Herbicide (Alachlor, Atrazine, Linuron and Paraquat) Residues in Deer Mice Inhabiting Conventional and Minimum Tillage Row-crop Fields

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The acreage of cropland in the United States incorporating conservation tillage methods has increased steadily from 14% in 1973 to over 24% in 1982 (12). During 1982, reduced tillage practices were utilized on 34% of Indiana's 13 million acres of cropland (10). Based primarily on economic advantages and improved technology, it is predicted that conservation tillage in some form will be used on 60% of the nation's cropland by the year 2010 (12).

Although a variety of practices qualify as reduced or conservation tillage, all have in common less disturbance to the soil with greater amounts of crop residues left on the soil surface. In most situations, chemical control of weeds substitutes for mechanical tillage. With minimum tillage (also referred to as no-till and zero tillage), weed control is solely by herbicides, and chemical applications and planting can be combined in the same operation.

The environmental consequences of this shift in agricultural practice are just beginning to be explored. With minimum tillage, soils are less prone to compaction and soil loss on sloped land can be reduced by as much as 90% (12). Besides maintaining soil productivity, reduced soil erosion should result in decreased siltation of waterways and decreased air-borne soil particles. Benefits to wildlife from conservation tillage have also been envisioned and recent studies bear this out (11, 23, 25).

A potentially detrimental impact of conservation tillage practices is the greater use of chemical pesticides. With reduced tillage, more vegetation residue remains on the soil surface interfering with herbicide incorporation. Thus, chemical application rates may need to be increased to maintain their effectiveness. Contact herbicides, such as paraquat and glyphosate, are unique to minimum tillage operations and should be the focus of research attention.

The purpose of this study was to determine if 4 commonly used herbicides or their metabolites could be detected in deer mice (*Peromyscus maniculatus*), the most common inhabitant of cultivated cropland in Indiana and much of the Midwest (17). A secondary objective was to determine if these herbicides may be having detrimental physiological effects on this rodent under natural field conditions. Alachlor, atrazine, and linuron were chosen because they are used extensively in the production of corn and soybeans, both in conventional and minimum tillage situations. Paraquat was chosen because it is the major contact herbicide used in zero tillage practices. Although these chemicals have been tested extensively on laboratory rodents and birds, few studies have examined the impacts of agricultural chemicals on wildlife species under natural field conditions (21).

Materials and Methods

Deer mice were taken from corn and soybean fields using snap traps baited with peanut butter and oats. All fields were commercially farmed and information about

TABLE 1. Agricultural pesticide use and deer mice trapped from cultivated fields in Scott County, Indiana, 1983. Capitalized chemicals are trade names.

Field	Chemical application rate (per acre)	Dates			Deer mice captured
		Spraying	Planting	Trapping	
Conventional corn					
CRA	alachlor (3 qts.) atrazine (2 lbs.) Amaze (7 lbs.)	1 Jun	2 Jun	13-14 Jul	3
LSm	atrazine (1.5 lbs.) butylate (4 qts.)	26 Apr	2 Jun	13-14 Jul	5
PFA	alachlor (2 qts.) atrazine (2 lbs.) carbofuran (15 lbs.)	9 Jun	9 Jun	19, 22 Jul	11
Conventional soybeans					
LAG	alachlor (3 qts.) linuron (2 qts.)	12 Jun	9 Jun	19, 22 Jul	14
HIG	alachlor (1 qt.) linuron (1 qt.)	1 Jun	25 May	14-15 Jul	22
No-till corn					
MON	alachlor (2 qts.) atrazine (2 lbs.) carbofuran (15 lbs.) paraquat (1.5 pts.)	1 Jun	19 May	3, 5 Aug	15
BRO-C	Bicep (3 qts.) carbofuran (9 lbs.) paraquat (2 qts.)	24 May	23 May	3 Aug	13
No-till soybeans					
BRO-S	Dual (1 qt.) linuron (0.5 qt.) paraquat (2 pts.)	3 Jul	3 Jul	3 Aug	5
KSm	alachlor (2 qts.) linuron (2 qts.) paraquat (1 pt.)	16 Jun	15 Jun	2 Aug	5

planting and spraying dates and chemicals used (Table 1) were obtained directly from farmers. Fields were located in Scott County of southeastern Indiana, where soils are primarily silt loams derived from glacial till. The topography is flat to moderately rolling.

