Table VIII. Volatilization Half-Lives of Fluridone and Desphenylfluridone in Outdoor Ponds Using Equations of Mackay and Leinonen (1975)

compound	H, atm m^3 mol^a	$1/(KL)$, m/h^b	$t_{1/2}$, days
fluridone	1.0×10^{-6}	3537.4	50.8
desphenylfluridone	1.1×10^{-5}	290.5	4.2

[a] Henry's constant calculated as described by Mackay et al. (1979). [b] $1/(KL) = 1/k_L + RT/(Hk_G)$ (m/h^{-1}), where k_L = liquid transfer coefficient (m/h) and k_G = gas transfer coefficient (m/h), R = gas constant [m^3 atm/(mol K)], and T = K (298). For fluridone, $k_L = 0.073$ and $k_G = 7.017$ m/h; for desphenylfluridone, $k_L = 0.0834$ and $k_G = 8.002$ based on published values for H_2O and CO_2 transfer (Mackay et al., 1979).

reduced somewhat by the proportion of fluridone sorbed to bottom sediments, aquatic plants, and seston (Mackay et al., 1979).

The persistence of [*carbonyl*-^{14}C]fluridone in pond sediment was much shorter than that observed in sediment–water systems in the laboratory. Previous work with unlabeled fluridone (Muir et al., 1980) indicated half-lives of greater than 12 months under field conditions which was similar to that observed in laboratory studies. The timing of the herbicide application to the artificial ponds may be an important factor in explaining the differences between the field experiments. Previous field applications were made in mid July while the experiments with radiolabeled fluridone were started in mid-June which allowed an additional 4–6 weeks of high water temperatures and long daylight hours. The $t_{1/2}$ in water of fluridone applied in mid-June was about half of that observed in the mid-July treatment (3.5 vs. 7.0 days). While fluridone appeared to be quite persistent in light-restricted culture flask experiments, the susceptibility of the herbicide to photodegradation permitted relatively rapid disappearance under actual field conditions.

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Determination of Urinary Residue Levels of the N-Dealkyl Metabolites of Triazine Herbicides

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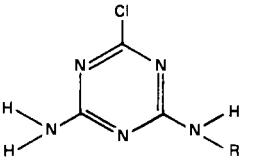
Analytical methodology for monitoring human exposure to the triazine herbicides atrazine, simazine, and propazine is needed because of the widespread use of these compounds in agriculture. In animals, the chief urinary metabolites that have been reported are the corresponding N-dealkyl triazines 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (I) and 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (II). An analytical procedure for determining urinary levels of these metabolites by gas chromatography is presented. A third metabolite, 2-chloro-4,6-diamino-*s*-triazine (III), found in the urine of rats dosed with the three parent compounds, is also reported here. This compound appears to be the major metabolite in rats.

A number of nitrogenous compounds find their way into the environment, among which are the triazine herbicides. Although the toxicity of these compounds to mammals is generally low (2000–5000 mg/kg oral LD_{50} in rats), they are used in such large quantities that indirect analytical methodology is needed to assess exposure of agricultural workers. The metabolism of the triazine herbicides appears to be rather complicated, but various researchers have reported that the major animal metabolites arise from N-dealkylation to produce compounds with the general structure



(Bakke et al., 1967, 1971, 1972; Böhme and Bär, 1967; Robbins et al., 1968; Hutson et al., 1970; Larson et al., 1971; Crayford and Hutson, 1972; Dauterman and Muecke, 1974; Larsen and Bakke, 1975). The general indication is that

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N-deethylation occurs more readily than N-deisopropylation.

Much of the published metabolic work has employed radiolabeled triazines. Isolation and identification of the metabolites required methods which were not readily adaptable to the indirect analysis of large numbers of samples collected from agricultural workers following dermal and inhalation exposure. A more promising method, developed by Böhme and Bär (1967), employed solvent extraction of urine followed by thin-layer chromatography (TLC) to separate and identify the metabolites. Thin-layer chromatography is not the most satisfactory quantitative technique available. The development of a method using gas chromatography for quantitative would provide sensitive, reasonably specific, and rapid analysis of the metabolites of triazine herbicides.

