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.

LITERATURE CITED

Bartha, R., *J. Agric. Food Chem.* **19**, 385 (1971).
Flashinski, S. J., Lichtenstein, E. P., *Can. J. Microbiol.* **20**, 399 (1974).
Fuhremann, T. W., Lichtenstein, E. P., *Toxicol. Appl. Pharmacol.* **22**, 628 (1972).
Graetz, D. A., Chesters, G., Daniel, T. C., Newland, L. W., Lee, G. B., *J. Water Pollut. Control Fed.* **42**, R 76 (1970).
Hsu, T-S., Bartha, R., *Soil Sci.* **116**, 444 (1974).
Katan, J., Fuhremann, T. W., Lichtenstein, E. P., *Science* **193**, 891 (1976).
Ko, W. H., Farley, J. D., *Phytopathology* **59**, 64 (1969).
Lichtenstein, E. P., Fuhremann, T. W., *Science* **172**, 589 (1971).
Lichenstein, E. P., Katan, J., Anderegg, B. N., *J. Agric. Food Chem.* **25**, 43–47 (1977).
Lichtenstein, E. P., Schulz, K. R., *J. Econ. Entomol.* **57**, 618 (1964).
Lichtenstein, E. P., Schulz, K. R., Fuhremann, T. W., *J. Agric. Food Chem.* **20**, 831 (1972).
Mick, D. L., Dahm, P. A., *J. Econ. Entomol.* **63**, 1155 (1970).
Miller, C. W., Zuckerman, B. M., Charig, A. J., *Trans. Am. Fish. Soc.* **95**, 345 (1966).
Rao, A. V., Sethunathan, N., *Arch. Microbiol.* **97**, 203 (1974).
Sethunathan, N., Yoshida, T., *J. Agric. Food Chem.* **21**, 504 (1973).
Van Alfen, N. K., Kosuge, T., *J. Agric. Food Chem.* **24**, 584 (1976).
Wang, C. H., Broadbent, F. E., *J. Environ. Qual.* **2**, 511 (1973).
Zuckerman, B., Deubert, K., Mackiewicz, M., Gunner, H., *Plant Soil* **33**, 273 (1970).

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

Shahamat U. Khan* and Paul B. Marriage

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,

Chemistry and Biology Research Institute, Research Branch, Canada Agriculture Ottawa, Ontario, Canada K1A 0C6 (S.U.K.) and Research Station, Canada Agriculture, Harrow, Ontario, Canada N0R 1G0 (P.B.M.).

Table I. Common Names, Chemical Names, Retention Times, and Thermionic Response of *s*-Triazines and Metabolites

Common name	Chemical name	Peak no. (Figure 1)	Retention time, min	0.5 fsd,[a] ng
Atratone	2-Methoxy-4-ethylamino-6-isopropylamino-*s*-triazine	1	4.7	8.0
Atrazine	2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine	2	6.7	4.7
Deethylatratone[b]	2-Methoxy-4-amino-6-isopropylamino-*s*-triazine	3	7.9	11.1
Deisopropylatratone[b]	2-Methoxy-4-ethylamino-6-amino-*s*-triazine	4	9.9	22.5
Deethylatrazine	2-Chloro-4-amino-6-isopropylamino-*s*-triazine	5	13.6	5.2

[a] 50% full-scale deflection. [b] Prepared by methylation of the corresponding hydroxy analogues with diazomethane.

1972; Kaufman and Blake, 1970), and ring cleavage (Dupuis et al., 1970). Phytotoxicity of the herbicide is destroyed by hydroxylation at the 2 position but not by dealkylation of either of the two alkylamino groups (Kaufman and Blake, 1970). Sirons et al. (1973) observed that atrazine applied in the field to a soil was converted into deethylatrazine (2-chloro-4-amino-6-isopropylamino-*s*-triazine) as a major and deisopropylatrazine (2-chloro-4-ethylamino-6-amino-*s*-triazine) as a minor phytotoxic metabolite. They concluded that dealkylation process was the most important pathway associated with the persistence and herbicidal activity of atrazine in soils. In a greenhouse experiment Beynon et al. (1972) investigated the breakdown of atrazine in soil sampled 32 and 70 days after treatment. They reported that hydroxylation of C–Cl bond predominated although dealkylation reactions were also evident. Best and Webber (1974) also investigated the breakdown of atrazine over a 5-month period in a soil under greenhouse conditions. Hydroxyatrazine was found to be the major metabolite along with trace amounts of monodealkylation products.