Conventionally tilled corn and soybean fields had been plowed or disked in the spring before planting. No-till cornfields were slot-planted directly into the previous year's residues. A slot-planter uses a knife-like implement to make a narrow furrow in which the seed is deposited. No-till soybeans had been planted to winter wheat the previous fall, and were slot-planted with soybeans directly into residues following wheat harvest in early summer. More detailed crop histories are given elsewhere (6).

Deer mice were trapped over a 4-night period in each field, and mammals captured were individually bagged, labeled, frozen, and transported to laboratories at Indiana University Southeast. They were analyzed using thin-layer chromatographic (TLC) techniques described below. It was necessary to pool 3-6 mice to obtain enough material for each analysis. For histological analyses, mouse tissues were fixed in alcoholic formalin, and later dehydrated, cleared, and embedded in paraffin blocks. Sections obtained from these blocks were attached to slides, stained with H & E, and mounted with Permount. The sections were then visually scanned for evidence of histological abnormalities.

Alachlor (= Lasso)

Alachlor (2-chloro-2,6 diethyl-N-(methoxymethyl)-acetanilide) is a preemergent herbicide manufactured by the Monsanto Corporation for the control of annual grasses and certain broadleaf weeds in soybeans and corn. The concentrate most commonly available at retail outlets contains 4 lbs. of alachlor per gallon. Following application, the active ingredient persists in the soil for 6-10 weeks (5).

For alachlor, whole, skinned mice were homogenized in a blender and extractions obtained using Method 1A of the Pesticide Analytical Manual, Vol. II (19). The 2,6 diethylanilide residue obtained in this manner was concentrated "in vacuo" to 0.5 ml, subsequently dissolved into 10μl of chloroform, and spotted on fluorescent silica gel TLC plates. Using a solvent system of 4:1 benzene-ethyl acetate, principal yellow spots would appear at Rf 0.85 if alachlor was present in the tissue samples.

Atrazine (= AAtrex)

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a substitute s-triazine available from CIBA-GEIGY Corporation in several trade name formulations such as AAtrex 4L, is a selective herbicide utilized for season-long weed control in corn and other crops. The retail product contains 4 lbs. of atrazine per gallon.

For atrazine analysis, homogenized mouse livers were extracted with anhydrous methanol, and the resulting filtered solution was hydrolyzed with 1N HCl (23). Following separation into phases with the addition of n-hexane, the aqueous bottom layer was drawn off, neutralized with NH_4OH, and then evaporated on a rotavapor to 0.5 ml. The resulting residue was dissolved into 10μl of chloroform, spotted on fluorescent silica gel TLC plates, and developed in a solvent system consisting of 4:1 benzene-ethyl acetate. Atrazine, if present, would be located at the Rf value of 0.27. The limit of detection using this method was 0.03 ppm with approximately 70% recovery of parent material.

Linuron (= Lorox)

Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), distributed by DuPont for selective weed control, is retailed as Lorox Weed Killer, a wettable powder containing 50% linuron, and Lorox L Weed Killer, an aqueous suspension containing 41% linuron (13).