EXPERIMENTAL PROCEDURES

Glassware and Reagents. Chromatographic columns were Chromatoflow Series D, 9 mm × 25 cm, with a Teflon valve (Pierce Chemical Co.) or the equivalent.

Silica gel, Woelm, Activity Grade I, was dried at 130 °C for at least 24 h. The activated silica gel was partially deactivated by adding 20% water (v/w) to the silica gel in a screw-capped tube. The tube was turned at about 50 rpm on a rotary mixer for at least 1 h before the silica gel was used.

Sodium sulfate and sodium chloride were extracted overnight with hexane in a Soxhlet apparatus, then dried in a hood, and stored at 130 °C.

Standards. Atrazine, simazine, propazine, 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (I), and 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (II) were obtained from Ciba Geigy (Greensboro, NC). Standard 2-chloro-4,6-diamino-*s*-triazine (III) was purchased from Aldrich Chemical Co. (Milwaukee, WI).

Equipment. Analysis of the samples was accomplished with a Tracor MT-222 gas chromatograph equipped with a 702 N–P detector (NPD). The detector was operated at a polarizing voltage of 20 V and a hydrogen flow rate of 2.5 mL/min. The detector background current was 1.5×10^{-11} A. A borosilicate glass column (1.8 m × 4 mm i.d.) was packed with 3% OV-101 on Carbowax 20M support bonded Chromosorb W (Moseman, 1978).

The column temperature was 155 °C for analysis of I and II and 175 °C for analysis of III. The carrier flow was 60 mL/min.

Method. A 1.0–5.0-mL volume of urine was pipetted into a glass-stoppered centrifuge tube. A volume of diethyl ether equal to twice the volume of urine was added to the tube. Sufficient sodium chloride was added to saturate the aqueous phase, and the tube was agitated for 1 min on a vortex mixer. The sample was centrifuged, the ether layer transferred to a second tube, and the extraction repeated with an additional volume of ethyl acetate. The combined extracts were concentrated under a stream of nitrogen for GC quantification of III. The extract was further concentrated to a volume of 0.2 mL for cleanup prior to analysis of I and II.

A chromatographic column was prepared by using 2 g of the deactivated silica gel. The adsorbent was topped with 1–2 cm of granular anhydrous sodium sulfate and rinsed with about 20 mL of hexane. The concentrated urine extract was diluted with 1 mL of hexane and transferred to the column. The tube was rinsed with several 2-mL volumes of 20% ether in hexane, and the rinses were added successively to the column. Finally, a volume of 20% ether in hexane was added to the column to give a total of 25 mL of solvent mixture (fraction 1). This fraction was discarded. A 25-mL tube was placed under the column and elution continued with 25 mL of 50% ether in hexane. This eluate (fraction 2) contained the two metabolites I and II. The eluate was concentrated to an appropriate volume and analyzed by gas chromatography.

Animal Feeding. Male Charles River rats averaging 300 g were housed in pairs in stainless steel metabolism cages in a controlled-climate room. Standard laboratory chow and water were available ad libitum.

Separate dosing mixtures were prepared by suspending weighed quantities of each triazine herbicide in peanut oil in volumetric containers. Simazine, atrazine, and propazine mixtures were prepared containing 50, 5, 0.5, and 0.005 mg/mL. The mixtures were agitated to obtain a uniform suspension immediately prior to withdrawing the volume to be administered.

Urine was collected in glass bottles over 24-h intervals. One control sample was collected from each cage during the 24 h prior to administration of the first dose. Three additional cages (six animals) were maintained as controls during the course of the dosing study, and urine was collected over 24-h intervals.

The animals were dosed by gavage with 1.0 mL of the appropriate oil suspension. The doses were repeated twice at 24-h intervals. Clean urine bottles were immediately placed under the cages, and urine was collected for 24 h after each dose. Following the last dose, urine was collected from all animals over 48-h intervals for 4 days. The urine samples were frozen until analyzed.

