drolysis, hydroxylation, and oxidation, followed by conjugation.

Based on the metabolic profiles obtained with five species, the metabolic pathways found with mammalian systems (Sullivan et al., 1972a,b) are operative also in fish species. The most significant conjugated metabolite found in all fish species studied had chromatographic characteristics of 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide. Hydrolysis studies by β-glucuronidase of this fraction obtained from the kissing gourami had shown that this peak may have contained the glucuronide of the 3,4 isomer also.

LITERATURE CITED

Chin, B. H., Eldridge, J. M., Sullivan, L. J., *Clin. Toxicol.* 7, 37 (1974).
Chin, B. H., Eldridge, J. M., Anderson, J., Sullivan, L. J., *J. Agric. Food Chem.*, in press (1979).
Creaven, P. J., Davies, W. H., Williams, R. T., *Life Sci.* 6, 105 (1967).
Creaven, P. J., Parke, D. V., Williams, R. T., *Biochem. J.* 96, 879 (1965).
Dorough, H. W., Casida, J. E., *J. Agric. Food Chem.* 12, 294 (1964).
Knaak, J. B., *Bull. World Health Org.* 44, (1971).
Knaak, J. B., Tallant, M. J., Bartley, W. J., Sullivan, L. J., *J. Agric. Food Chem.* 13, 537 (1965).
Sullivan, L. J., Chin. B. H., Carpenter, C. P., *Toxicol. Appl. Pharmacol.* 22, 161 (1972a).
Sullivan, L. J., Eldridge, J. M., Knaak, J. B., Tallant, M. J., *J. Agric. Food Chem.* 20, 980 (1972b).
Trowell, O. A., *Exp. Cell Res.* 16, 118 (1959).

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Uptake of Glyphosate and *N*-Nitrosoglyphosate from Soil by Oat Plants

Absorption of glyphosate and *N*-nitrosoglyphosate from soil treated with high rates of these compounds and their translocation in oat plants was observed in a greenhouse experiment. *N*-Nitrosoglyphosate moved more readily into the root and subsequently into the shoot of oat plants than glyphosate. Formation of *N*-nitrosoglyphosate in soil and its uptake by plants under normal field conditions is not expected.

The herbicide glyphosate [*N*-(phosphonomethyl)glycine] provides effective control of most herbaceous perennial weeds through postemergence application (Spurrier, 1973). For this purpose it is applied to weed foliage at rates up to 4.2 kg/ha, often as a spot treatment. Formation of the *N*-nitroso derivative of glyphosate in various nitrite-treated soils under in vitro conditions has been recently demonstrated in the laboratory (Khan and Young, 1977). The *N*-nitrosoglyphosate formed was persistent in soil up to about 4 months. The formation of the *N*-nitroso derivative of glyphosate in soil at average recommended rates of application is not expected under normal field conditions due to the high rates of glyphosate and nitrite required for its production. However, in instances when nitrite accumulates temporarily in high concentrations (Chapman and Liebig, 1952), the use of exceptionally high levels of glyphosate raises the possibility of the herbicide undergoing N-nitrosation in soil (Khan and Young, 1977) and being subsequently taken up by plants. The herbicide glyphosate can be taken up by plant roots and translocated to the shoots (Sprankle et al., 1975). It has been shown that *N*-nitrosodimethylamine is stable in soil (Tate and Alexander, 1975, 1976) and can be translocated into vegetable crops (Dean-Raymond and Alexander, 1976).

The investigation reported here is an extension of the study on possible formation of *N*-nitroso derivatives of pesticides in soil. Its purpose was to investigate whether uptake of *N*-nitrosoglyphosate by plants would occur from soil and to compare this uptake with that of glyphosate.

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade and used as received. Glyphosate, as its isopropylamino salt (96.7% purity), was obtained from Monsanto Commercial Products Co. (St. Louis, MO).

Synthesis of *N*-Nitrosoglyphosate. To a stirred solution of 1.157 g of glyphosate isopropylamine salt in 50 mL of 0.12 N hydrochloric acid was added 3 g of sodium nitrite. The stirring was carried out in the dark overnight. The *N*-nitrosoglyphosate thus prepared (yield = 1.0 g) was kept in the dark and refrigerated when not in use. The purity of the compound was checked by its TLC properties and UV spectra (Young et al., 1977). Many *N*-nitrosamines are potent carcinogens. Although the carcinogenic properties of *N*-nitrosoglyphosate are unknown at this time, safety precautions such as those outlined in the National Cancer Institute Safety Standards for Research Involving Chemical Carcinogens to prevent skin contact and inhalation must be exercised at all times.