For these extractions, whole, skinned mice were homogenized in a blender, initially extracted with anhydrous ether, and hydrolyzed with 1N HCl (4). The resulting solution was then adjusted to a pH greater than 11 with NaOH, and from this solution, p-chloroaniline was re-extracted into a small volume of ether, dried with magnesium sulfates, and then evaporated almost to dryness with a rotavapor. The resulting concentrate was dissolved in 9:1 petroleum ether-acetone, spotted on fluorescent silica gel plates, and subsequently developed in the following solvent system: 35 ml methanol, 17.5 ml isoamylalcohol, 35 ml benzene, and 12.5 ml 2N HCl. If present, the p-chloroaniline derivative of linuron would appear at the Rf value of 0.85. The reported lower limit of detectability is 0.1 micrograms (4). Our recorded percent recovery of standard reference materials was 91.7%.

Paraquat

Paraquat (1,1'-dimethyl-4,4 dipyridilium dichloride), a non-selective contact herbicide, is distributed by Chevron as Paraquat CL containing 2 lbs. of the paraquat cation per gallon.

Whole, skinned, homogenized mice were extracted according to methods published in the Pesticide Analytical Manual, Vol. II (19). The filtered solution was then concen-

trated "in vacuo" to 0.5 ml, spotted on fluorescent silica gel plates, and developed in the following sequential solvent system which was designed specifically for the detection of paraquat and its metabolites (1). The TLC plate remained in Solvent A which consisted of 1:1:2:1 benzene-amyl alcohol-methanol-1N HCl for seven minutes and was then immediately placed in Solvent B which consisted of 40:9:1 acetonitrile-H_2O-ammonia until the solvent had traveled the entire length of the TLC plate. Paraquat, if present, remains at Rf 0.19. Related compounds have been reported at the following locations (1): QUINA-Rf 0.34, monopyridone-Rf 0.49, monoquat-Rf 0.54, and dipyridone-Rf 0.79.

Results and Discussion

A total of 76 deer mice was utilized in 17 assays for residues of the 4 herbicides (Table 2). Five assays indicated the presence of herbicides or their metabolites. Nine additional mice were histologically examined for lung and liver damage, and 2 individuals showed evidence of liver abnormalities (Table 2).

TABLE 2. Summary of herbicide residue determinations and histological examinations of deer mouse livers.

Herbicide	No. fields represented	No. mice sampled	Frequency of samples[a] with herbicide residues	Frequency of liver damage
Alachlor	6	32	2/7	0/4
Atrazine	3	18	0/4	0/2
Linuron	2	11	0/3	0/2
Paraquat	2	15	3/3[b]	2/3

[a]Pooled samples include 3-6 individual deer mice.
[b]Metabolites uncertain.

Alachlor

Alachlor residues were detected in mice from two fields, a conventionally tilled cornfield (CRA) and a no-till soybean field (KSm). UV spectrophotometric analyses of the sample residues revealed principal wavelength peaks at 234 nm. Using p-chloroaniline as a reference, the recorded residues were calculated to be 0.0003 ppm (CRA) and 0.0001 ppm (KSm). These recorded levels are extremely low and far below the reported sensitivity levels of 0.01 to 0.02 ppm for this compound (18).

Toxicology studies (5) of the effects of alachlor on rats indicated relatively high acute oral LD50s ranging from 100 mg/kg to 5800 mg/kg for various formulations of the retail product Lasso. Alachlor produced tumors in some laboratory mice when fed at levels greater than 260 mg/kg/day over the entire lifetime of the experimental animals (5). All 4 deer mice histologically examined from fields in which alachlor was used showed normal liver appearance. The presence of detectable alachlor in some individuals is unlikely to create an environmental problem due to the large dosages required to induce tumors. In conventional as well as reduced tillage fields, it is improbable that these levels would ever be approached.

Atrazine

Atrazine residues were not detected in any samples, nor did the livers of 2 mice examined appear abnormal. The acute oral toxicity of atrazine (AAtrex 4L) in rats was 1886 mg/kg in males and 1075 mg/kg in females (8). Besides this relatively high level of toxicity, atrazine is rapidly excreted from the body (8) posing little threat to nontarget rodents and their predators. Atrazine accounts for almost 25% of all herbicides applied to crops in the United States, and may be metabolically transformed

by both plants and animals into a mutagenic substance (12, 20). Atrazine has been shown to affect the behavior of rats by altering their circadian rhythms (18).