Fortification and Recovery of Triazine Metabolites. Recovery of triazine metabolites (I–III) from silica gel was determined by adding 0.5 mg of each standard to a silica gel column prepared as previously described. The column was eluted first with 25 mL of 20% ether in hexane (fraction 1), then with 25 mL of 50% ether in hexane (fraction 2), and finally with 25 mL of ether (fraction 3). Each fraction was analyzed by gas chromatography.

Control human urine samples were fortified with 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (I), 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (II), and 2-chloro-4,6-diamino-*s*-triazine (III) at 0.1, 1.0, and 10.0 ppm. The fortified urine was analyzed as previously described.

RESULTS AND DISCUSSION

The two metabolites I and II were completely extracted from urine with diethyl ether, but the extraction was incomplete if ethyl acetate was the extracting solvent. Conversely, extraction of metabolite III was incomplete using diethyl ether but complete using ethyl acetate. Complete recovery of the three metabolites could be achieved by extracting first with diethyl ether, followed by extraction with ethyl acetate.

Triplicate recoveries of the three metabolites from silica gel columns were determined. The average recoveries of metabolites I and II were 105.4% (104.9–106.3%) and 96.2% (95.8–96.5%), respectively. I and II eluted from silica gel completely in fraction 2 (25 mL of 50% ether in hexane). Metabolite III was not eluted from silica gel in any of the three fractions nor by an additional 25 mL of methanol.

Table I shows the recoveries of the three metabolites from fortified human urine. Metabolite III was quantified at all three levels of fortification without cleanup. The retention time of the compound was sufficiently long that it was well separated from urine coextractives (Figure 1). The two higher fortification levels of metabolites I and II (1.0 and 10 ppm) were also quantified without cleanup. At these levels, there was no significant interference from

Table I. Recovery of Triazine Metabolites from Fortified Human Urine Samples

spiking level, ppm	metabolite	% recovery 1	2	3	av recovery, %
10	I	98.6	96.7	98.6	98.0
10	II	97.3	97.3	97.3	97.3
10	III	97.5	99.3		98.4
1	I	89.7	93.0	94.2	92.3
1	II	94.0	97.8	98.9	96.9
1	III	94.8	90.4		92.6
0.1	I	89.3	92.6	90.2	90.7
0.1	II	81.5	85.3	84.4	83.7
0.1	III	89.7	85.8		87.8

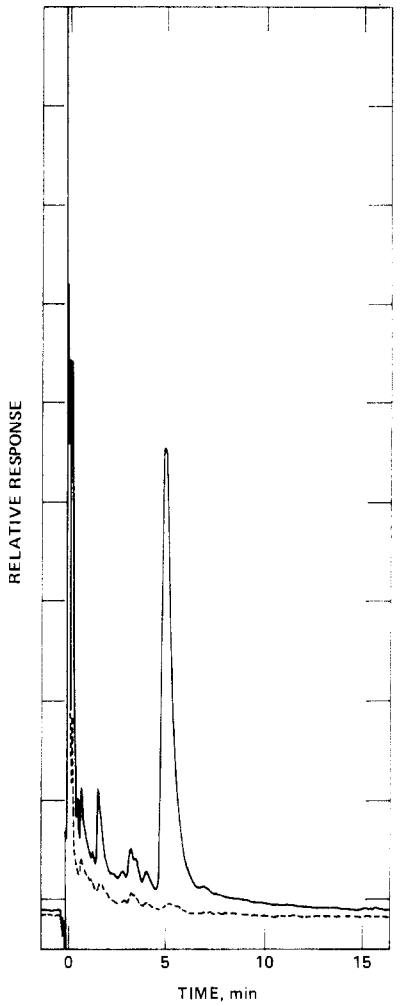

Figure 1. Gas chromatograms of extracts of urine control (---) and urine sample fortified with 0.1 ppm of 2-chloro-4,6-diamino-*s*-triazine (—).

urine coextractives. At the fortification level of 0.1 ppm, the extracts were cleaned up by silica gel chromatography prior to quantification. Figure 2 shows a representative chromatogram of an extract of human urine fortified at 0.1 ppm with I and II, following cleanup by silica gel column chromatography.