The present study is a continuation of the investigation on the persistence of atrazine in an orchard soil after nine consecutive annual applications (Marriage et al., 1975). Its purpose was to investigate whether degradation products of atrazine would persist in this soil until atrazine residues had decreased sufficiently to allow planting of susceptible crops and whether uptake of such products by plants would occur. The metabolites in soil samples taken from the field plots in 1973 and 1974 were identified and their uptake by oat plants was determined.

EXPERIMENTAL SECTION

Orchard Treatment. The peach orchard was planted in April 1963 and atrazine was applied each year from 1963 to 1971 at 4.5 kg/ha of active ingredient (Marriage et al., 1975). The soil type was Fox sandy loam with a pH of 5.3 and 1.8% organic matter. The control plots were hand-weeded. The experiment contained four replicates. The trees were cut to leave stumps in Dec 1969, and stumps were removed from the orchard in the winter of 1971. In the spring of 1972, 1973, and 1974 the land was disked lengthwise with respect to the plots and oats were planted in early April of each year.

Soil Samples. Soil samples were collected from the 0–15 cm layer in March 1973 and Oct 1974. For both samplings, two subsamples of approximately 100 cm^2 area were taken as close as possible to the midpoint between the location of the former middle and guard trees in each plot and pooled. The samples were air-dried, screened, passed through a 20-mesh sieve, and stored at −20 °C.

Greenhouse Experiment. Oats (cultivar "Gray") were grown in soil samples collected from the treated and hand-weeded plots in 1973 and 1974, as well as in check soil samples fortified with analytically pure hydroxyatrazine at 0.5, 1.0, and 2.0 ppm levels. Fourteen oat seeds were planted in 150 g of soil at the surface in perforated aluminum plates (9 cm diameter) and after germination were thinned to ten uniform sized plants. Plates were watered daily by shallow subirrigation, and after 2 weeks roots and shoots were removed. The experiment was replicated six times in a greenhouse with temperature 30 °C (day) and 20 °C (night). Supplemental light was provided to give a day length of approximately 15 h.

The fresh weight of shoots was determined, and the samples were freeze-dried. The root samples were vacuum-dried at 40 °C and weight determined. The soil samples were air-dried. All samples were stored at −20 °C until they were analyzed.

Chemicals. All solvents were of pesticide grade and used as received. Reference standards of atrazine and metabolites (Table I) were gifts from Ciba-Geigy Limited, Switzerland.

Determination of Residues. (*1*) *Soil.* The air-dried soil (100 g) sample was extracted with 200 mL of methanol in a mechanical shaker for 2 h and then filtered under suction. The sample residue was washed with methanol (3 × 75 mL), and the combined filtrate evaporated to dryness on a rotary evaporator at room temperature. The dried residue was dissolved in several portions of chloroform (5–10 mL) and placed on an acidic alumina column (24 × 70 mm, 20 g of acidic aluminum oxide, Woelm, activity I) topped with 10 mm of anhydrous Na_2SO_4 and prewashed with chloroform. The column was first eluted with 250 mL of dried (anhydrous Na_2SO_4) chloroform (eluate I) and then with 250 mL of methanol (eluate II).

Eluate I which contained atrazine and 2-chloro metabolites was concentrated to about 10 mL on a rotary evaporator at room temperature and finally taken to dryness with a stream of dry air. The residue was dissolved in hexane, and an aliquot of this solution was injected into the gas chromatograph.

Eluate II which contained 2-hydroxy metabolites was concentrated to about 5 mL on a rotary evaporator at room temperature and methylated by adding an excess of freshly prepared diazomethane solution (prepared from Diazald, Aldrich Co. Inc., Milwaukee, Wis.) until the yellow color persisted. The flask was stoppered, and the contents were allowed to stand at room temperature for about 3 h with occasional shaking. The mixture was taken to dryness in a stream of dry air, the residue dissolved in hexane, and an aliquot analyzed by gas chromatography.

(*2*) *Plant.* Both root and shoot samples were finely ground and pooled within each treatment. The sample was blended at a high speed with dried chloroform (1:100, w/v) for 5 min. The mixture was filtered under suction and the sample residue washed with 100 mL of chloroform. The combined filtrate was concentrated to about 5 mL on a rotary evaporator (extract I), transferred to an acidic alumina column, eluted with chloroform, and processed as described before.