Linuron

Linuron was not detected in any tissue samples. Dupont (13) reported an acute oral LD50 for linuron of 1906 mg/kg for male mice and 2873 mg/kg for females. Linuron fed to mice at dietary levels of 50 and 150 ppm for two years produced no measurable chronic effects, but an extremely high dietary level of 1500 ppm produced hepatocellular adenomas in female mice (13). In a reproduction study, linuron produced a high incidence of deformed embryos at a feeding rate of 200 mg/kg (14). No general toxic, reproductive, or teratogenic effects were noted in a 3-generation rat study at a dietary level of 125 ppm (13).

Paraquat

In utilizing the sequential TLC procedure for the detection of paraquat and its metabolites, no immediate evidence of paraquat was found at Rf 0.19-0.21. However, identical streaks with an Rf range from 0.54-0.72 were noted on plates developed from a no-till corn (BRO-C) and a no-till soybean field (BRO-S) (Figure 1). In addition, the tissue extractions from the no-till cornfield produced plates with compact spots located at Rf 0.89. The known degradation products of paraquat falling in this range would be monoquat (Rf 0.54) and dipyridone (0.79). Considering that definite resolution was not obtained, it cannot be stated conclusively that these represent paraquat metabolites. The livers of 2 of 3 additional mice examined from both fields showed signs of damage, mainly changes in appearances of fatty cells which is a characteristic response of the liver to a wide variety of toxic compounds, including paraquat (24).

In contrast to the relatively low toxicities of the other herbicides considered in this study, paraquat is moderately toxic and known to damage epithelial tissues of the skin, nails, cornea, liver, kidney, the gastrointestinal tract, and the respiratory tract (24). Such injuries may be reversible in all but the lung where a severe pulmonary reaction to paraquat in often fatal (24). Intraperitoneal injections were toxic at 17-21 mg/kg in rats. Acute oral mammalian toxicities ranged from an LD50 of 5 mg/kg in hares (15) to 115 mg/kg in rats (24). This difference due to the mode of administration is attributed to poor absorption of paraquat through the gastro-intestinal tract (9). Parquat is also a strong skin irritant with a reported acute dermal LD50 of about 85 mg/kg in rats (24). Deer mice we examined displayed no skin lesions or loss of hair. Chronic administration of small doses of paraquat produced no clinical signs for several weeks (24). Thereafter, signs of illness developed in the form of anorexia, weight loss, and dyspnea. The animals usually died within 10 days of the onset of the symptoms (24). Paraquat has been noted to have many mutagenic and embryotoxic properties (3, 24). Additional gross and microscopic morphological changes arising from paraquat ingestion in rats included: loss of body weight, teratogenic effects in embryos, damage to the liver and kidney, lung weight increase and considerable pulmonary fibrosis, smaller spleen and thymus, heavier adrenals with abnormal histology, lowered white and red blood cell counts, degenerative changes in the testes, corneal opacification, and other changes as well (2, 24).

Conclusions

The lack of detectable concentrations of atrazine and linuron or their metabolites, the low frequency and extremely low concentrations of alachlor, and the absence of apparent liver damage in deer mice taken from fields in which these herbicides were used, indicate little cause for immediate environmental concern about their regulated

