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Determination of s-Triazine Herbicide Residues in Urine: Studies of Excretion and Metabolism in Swine as a Model to Human Metabolism

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The excretion and metabolism of atrazine and procyazine were studied in pigs. Pigs were used because of their close modeling of human metabolism. Young (3–5 months) Pittman–Moore miniature pigs were dosed under anesthesia via a stomach tube with 0.1 g of the commercial grade herbicide dissolved in ethanol. Urine samples were collected from a drain in the floor of the metabolic cages. The urine samples were extracted and subsequently analyzed by gas chromatograph using the Hall electrolytic conductivity detector in the nitrogen mode. A column clean-up procedure using deactivated alumina was employed to further purify the urine extracts for analysis using electron-capture or mass spectrometric detectors. Atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and its metabolites were detected in the urine for slightly more than 24 h. Procyazine (2-[(4-chloro-6-cyclopropylamino)-s-triazin-2-yl)-amino]-2-methylpropionitrile) and its metabolites were not detected in the urine after about 10 h. Gas chromatographic/mass spectrometric analysis of pig urine extracts showed conclusively that the parent herbicide was being excreted. In addition, deethylatrazine [2-chloro-4-(amino)-6-(isopropylamino)-s-triazine] was identified as an atrazine metabolite, and several other compounds were identified as impurities in the dosing samples. If the findings of this research are extrapolated to monitoring studies in man, s-triazine herbicide may be expected in the urine within 24 h of exposure and primarily as the parent compound.

Because of their extreme popularity, the metabolism and degradation of the s-triazine herbicides has been extensively studied in recent years. The s-triazine herbicides have been shown to be excreted in the urine and feces and are not retained for long periods of time in the body tissues. Radiolabeled atrazine was found to be excreted in the urine (65.5%) and feces (20.3%) of rats after 72 h (Bakke et al., 1972). Less than 0.1% of the dose was detected in the expired air, indicating that the s-triazine ring was not metabolized to carbon dioxide. Analysis of body tissues indicated that 15.8% of the radioactivity was retained with high concentrations in the liver, kidney, and lung tissues, with low concentrations in the muscle and fat. Propazine (^{14}C-ring-labeled) was eliminated from lactating goats (Robbins et al., 1968) at levels of 43% in the urine and 41.5% in the feces, with a maximum milk concentration of 1.5 ppm after approximately 8 h. Residue levels (0.3–1.5 ppm) of radioactive propazine and/or metabolites were found in the blood, brain, heart, kidney, liver, lung, muscle, spleen, and udder at 72 h. No radioactivity was detected in the omental fat and kidney fat.

Cyanazine (^{14}C-ring-labeled), a homologue of the procyazine studied here, was excreted by rats in the urine (approximately 40%) and feces (approximately 47%), with only 3% of the compound remaining in the animal after 4 days (Hutson et al., 1970). The excretion of radioactive material from ethyl-labeled cyanazine dosing was: urine, 17%; feces, 26.3%; carbon dioxide, 48%; and remaining in the carcass, 5%.

In a study of a lactating dairy cow (Bakke et al., 1971), [^{14}C]sumitol was found to be excreted in the urine (76.1%), feces (10.6%), and milk (2.4%). The liver and spleen were found to contain detectable radioactivity, while the brain, leg muscle, adrenal gland, perirenal fat, omental fat, tailhead fat, kidney, heart, and blood contained minimal concentrations at sacrifice after 120 h. Within 48 h of administration of atrazine, propazine, simazine, prometone, and sumitol, most of the ring-labeled radioactivity had appeared in the urine of cows (Thacker, 1971). Approximately 2% of the atrazine (St. John, 1965) and approximately 1% of the simazine fed to cows were recovered unmetabolized in the urine. No residues were detected in the milk by colorimetric assay.

The persistence of the s-triazine residues in animal tissues has been studied. Radiolabeled propazine was detected in the tissues of rats 12 days after dosing (Bakke et al., 1967) and 7.5% of the radioactivity was found in rat carcasses 72 h after dosing with cyprazine. The nonchlorinated s-triazines were eliminated more quickly than the chlorinated s-triazines. Low or undetectable levels of tissue radioactivity were observed 72 h after the dosing of rats with ^{14}C-labeled 2-hydroxyatrazine (Bakke et al., 1967), sumitol, its 2-hydroxy analogue, and its two dealkyl

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analogues (Larson and Bakke, 1971).