The insoluble residue of root or shoot tissue was transferred into the blender and blended at high speed with methanol (1:100, w/v) for 5 min. The mixture was

Table II. Recovery of Atrazine and Metabolites from Fortified Samples of Soils and Plant Shoots and Roots[a]

Sample	Fortification, ppm	Recovery, %				
		Atrazine	Deethylatrazine	Hydroxyatrazine	Deethylhydroxyatrazine	Deisopropylhydroxyatrazine
Soil	0.05	96.5 ± 1.5	81.0 ± 2.2	68.3 ± 4.9	72.5 ± 3.5	73.0 ± 1.6
	0.01	92.5 ± 2.4	86.0 ± 4.8	70.0 ± 2.1	73.0 ± 4.3	74.0 ± 5.5
Shoots	0.10			76.0 ± 5.4	69.0 ± 6.7	72.5 ± 7.8
Roots	0.10			71.0 ± 6.9	77.9 ± 4.5	69.0 ± 2.1

[a] Mean values for triplicate samples with standard errors.

filtered under suction, and the insoluble sample residue was blended again with methanol (1:100, w/v) and finally with water (1:100, w/v). Methanol was removed from the combined filtrate and the water phase concentrated to a smaller volume on a rotary evaporator (extract II). An XAD-2 column (20–50 mesh, 2.4 × 25 cm) was prepared and cycled between methanol and water three times and finally washed exhaustively with water. Extract II was transferred into the XAD-2 column and washed with one column volume of water. The nonionic polymeric adsorbent XAD-2 adsorbed most of the polar metabolites. However, to ensure complete adsorption the aqueous eluate was recycled through the column. The column was then eluted with 100% methanol (200 mL), and the eluate was concentrated to about 20 mL on a rotary evaporator at room temperature and divided into two parts, eluate III and eluate IV, for determining free and conjugated metabolites. Eluate III was methylated with diazomethane and processed as described for eluate II. Eluate IV was transferred into a graduated centrifuge tube, taken to dryness in a stream of dry air, and the residue was dissolved in 15 mL of water. The content of the tube was subjected to hydrolysis by heating at 100 °C for 4 h with 5 mL of concentrated HCl. The mixture was evaporated to dryness under vacuum, redissolved in a small volume of methanol, methylated with diazomethane, and processed as described for eluate II.

Performance of the Method. The recoveries of the residues by the methods used were determined by adding known amounts of the compounds to soil or oat samples. The soil used was obtained from control plots which had not been treated with atrazine. The air-dried ground soil (50 g) sample was fortified with a mixture of atrazine, deethylatrazine, hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine at 0.05 and 0.01 ppm levels. The solvent (methanol) was allowed to evaporate and the sample mixed thoroughly. Further processing of the soil sample was done as described above.

The shoot and root samples were obtained from oat plants grown in control soil. The freeze-dried shoot and oven-dried root samples were finely ground and fortified (1 g) with a mixture of hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine at a 0.1 ppm level. The solvent was allowed to evaporate and the sample mixed thoroughly. Further processing of the samples was done as described above with one exception. The methanol eluate from the XAD-2 column was not divided into two parts, rather the whole eluate was taken to dryness, the residue was dissolved in 15 mL of water and subjected to hydrolysis as described above for eluate IV.

All samples were extracted and analyzed in duplicate or triplicate and average values are reported. Residue levels in soil are reported on an oven-dry basis, in shoots on fresh weight basis, and in roots on a vacuum oven-dry (40 °C) basis. The results reported here are not corrected for recovery.

Gas Chromatography (GC). The gas chromatograph was a Pye series 104, Model 64, fitted with an alkali flame ionization detector having an RbCl annulus. The column was a 1.5 m × 0.4 cm i.d. glass tube packed with 3% Carbowax 20M coated on 80–100 mesh Chromosorb WHP. The operating conditions were: on-column injections; injector port temperature control turned off, column and detector temperatures, 220 and 270 °C, respectively. The nitrogen carrier gas, hydrogen, and air flow rates were 60, 35, and 300 mL/min, respectively.