Soil Treatment and Greenhouse Experiment. (*a*) *Preliminary Experiment.* Fox sandy loam soil (300 g), previously steam sterilized to prevent microbial herbicide degradation, was spread evenly in individual 21 × 29 cm plastic trays. The soil was sprayed with successive applications of glyphosate solution (1% AI) to achieve four replicates of soil concentrations of 25–625 ppmw. The

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Table I. Effect of Glyphosate and *N*-Nitrosoglyphosate on Shoots and Roots Yield (g) of Oat Plants Grown in the Treated Soil[a]

treatment level, ppmw	glyphosate		*N*-nitrosoglyphosate	
	root	shoot	root	shoot
0	1.94 ± 0.06	1.73 ± 0.09	1.94 ± 0.06	1.73 ± 0.09
5	2.19 ± 0.10	1.81 ± 0.15	2.25 ± 0.34	1.78 ± 0.08
10	2.02 ± 0.03	1.66 ± 0.05	2.76 ± 0.07	1.65 ± 0.10
25	1.91 ± 0.13	1.81 ± 0.11	1.92 ± 0.12	1.73 ± 0.07
50	2.55 ± 0.42	1.59 ± 0.08	1.82 ± 0.10	1.78 ± 0.12
100	2.16 ± 0.16	1.36 ± 0.09	1.87 ± 0.12	1.76 ± 0.09
200	1.78 ± 0.01	0.16 ± 0.05	1.72 ± 0.14	1.68 ± 0.08
300	1.27 ± 0.11	0.08 ± 0.04	1.46 ± 0.03	1.19 ± 0.02

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

samples were dried overnight, mixed thoroughly, and transferred to small aluminum pie plates (9-cm diameter) with perforated bottoms. Fourteen oat (*Avena sativa* L.) seeds (cultivar Gary) were planted at the soil surface and after germination were thinned to ten uniform size plants. Plates were watered daily by shallow subirrigation and after 2 weeks roots and shoots were removed. The roots were washed in distilled water and surface dried. The fresh weight of roots and shoots was determined. The experiment was conducted in a greenhouse with temperatures 25–30 °C (day) and 20 °C (night). Supplemental light was provided to give a day length of approximately 15 h.

(*b*) *Final Experiment.* A bioassay similar to the preliminary experiment was carried out with soil concentrations of glyphosate and *N*-nitrosoglyphosate from 5 to 300 ppmw. The experimental details were similar to those described in the previous section except that instead of spraying, 25 mL of appropriate solutions were applied over the surface of the 300 g of soil in large polyethylene bags.

Extraction and Analysis. Fresh root or shoot samples were ground to a paste in 5 mL of water and transferred to a beaker. The material was boiled with 150 mL of water for 1 h and filtered while hot. The sample residue was washed with 100 mL of water. The combined filtrate was concentrated to about 10 mL on a rotary evaporator and washed three times with 40-mL portions of methylene chloride. The aqueous phase was then used for the analysis of glyphosate or *N*-nitrosoglyphosate.

(*a*) *Glyphosate.* The aqueous phase was treated with sodium nitrite at pH 3.0 to prepare the *N*-nitroso derivative, followed by column and thin-layer chromatography and finally fluorescence detection as described by Young et al. (1977).

(*b*) *N-Nitrosoglyphosate.* The aqueous phase was evaporated to near dryness under reduced pressure and the residue dissolved in 20% water in acetonitrile. The material was transferred to a 6 × 1 cm Florisil column (60–100 mesh, PR grade, moisture content 0.8%, 3 g, prewashed with 20% water in acetonitrile). The column was first eluted with 60 mL of 20% water in acetonitrile and then with 60 mL of 60% water in acetonitrile. The first eluate was discarded and the second eluate was evaporated to near dryness. The material was dissolved in 2–5 mL of 50% water in methanol. Aliquots containing *N*-nitrosoglyphosate and an aliquot of the sample were spotted on Quantum LK6D (250-μm thickness) TLC plates developed in 95% ethanol–benzene–water (4:1:1), dried, irradiated for 20 min with UV light. The concentration of *N*-nitrosoglyphosate was determined as described by Young et al. (1977). The identity of *N*-nitrosoglyphosate was confirmed by comparison of TLC properties with those of the authentic compounds using different solvent systems for chromatogram development (Young et al., 1977).

