and underwent detoxification in plant tissues by conjugation.

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is a widely used selective herbicide for the control of annual grasses and broad-leaved weeds in corn, sorghum, and orchards. It is also used as nonselective herbicide for vegetation control in noncrop land. Although atrazine can persist in some soils for more than 1 year in quantities sufficient to damage susceptible crops (Burnside et al., 1969), accumulation of the herbicide in the soil normally does not occur, even upon repeated application (Fryer and Kirkland, 1970). Marriage et al. (1975) investigated atrazine persistence in an orchard soil which recieved nine consecutive annual applications from 1963 to 1971 of relatively high rates of the herbicide (4.5 kg/ha). Chemical analysis of surface soil samples taken 139 days after the last application in 1971 showed low levels of atrazine (0.4 ppm). These observations led them to conclude that under local conditions in southern Ontario atrazine should not pose a residue accumulation hazard even in situations where it is applied at high rates for many years. This may be true, however, only under certain soil and environmental conditions. Burnside et al. (1971) observed that atrazine residues increased with successive applications over 3 years on several loam soils.

It has been shown that metabolism of atrazine in soil involves hydroxylation (Harris, 1967; Skipper et al., 1967; Skipper and Volk, 1972; Armstrong et al., 1967; Armstrong and Chesters, 1968; Obien and Green, 1969; Zimdahl et al., 1970), dealkylation (Skipper et al., 1967; Skipper and Volk,

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