Gas Chromatography–Mass Spectrometry (GC–MS). A Finnigan Model 3100 mass spectrometer connected to a Finnigan Model 9500 gas chromatograph by means of a jet separator was used. The mass spectrometer was interfaced with a Model 6100 computer-controlled data acquisition system. A 1.5 m × 0.4 cm i.d. glass column packed with 3% Carbowax 20M coated on 80–100 mesh Chromosorb WHP was used for gas chromatographic separation. The mass spectra were recorded at 70 eV. A synthetic mixture of the suspected metabolites was prepared from the reference compounds and the mass spectra obtained. The soil and plant samples were analyzed under identical conditions and the mass spectra of the metabolites were compared with those of the reference compounds.

RESULTS AND DISCUSSION

The gas chromatographic response of a synthetic mixture of atrazine and metabolites is shown in Figure 1. The column separated all the compounds with good resolution. Dealkylated analogues of hydroxyatrazine were converted to the corresponding methoxy derivatives prior to gas chromatography. Under the GC conditions described, the compounds gave a 50% full-scale deflection (0.5 fsd) in the 4.7- to 22.5-ng range (Table I).

The performance of the method was tested for the determination of residues in soil samples at 0.01, 0.05, or plant samples at 0.1 ppm levels. Recoveries from the fortified soil, shoot, and root samples ranged from 68 to 96, 69 to 76, and 69 to 78%, respectively (Table II). The recoveries of hydroxyatrazine and its dealkylated analogues were low due to poor efficiency of the methylation. Preliminary experiments showed that hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine were converted to methoxy analogues in 50 to 80% yield. Thus, the data reported for residues of hydroxy metabolites should only be regarded as qualitative. However, it should be pointed out that in preliminary experiments little loss of methoxy analogues was observed during the extraction and column clean-up procedures.

Gas chromatographic analyses of extracts of experimental samples showed peaks having retention times identical with those of reference standards. Typical gas chromatograms of extracts of soil, shoot, and root samples are shown in Figure 2. A few unknown peaks appeared in the chromatograms but they did not interfere with the peaks of atrazine or metabolities. The identities of the compounds in the extracts were confirmed by cochromatography with authentic standards and finally by GC–MS analysis. A GC–MS of the peak with retention

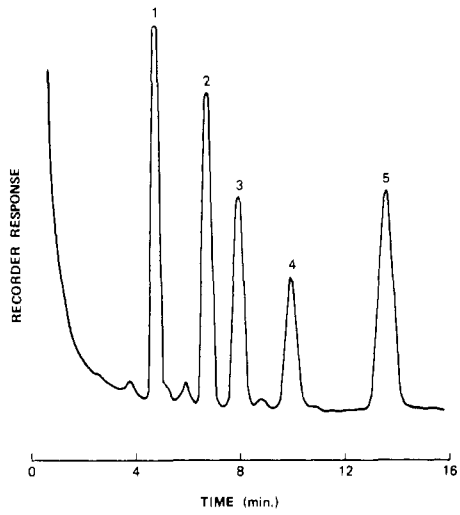

Figure 1. Gas chromatograms of: (1) atratone; (2) atrazine; (3) deethylatratone; (4) deisopropylatratone; and (5) deethylatrazine.