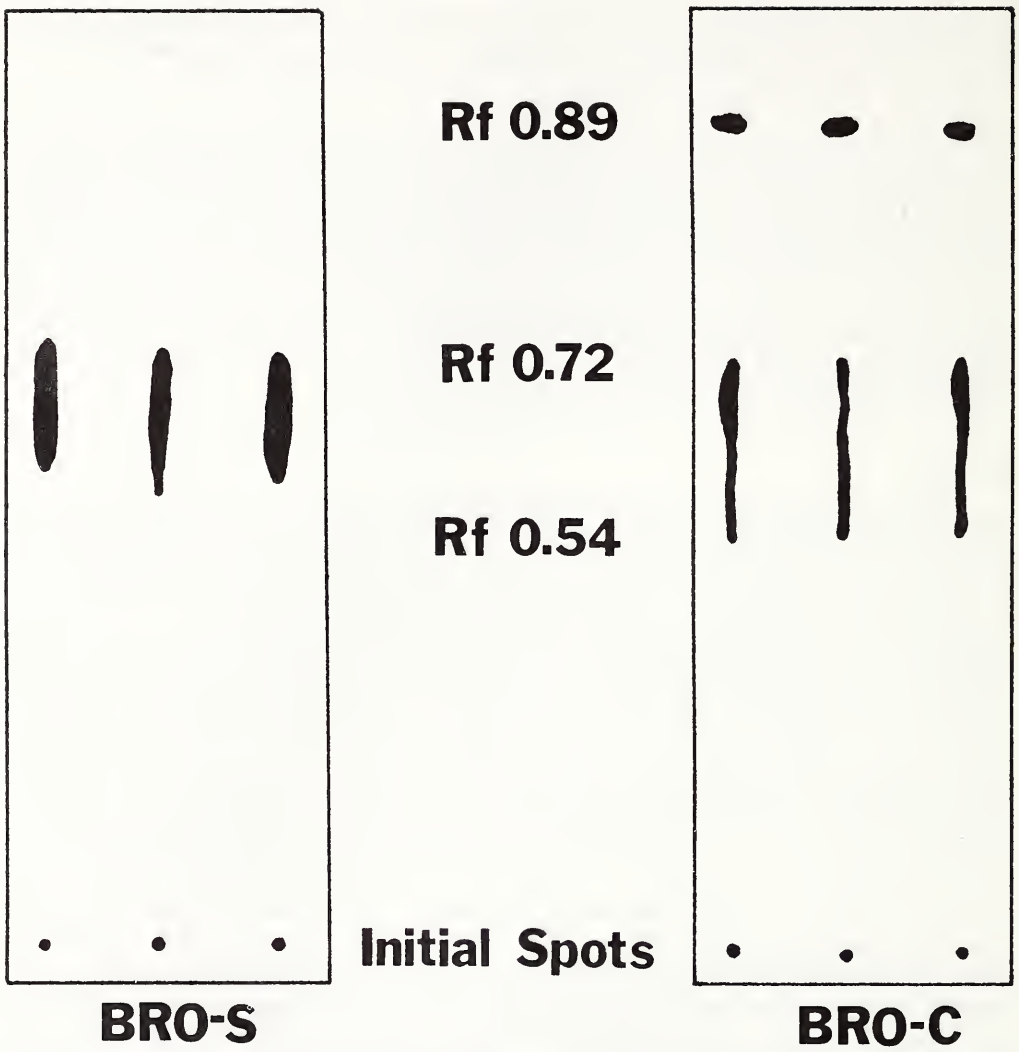

FIGURE 1. Tracings of TLC plates from tissue sample extractions of deer mice taken from 2 paraquat-treated fields (BRO-S, BRO-C) indicating metabolites of uncertain origin. Paraquat, if present, would have migrated directly above the initial spots.

use on agricultural lands. Unlike many insecticides, herbicides rarely persist i n the environment for more than a few days or weeks (16). The results for paraquat, however, suggest that metabolites may be present in deer mice 31-71 days after field application and may be responsible for observed liver damage. This could result in elevated rates of mortality in deer mouse populations in no-till fields, although population levels and short-term mortality rates were found to be similar in conventional and minimum tillage fields in southern Indiana (6, 7).

Further research is warranted to determine the exact origin of metabolites found in this study, as well as to obtain better estimates of the incidence of liver damage in deer mice inhabiting row-crop fields. It would seem prudent to encourage use of alternative contact herbicides (e.g., glyphosate) for no-till farming that may pose less risk.

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