Dealkylation of the amino group has been shown to be a common metabolic reaction of the *s*-triazine herbicides in rats (Larson and Bakke, 1971; Larson and Bakke, 1975; Bakke and Price, 1973; Böhme and Bär, 1967), sheep (Robbins et al., 1968), and cows (Bakke et al., 1971; Thacker, 1971). Oxidation of an *N*-alkyl sidechain to the carboxylic acid (Bakke et al., 1972; Larson and Bakke, 1975; Böhme and Bär, 1967) and alcohol (Robbins et al., 1970; Shimabukuro, 1966; Larson and Bakke, 1975) and hydrolysis of the 2-chloro group (Robbins, 1970; Larson and Bakke, 1975) have been observed in rats.

Two major rat urinary metabolites of cyanazine were identified by mass spectrometry and NMR (Hutson et al., 1970) as *N*-acetyl-*S*-4-amino-6-(1-methyl-1-cyanoethylamino)-*s*-triazinyl-2-*l*-cysteine and 2-chloro-4-amino-6-(1-methyl-1-cyanoethylamino)-*s*-triazine (deethylcyanazine). These metabolites represented 40 and 15%, respectively, of the total urinary radioactivity.

Conjugation of several 2-chloro-*s*-triazines by glutathione was observed (Dauterman and Muecke, 1974) in in vitro studies with rat liver subcellular fractions. In addition, N-dealkylation was observed. No hydrolysis at the 2-position was reported.

In order to assess the environmental impact of pesticides, their usage, and their long-term health effects, monitoring studies of the pesticide residues in the field must be carried out. Until recently, analytical methodology has been severely limited, resulting in narrowly focused monitoring programs for selected classes of compounds. The development of methodology for identification of *s*-triazines and their metabolites in residue samples by nonradiochemical techniques would permit monitoring studies to further assess the environmental impact and human effects of these compounds.

The purpose of this study is to evaluate a residue method for the identification of *s*-triazines and their volatile metabolites using swine as a model system for potential human exposure.

EXPERIMENTAL SECTION

Chemicals. Analytical reference standard atrazine was obtained from Quality Assurance Section, Environmental Toxicology Division, HERL, EPA, Research Triangle Park, NC. Atrazine and procyazine were obtained from the Botany Department of Iowa State University, Ames, IA, as the 80W commercial preparation.

A sample of the technical procyazine was purified by recrystallization from acetone: mp 169–170 °C.

Anal. Calcd for $C_{10}H_{13}N_6Cl$: C, 47.52; H, 5.15: N, 33.27. Found: C, 47.60; H, 5.31; N, 32.85.

Sample Preparation. Samples were prepared for GC analysis using an extraction procedure developed in these laboratories (Erickson, 1978a) for work with the HECD in the nitrogen mode. The urine samples were sufficiently free from interference so that a column cleanup was unnecessary. However, for GC/mass spectrometric analysis, the background level was critical and it was necessary to clean up the samples using a deactivated alumina column (Montgomery et al., 1969; Kahn and Purkayastha, 1975; Purkayastha and Cochrane, 1973; Young and Chu, 1973; Lawrence, 1974; Schroeder, et al., 1972; Johnson, 1975). Alumina (Fisher Chemical Co. 80–200 mesh) dried at 180 °C for 24 h, was deactivated by the addition of 10% distilled water with thorough mixing. A column, 5 × 0.8 cm i.d., was prepared and topped with 1 cm of Na_2SO_4. The column was wetted with 10 mL of hexane and the sides tapped to remove trapped air. A small amount of the concentrated urine extract was added

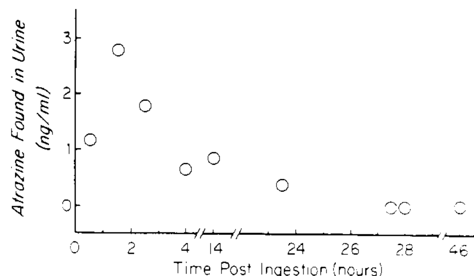

Figure 1. Excretion profile of atrazine in pig urine.

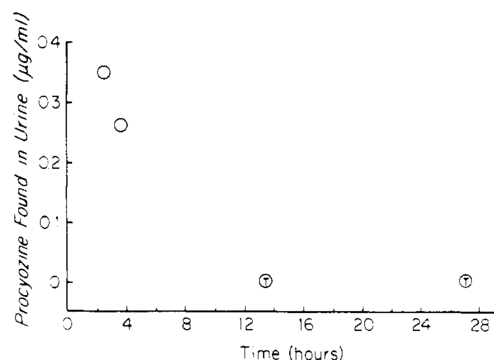

Figure 2. Excretion profile of procyazine in pig urine.

to the column and allowed to elute. Portions (25 mL) of 90:10 hexane/ethyl ether and 50:50 hexane/ethyl ether were eluted through the column and fractions collected at 5–10-mL intervals. The *s*-triazines were found to elute with the 50:50 hexane/ethyl ether mixture. A number of early eluting peaks were observed in chromatograms of the 90:10 eluates.