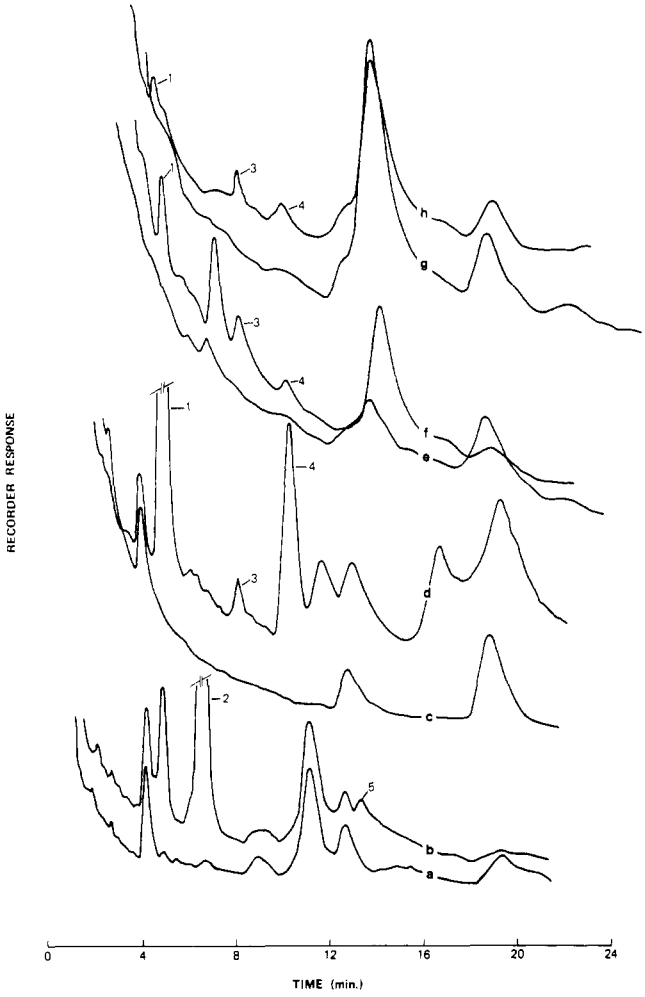

Figure 2. Gas chromatograms of extracts of field treated soils (1973 samples) and oat plant tissues (after hydrolysis) grown in this soil in the greenhouse: (a) check soil (chloroform eluate); (b) treated soil (chloroform eluate); (c) check soil (methanol eluate); (d) treated soil (methanol eluate); (e) check shoots; (f) treated shoots; (g) check roots; and (h) treated roots.

time of 6.7 min (peak 2, Figure 2) observed in the gas chromatograms of soil extracts showed a molecular ion at m/e 215, a chlorine isotopic peak ($M^{+\cdot}$ + 2), a base peak at m/e 200 ($M^{+\cdot} - CH_3$), and an ion at m/e 173 ($M^{+\cdot} - CH_3CH{=}CH_2$). The spectrum was consistent with the

Table III. Residues of Atrazine and Metabolites in a Sandy Loam Soil from Field-Treated Plots

Compound	Residues, ppm[a] 1973	1974
Atrazine	0.025	0.011
Deethylatrazine	T[b]	
Hydroxyatrazine	0.220	0.088
Deethylhydroxyatrazine	0.018	0.011
Deisopropylhydroxyatrazine	0.183	0.190

[a] Oven-dry basis. [b] <0.005 ppm.

mass spectrum of authentic atrazine. The compound represented by a GC peak at a retention time of 13.6 min (peak 5, Figure 2) showed a molecular ion at m/e 187, a chlorine isotopic peak ($M^{+\cdot}$ + 2), a base peak at m/e 172 ($M^{+\cdot} - CH_3$), and an ion at m/e 145 ($M^{+\cdot} - CH_3CH{=}CH_2$). The mass spectrum of this compound was identical with that of authentic deethylatrazine. The mass spectra of compounds represented by GC peaks at retention times of 4.7, 7.9, and 9.9 min (peaks 1, 3, and 4, Figure 2) exhibited molecular ions at m/e 211, 183, and 169, respectively. Furthermore, the molecular ions decomposed with loss of CH_3 ($M^{+\cdot} - 15$), $CH_3CH{=}CH_2$ ($M^{+\cdot} - 42$), and C_2H_4 ($M^{+\cdot} - 28$) to give the m/e 196 and 169, m/e 168 and 141, and m/e 154 and 141 ions, respectively. Further fragmentation was analogous to that observed for authentic 2-methoxy derivatives of hydroxyatrazine, deethylhydroxyatrazine, and deisopropylhydroxyatrazine. In view of the foregoing, compounds represented by peaks 2, 5, 1, 3, and 4 (Figure 2) in the gas chromatograms of extracts from soil, shoot, and root samples were identified as atrazine, deethylatrazine, atratone, deethylatratone, and deisopropylatratone, respectively.

The possible reactions involved in the metabolism of atrazine in soil are summarized in Figure 3. The metabolites of atrazine (I) are the hydroxylated analogue of I, namely hydroxyatrazine (II) partially N-dealkylated intermediates of I and II, including III, IV, V, and VI, further N-dealkylation of which will lead to the formation of compounds VII and VIII. Both of these compounds may then undergo side chain modification, deamination, or ring cleavage reactions with the oxidation of the final product resulting in the liberation of CO_2. In the present study residues of atrazine (I) and metabolites II, III, V, and VI were found to be present in the orchard plot soil (Table III) even though the samples were taken 2 and 3.5 years after the last application of the herbicide. The concentration of metabolites II and VI was considerably greater than the parent compound I. Beynon et al. (1972) also observed that the breakdown of atrazine (I) in a soil treated at 1.5 kg/ha in the greenhouse was mainly by hydrolysis resulting in the formation of II, together with small amounts of dealkylated compound IV and VII. Sirons et al. (1973) noted that atrazine (I) applied in the field was converted to III as a major and IV as a minor phytotoxic metabolite. These workers pointed out that isolation and identification of hydroxy analogues in soil was hindered due to analytical difficulties. Whether atrazine in soil is first hydroxylated followed by dealkylation or vice versa has not been resolved. However, results of this study indicated that both reactions were partially active as evidenced by the presence of residues of metabolites containing both 2-chloro and 2-hydroxy moieties (Table III). It is also evident that metabolic dealkylation occurred on either of the alkylamino groups. The subsequent dealkylation of III and V or VI would be expected to result in the formation of VII and VIII, respectively. The existence of such compounds has been