The levels of detection were limited by the presence of interfering coextractives, the amounts of which were considerably greater in rat than in human urine extracts. In rat urine, metabolites I and II could be detected down to 0.05 ppm and metabolite III down to 0.01 ppm. In human urine, the limit of detection for each of the three metabolites was 0.005 ppm.

Feeding studies were conducted primarily to obtain urine containing biologically incorporated metabolites of triazine herbicides. By feeding known amounts of herbicides to rats, it was possible to estimate the degree of conversion to each of the metabolites.

Table II. Percent of Administered Dose Excreted as Metabolites I and II

herbicide	dose level, mg (1 rat)	total μM fed (2 rats)	μM excreted (2 rats)	% of dose excreted
simazine	50	1485	41.45	2.79
	5	148.5	0.77	0.52
	0.5	14.85	0.052	0.35
atrazine	50	1389	50.9	3.67
	5	138.9	0.382	0.27
propazine	50	1305	7.01	0.54
	5	130.5	0.106	0.081

Table III. Levels of Metabolites I and II in Urine of Rats Exposed to Triazine Herbicides

herbicide	day	metabolite	ppm of metabolite in urine 50 mg	5 mg	0.5 mg
simazine	1	I	29.8	0.87	0.12
	2	I	35.4	0.82	0.12
	3	I	229	1.82	0.068
atrazine	1	I	9.3	0.28	ND[a]
		II	34.4	0.40	ND
	2	I	7.6	0.11	ND
		II	32.0	0.43	ND
	3	I	36.2	0.20	ND
		II	156	0.94	ND
propazine	1	II	12.6	0.16	ND
	2	II	19.7	0.26	ND
	3	II	9.85	0.22	ND

[a] ND = not detected.

Three herbicides, simazine, atrazine, and propazine, were chosen as being representative of the triazine herbicides. By production volume, they are among the most widely used triazine herbicides. The rats were dosed at five different levels to obtain a wide range of exposure. The principal urinary metabolites were expected to be I from simazine, I and II from atrazine, and II from propazine.

Analysis of the urine samples from this study revealed that only a small percentage of the administered herbicide was excreted as I and/or II (Table II). Table III shows the actual levels of metabolites I and/or II found in the urine of rates exposed to simazine, atrazine, and propazine. These metabolites were readily detected in urine following exposure to 50 and 5 mg/day. Rats exposed to 0.5 mg/day of simazine excreted measurable amounts of metabolite I, but rats exposed to atrazine and propazine at that level did not have detectable levels of either metabolite in their urine.

The urine extracts all contained another peak which was not present in the urine of control animals. It was subsequently identified as 2-chloro-4,6-diamino-*s*-triazine (III) by comparison of mass spectrum and GC retention time of the unknown with an authentic standard of III. It was necessary to repeat the feeding study to obtain fresh urine containing metabolite III because the metabolites degrade rapidly when urine is stored for an extended time or is thawed and refrozen. In this second study, rats were exposed to the four lower levels of triazine herbicides, 5, 0.5, 0.05, and 0.005 mg/day. These urine samples were analyzed solely for metabolite III.

Table IV shows the levels of metabolite III found in the urine of rats exposed to the three herbicides. This metabolite was excreted at detectable levels in rats exposed to 5 and 0.5 mg/day of all three herbicides. Data from day 2 are missing because a malfunction of the automatic watering system caused the urine bottles to overlow.

Table IV. Levels of Metabolite III in Urine of Rats Exposed to Triazine Herbicides

	simazine		atrazine		propazine	
	day 1	day 3	day 1	day 3	day 1	day 3
			5.0 mg of Herbicide			
ppm in urine	8.33	51.5	15.9	28.8	8.82	10.6
μM fed/day	24.9	24.9	22.3	22.3	21.8	21.8
μM excreted	0.975	4.53	0.709	7.12	0.727	2.09
% excreted	3.9	18.2	3.18	31.9	3.33	9.59
			0.5 mg of Herbicide			
ppm in urine	0.645	0.42	0.43	0.858	0.040	1.66
μM fed/day	2.49	2.49	2.38	2.38	2.35	2.35
μM excreted	0.0345	0.0391	0.0685	0.102	0.00804	0.400
% excreted	1.38	1.57	2.89	4.29	0.342	17.0