Performance of the Method. Shoot and root samples were obtained from oat plants grown in untreated soil. The shoot or root sample (3.0 g) was ground to a paste and fortified with glyphosate or *N*-nitrosoglyphosate at 5, 10, 25, and 80 ppm levels. The samples were processed as above and recoveries determined.

RESULTS AND DISCUSSION

Both experiments indicated severe injury of glyphosate to plants at a soil concentration of 300 ppmw or higher with shoots failing to develop. From 50 to 300 ppmw, increasing visible effects were observed including stunting of plants, leaf folding, and chlorotic striping of leaves. No injury was observed below 50 ppmw concentration. Glyphosate was more phytotoxic than *N*-nitrosoglyphosate to oat plants grown in the treated soil (Table I). Effects of *N*-nitrosoglyphosate were similar to those observed with glyphosate but did not occur until 200–300 ppmw. Shoot weight was reduced with increased soil concentrations of glyphosate and *N*-nitrosoglyphosate, reflecting the severity of observed response. The reduction in weight of roots tended to be much less severe than that of shoots in oat plants treated with glyphosate. The recoveries from the fortified shoot and root samples are shown in Table II. While the recovery data are not a true indication of extraction efficiency as applied to field treated samples, it does show that little loss of the chemical was experienced during the working procedure.

Earlier studies have demonstrated that glyphosate can be absorbed from soil and translocated into plants (Sprankle et al., 1975). Present data demonstrate that *N*-nitrosoglyphosate is not strongly retained by the soil but moves more readily into the root and shoot of oat plants than glyphosphate (Table III). Furthermore, *N*-nitrosoglyphosate was detected in roots at 5 ppmw or higher soil concentrations but was not detected in the shoot until the soil concentration reached 25 ppmw. Glyphosate was found in both roots and shoots of oat plants at 50 ppmw, the same concentration at which very slight visible effects occurred. Glyphosate amounts in roots and shoots from the preliminary experiment were similar to those in Table III at corresponding concentrations. The amount of either compound remaining unabsorbed on the root surface should be low as a result of soil rather than hydroponic uptake and the water solubilities of the compounds.

The formation and stability of *N*-nitroso pesticides in soil and their uptake plants has been the subject of several recent investigations. *N*-Nitrosodimethylamine has been shown to be stable in soil (Tate and Alexander, 1975, 1976) and can be translocated from soil into vegetable crops (Dean-Raymond and Alexander, 1976). Our earlier findings showed that *N*-nitrosoglyphosate can be formed and

Table II. Recoveries (%) of Glyphosate and *N*-Nitrosoglyphosate from Fortified Root and Shoot Samples[a]

sample	glyphosate added, ppmw[b]				*N*-nitrosoglyphosate added, ppmw			
	5	10	25	80	5	10	25	80
shoot	76.3 ± 5.7	83.0 ± 4.3	85.0 ± 3.6	92.2 ± 3.0	77.7 ± 6.1	83.2 ± 2.2	87.6 ± 2.0	89.9 ± 2.5
root	77.5 ± 4.8	83.8 ± 4.5	87.0 ± 5.8	96.5 ± 1.7	78.8 ± 4.4	81.5 ± 3.8	84.0 ± 4.9	97.8 ± 1.4

[a] Mean values for triplicate samples with standard errors. [b] Fresh-weight basis.

Table III. Residues (ppmw on Fresh Weight Basis) of Glyphosate and N-Nitrosoglyphosate in Roots and Shoots of Oat Plants Grown in the Treated Soil[a]

treatment level, ppmw	glyphosate		N-nitrosoglyphosate	
	root	shoot	root	shoot
0	ND[b]	ND	ND	ND
5	ND	ND	4.9 ± 0.2	ND
10	ND	ND	9.1 ± 0.2	ND
25	4.8 ± 0.3	ND	21.3 ± 0.9	4.4 ± 0.2
50	8.6 ± 0.7	1.4 ± 0.1	40.3 ± 1.5	7.9 ± 0.6
100	17.0 ± 0.9	3.9 ± 0.1	72.7 ± 5.2	15.4 ± 0.5
200	39.1 ± 1.9	10.1 ± 0.3	135 ± 7.6	25.1 ± 1.1
300	59.8 ± 4.6	16.8 ± 1.0	213 ± 5.8	45.6 ± 1.2

[a] Mean values for triplicate samples with standard errors. The data are not corrected for recovery. [b] Not detected.

is persistent in soil (Khan and Young, 1977). The data presented in this paper indicate that N-nitrosoglyphosate can be assimilated by the roots of oat plants and translocated to the shoots.