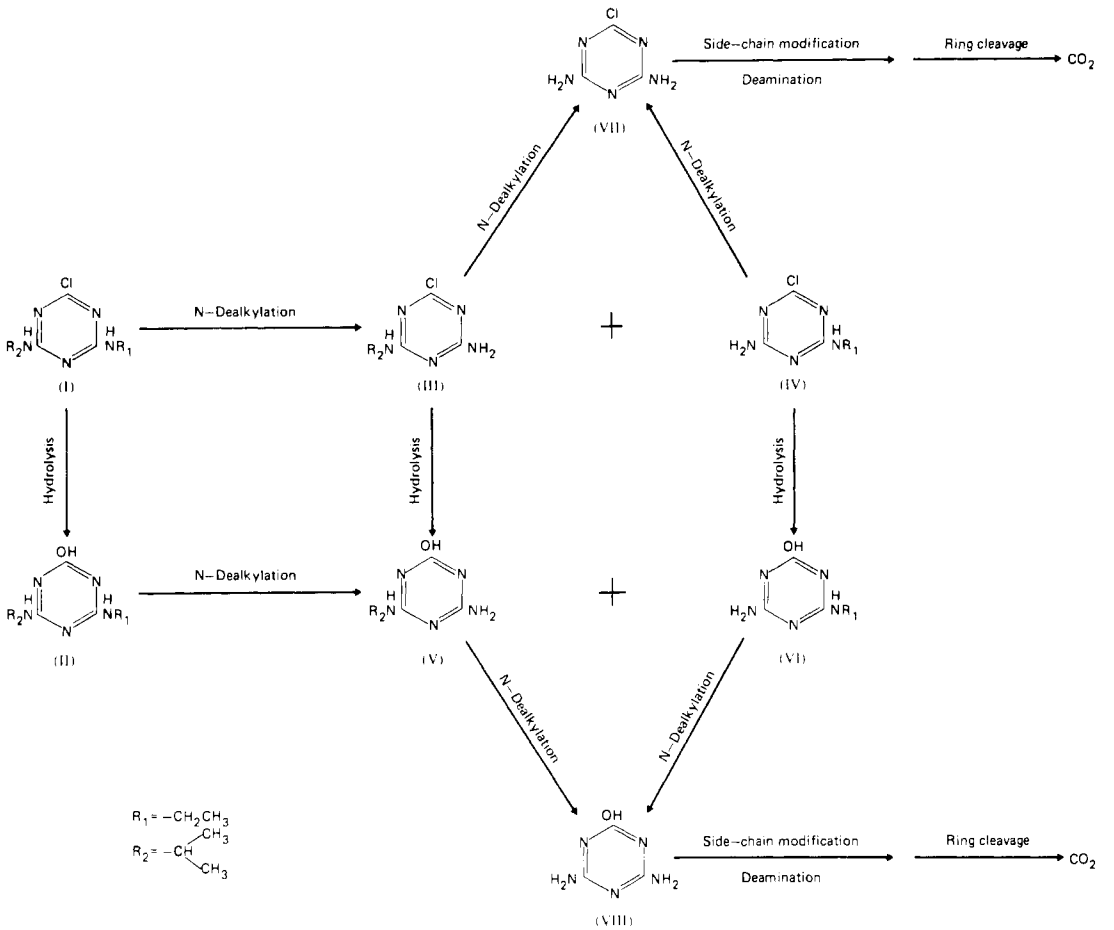

Figure 3. The possible breakdown products of atrazine in soil.