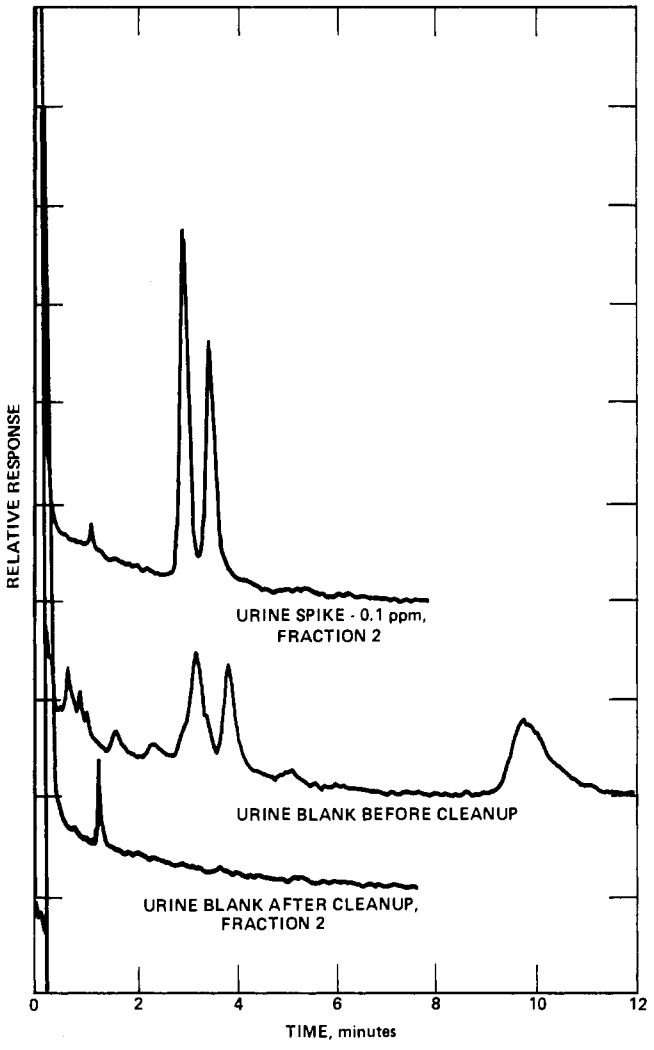

Figure 2. Gas chromatograms of urine extracts. Fortification levels were 0.1 ppm each of 2-chloro-4-amino-6-(ethylamino)-*s*-triazine (I) and 2-chloro-4-amino-6-(isopropylamino)-*s*-triazine (II).

No detectable levels of the three metabolites were found in the urine of rats exposed to 0.05 or 0.005 mg/day simazine, atrazine, or propazine nor in urine collected over the period 24–72 h following the last dose. The urine control rats was also free of detectable levels of the three metabolites.

The data reported in Table III support the observation that N-deethylation occurs more readily than N-deisopropylation. The highest level of mono-N-dealkylated metabolite resulted from exposure to simazine, which has ethyl substituents on both amino groups. Relatively low levels of metabolite II were found in urine of rats exposed to propazine which is isopropyl substituted. The intermediate compound, atrazine, with both an ethyl and an isopropyl substituent, was degraded primarily by N-deethylation to yield the isopropyl-substituted metabolite II as its principal metabolite.

The levels of metabolite III resulting from loss of the second alkyl group are not as readily explained by this rationale. They are uniformly higher levels of I and/or II, but no clear pattern emerges.

Although this work did not constitute a study of the metabolism of triazine herbicides, an interesting observation should be noted. In most cases, the levels of all metabolites excreted in the urine increased following repeated exposure, indicating that chronic exposure may result in substantially higher levels of urinary metabolites than would be the case following a single exposure. This may prove of value in evaluating urinary metabolite levels of agricultural workers chronically exposed to triazine herbicides.

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