Whether the nitrosation of glyphosate in soil to form N-nitrosoglyphosate will occur under natural conditions is still a matter of conjecture. However, based on the previous study (Khan and Young, 1977) we do not expect the formation of detectable amounts N-nitrosoglyphosate in soil under normal field conditions. It was observed that high concentrations of the herbicide glyphosate and nitrite were essential to get measurable amounts of N-nitrosoglyphosate in soil, which amounts in turn were considerably lower than the concentrations of N-nitrosoglyphosate used in this study. Even though soil concentrations were extremely high, the observations that N-nitrosoglyphosate can be taken up by plants should prompt further research to determine whether such a possible hazard is in fact a reality with other pesticides.

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LITERATURE CITED

Chapman, H. D., Liebig, G. F., *Soil Sci. Soc. Am. Proc.* **16**, 276 (1952).
Dean-Raymond, D., Alexander, M., *Nature (London)* **262**, 394 (1976).
Khan, S. U., Young, J. C., *J. Agric. Food Chem.* **25**, 918 (1977).
Sprankle, P., Meggitt, W. F., Penner, D., *Weed Sci.* **23**, 224 (1975).
Spurrier, E. C., *Pans* **19**, 607 (1973).
Tate, R. L., Alexander, M., *J. Natl. Cancer Inst.* **54**, 327 (1975).
Tate, R. L., Alexander, M., *J. Environ. Qual.* **5**, 131 (1976).
Young, J. C., Khan, S. U., Marriage, P. B., *J. Agric. Food Chem.* **25**, 918 (1977).

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Identification of "Isodihydrolavandulol" by Application of Fourier Transform Nuclear Magnetic Resonance Spectroscopy to Gas Chromatography Eluates

"Isodihydrolavandulol" has been found to be a mixture of two major components. The FT-NMR spectra of each component were obtained on samples collected from a gas chromatograph. On the basis of the evidence from these spectra, the components were identified as 4-methyl-2-propyl-2-hexenol and 2-propyl-2-heptenol.

According to the literature (Arctander, 1969), "isodihydrolavandulol" is either 2-isopropyl-5-methyl-2-hexenol (1) or 2-isopropylidene-5-methylhexanol (2) (Figure 1). As shown in Figure 2a, "isodihydrolavandulol" is a mixture of two compounds whose identification is the subject of this communication.

The isomeric 2-hexenols have been described in the patent literature (Kallianos et al., 1972) as obtained from the base-catalyzed self-condensation of 3-methylbutyraldehyde, followed by sodium borohydride reduction of the unsaturated aldehydes. The mixture obtained in this manner consists predominantly of the cis and trans isomers of 1 and a small amount of 2-isopropyl-5-methyl-3-hexenol (3). As is discussed below, these structure assignments have been confirmed in this study. The gas chromatogram (Figure 2b) of this mixture was distinctly different from that of "isodihydrolavandol".

An unambiguous synthesis of 2-isopropylidene-5-methylhexanol (2) was carried out according to the scheme shown in Figure 3. As shown, the double bond was introduced regiospecfically by means of a Wittig–Horner reaction (Wadsworth and Emmons, 1961). The ^{1}H NMR spectrum of this alcohol (2) clearly showed the presence of the expected isopropylidene group; the gas chromatogram (Figure 2c) of this alcohol (2) did not coincide with either of the major peaks of "isodihydrolavandulol". Thus "isodihydrolavandulol" has neither of the structures by which it is commonly described.

The rapid development of Fourier transform NMR spectroscopy has made possible obtaining spectra on very small quantities of material. It appeared feasible to obtain usable spectra from samples collected from an analytical gas chromatograph. The details of the technique by which the spectra were obtained are presented in the Experimental Section; the interpretation of these spectra follows.

The structures of the three components of the mixture derived from 3-methylbutyraldehyde were confirmed by NMR spectroscopy. The proton and ^{13}C spectra of the two major components were consistent in all respects with cis and trans isomers of 2-isopropyl-5-methyl-2-hexenol (1).

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