Table IV. Weights of Shoots and Roots, and Residues of Atrazine and Metabolites in Oat Plants Grown in the Field-Treated and Fortified Control Soils in the Greenhouse

Soil	Fortification with hydroxyatrazine, ppm	Fresh weight[a] of shoots, g	Dry weight[a] of shoots, g	Dry weight[a] of roots, g	Residues, ppm: Shoots (fresh weight basis) Hydroxyatrazine	Deethylhydroxyatrazine	Desisopropylhydroxyatrazine	Roots (dry weight basis) Hydroxyatrazine	Deethylhydroxyatrazine	Desisopropylhydroxyatrazine
Control (1973)		1.80	0.28	0.30						
Field treated (1973)		1.96	0.26	0.42	0.564		0.409	1.030	0.634	0.653
Control (1974)		1.91	0.26	0.33						
Field treated (1974)		1.74	0.24	0.35	0.193		0.392	0.464	0.312	0.660
Control (1973)	0.5	2.15	0.26	0.39	0.236			0.195		
Control (1973)	1.0	2.15	0.30	0.43	0.330			0.670		
Control (1973)	2.0	2.10	0.30	0.40	0.514			0.950		

[a] Average of six replicates.

reported in the soil (Beynon et al., 1972; Ramsteiner et al., 1972; Esser et al., 1975). However, degradation of these metabolites in soils have been found to occur very rapidly (Wolf and Martin, 1975). It should also be pointed out that the detection of low levels of VII and VIII residues in soils can be hindered due to a poor response of the compounds on the AFID detector used in this study. Under the GC conditions used 40 and 25 ng of compounds VII and VIII (methoxy analogue) gave 50% full scale deflection (0.5 fsd), respectively. The corresponding value for atrazine (I) was 4.7 ng (Table I).

In the previous study (Marriage et al., 1975) analysis of soil samples from orchard plots taken after the last application of the herbicide in 1971 showed a rapid decrease in atrazine residues and the levels were low after 5 months (<0.40 ppm). However, no attempt was made to determine the residues of atrazine breakdown products in the soil. The data presented in Table III clearly demonstrate that

disappearance of atrazine in the treated orchard soil resulted in the formation of various metabolites some of which persisted over a number of years.

Atrazine (I) or its hydroxy analogue (II) absorbed by plants from nutrient solution under controlled conditions have been shown to be metabolized and detoxified via 2-hydroxylation, N-dealkylation, and gluthione conjugation (Shimabukuro, 1967; Shimabukuro et al., 1970; Ashton and Crafts, 1973). However, very little consideration has been given to the uptake of metabolites actually present in the field-treated soil. The greenhouse experiment in the present study was primarily designed to determine the uptake of residues by oat plants from the orchard soil sampled in 1973 and 1974. Oats was chosen as it does not readily degrade *s*-triazines (Ashton and Crafts, 1973). The data indicate that residues of II were absorbed by oat plants grown in the fortified soils (Table IV). The compound was present in the shoot and root samples in the conjugated form as no free hydroxyatrazine was detected in eluate III obtained by eluting XAD-2 column with 100% methanol and was released only after the hydrolysis of the latter (eluate IV). Similarly, metabolites II, V, and VI were absorbed by oat plants grown in treated orchard soils sampled in 1973 and 1974. All of these metabolites were also present in plant tissues in the form of conjugates and were only released in the extracts after hydrolysis. In this study no attempt was made to determine the nature of the conjugates. There was no atrazine or deethylatrazine in shoots or roots of plants grown in soil sampled in 1973 and their absence indicates that either the compounds were not absorbed or that they were hydroxylated after their uptake.

The results of this study show that the mechanism of atrazine metabolism in soil involves hydrolysis and N-dealkylation reactions. The metabolites may persist in soil for a considerable length of time after the cessation of long-term application of high rates of the herbicide. The investigation indicates that residues of metabolites can be absorbed by oat plants grown in the treated soil and are subject to conjugation in plant tissues. It is logical to suggest that even where atrazine is applied on an annual basis in corn, atrazine degradation products may persist beyond the growing season and be absorbed by various crops planted the following year.

ACKNOWLEDGMENT

The skilled technical assistance of H. Lie is much appreciated. The mass spectrometric analysis by S. I. M. Skinner is gratefully acknowledged. Atrazine and its metabolites used as reference standards in this study were gifts from Ciba-Geigy Limited, Switzerland.

LITERATURE CITED

Armstrong, D. E., Chesters, G., Harris, R. F., *Soil Sci. Soc. Am., Proc.* **31**, 61 (1967).
Armstrong, D. E., Chesters, G., *Environ. Sci. Technol.* **2**, 683 (1968).
Ashton, F. M., Crafts, A. S., "Mode of Action of Herbicides", Wiley, New York, N.Y., 1973, pp 310–346.
Best, J. A., Webber, J. B., *Weed Sci.*, **22**, 364 (1974).
Beynon, K. I., Stoydin, G., Wright, A. N., *Pestic. Biochem. Physiol.* **2**, 153 (1972).
Burnside, O. C., Fenster, C. R., Wicks, G. A., Drew, J. V., *Weed Sci.* **17**, 241–245 (1969).
Burnside, O. C., Fenster, C. R., Wicks, G. A., *Weed Sci.* **19**, 290–293 (1971).
Dupuis, G., Laanio, T., Marbach, P., Esser, H. O., *Int. Congr. Plant Protect., 7th, Abstr.*, 711 (1970).
Esser, H. O., Dupuis, G., Ebert, E., Marco, G. J., Vogel, C., in "Herbicides", Vol. 1, Kearney, P. C., Kaufman, D. D., Ed., Marcel Dekker, Inc., New York, N.Y., 1975, pp 129–208.
Fryer, J. D., Kirkland, K., *Weed Res.* **10**, 133 (1970).
Harris, C. I., *J. Agric. Food Chem.* **15**, 157 (1967).
Kaufman, D. D., Blake, J., *Soil Biol. Biochem.* **2**, 73 (1970).
Marriage, P. B., Saidak, W. J., Von Stryk, F. G., *Weed Res.*, **15**, 373 (1975).
Obien, S. R., Green, R. E., *Weed Sci.* **17**, 509 (1969).
Ramsteiner, K. A., Hörmann, W., Eberle, D., *Z. Pflanzenkr. (Pflanzenpathol.) Pflanzenschutz, Sonderheft*, **6**, 43 (1972).
Shimabukuro, R. H., *Plant Physyiol.* **42**, 1269 (1967).
Shimabukuro, R. H., Swanson, H. R., Walsh, W. C., *Plant Physiol.* **46**, 103 (1970).
Sirons, G. J., Frank, R., Sawyer, T., *J. Agric. Food Chem.* **21**, 1016 (1973).
Skipper, H. D., Gilmour, C. M., Furtick, W. R., *Soil Sci. Soc. Am. Proc.*, **31**, 653 (1967).
Skipper, H. D., Volk, V. V., *Weed Sci.* **20**, 344 (1972).
Wolf, D. C., Martin, J. P., *J. Environ. Qual.* 4, 134 (1975).
Zimdahl, R. L., Freed, V. H., Montgomery, M. L., Furtick, W. R., *Weed Res.* **10**, 18 (1970).

Received for review February 22, 1977. Accepted June 21, 1977. This paper is Contribution No. 967.

Herbicidal Activity of Novel Acrylamides

Tetsuji Iwasaki, Norioki Miyamoto, Tsuneyuki Takeno,* Mariko Oiwa, and Kyozaburo Tachibana

A novel family of compounds, *cis*-β-alkylsulfenyl-, *cis*-β-alkylsulfinyl-, and *cis*-β-alkylsulfonylamide, were prepared and screened for their herbicidal activities. *N*-*n*-Propyl-*cis*-β-*n*-butylsulfinylacrylamide was highly active against both crabgrass *Digitaria adscendens* and pigweed *Amaranthus ascendens*, activity being a function of chain length attached to the sulfur atom.

The intensive studies on the antimicrobial activities of the β-keto acrylic acids have been conducted owing to the similarity of their chemical structures to penicillic acids, which are well known as an antibiotic (Cumper and Walker, 1956; Hellström, 1957; Kirchner et al., 1949; Omura et al., 1974; Papa et al., 1948; Price and Oae, 1962; Walton, 1957). In view of the isoelectronic nature of the sulfinyl and carbonyl groups (Birkinshaw et al., 1936), we synthesized a number of β-alkylsulfenyl-, β-alkylsulfinyl-, and β-alkylsulfonylacrylic acids and their derivatives, including the esters and the amides, with the hope of finding a novel class of antimicrobial compounds. In the

Biological Research Center, Kao Soap Co., Ltd., Wakayama-shi 640, Japan.