Gas Chromatograph. A MT-221 gas chromatograph (Tracor, Inc., Austin, TX), fitted with a Model 310 Hall electrolytic conductivity detector (HECD) in the nitrogen mode was used. Details on the instrument and its operation have been discussed previously (Erickson, 1978a,b).

GC/Mass Spectrometer. A Finnigan quadrupole GC/mass spectrometer (Model 3200) was used to obtain GC/MS data. The instrument was operated under the following conditions. The GC column was 3% OV-101 (4 ft) operating at oven temperatures between 170–210 °C with inlet pressures between 4 and 10 psig. The mass spectrum was scanned between 100–350 m/e units with a scan time of 3 s.

Swine Treatment. Pittman–Moore mini-pigs (Lederle Laboratories) between 2 and 4 months old, weighing 30–50 lb, were used in the study. Under total anesthesia (pentobarbital), the pigs were dosed with ethanolic solutions of 0.1 g of commercial atrazine 80W, or procyazine 80W, using a stomach tube. This dosing method was not totally satisfactory since the anesthesia inhibited urination, but was the only viable method found. To promote urination, the pigs were allowed to drink only 0.5% salt water.

Immediately following dosing with the herbicide, the pig was placed in a clean cage. Urine samples were collected as often as a significant amount (>50 mL) had been excreted. Except for the samples collected overnight, care was taken to prevent contamination of the urine samples by feces and feed.

RESULTS AND DISCUSSION

Excretion Rates. The excretion profiles for atrazine and procyazine and some of their metabolites are shown in Figures 1 and 2. Concentrations were obtained by

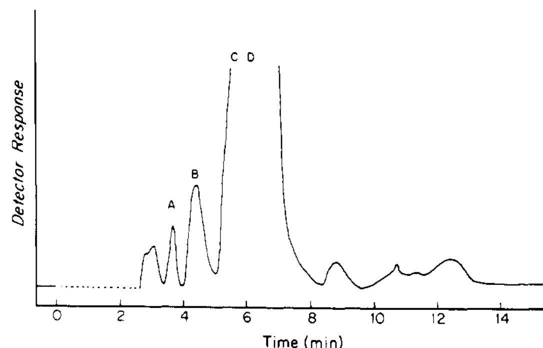

Figure 3. Total ion current gas chromatogram of GC/MS analysis of urine extract containing atrazine metabolites: (A) P = 177, (B) deethylatrazine, (C) atrazine, (D) atrazine and propazine (impurity). Conditions: Finnigan GC/mass spectrometer; column, 3% OV-101; column pressure, 4 psig; column temperature, 170 °C; injection, 5 μL.

GC/HECD analysis of urine samples from a single dosing of a pig. Atrazine and its metabolites were excreted in approximately 1 day. Procyazine and its metabolites were excreted very rapidly in the urine (<10 h).

Identification of Urinary Metabolites. The extract of a urine sample collected from an atrazine-dosed pig was shown to contain atrazine and two metabolites using gas chromatography with the HECD in the nitrogen mode. The gas chromatogram of this sample from GC/mass spectrometry, however, contained six peaks as shown in Figure 3. The mass spectrum of B is characterized by major peaks at m/e values of 189, 187, 174, and 172 with isotope ratios corresponding to a compound containing one chlorine. The compound was tentatively identified as deethylatrazine. Peak C was identified as atrazine by comparison of its mass spectrum with that of an authentic sample. The other compound (peak D), at much lower concentration, has a parent ion of 231, 229 and is not observed in the spectrum of atrazine standard. The mass spectral evidence indicates that this compound could be propazine or Pramitol. Gas chromatographic analysis of atrazine 80W (HECD in the nitrogen mode, 5% Reoplex 400 column) confirmed the presence of a small amount of propazine. The mass spectral patterns for the unlabeled peaks in Figure 3 are too weak for interpretation.

The extracts from pig urines following ingestion of procyazine were found to contain procyazine and at least two metabolites which were not present in a pre-dosed urine sample. The GM/MS characteristics (Figure 4) of this sample were obtained. Peak A, which was not observed on the HECD in the nitrogen mode in predose urines, has an assigned parent ion of 177 and does not appear to be an s-triazine. The mass spectrum of peak B results from two unidentifiable compounds, one of which is chlorinated with an apparent parent ion at 225 and may be a procyazine metabolite or impurity. Peak C contains a parent ion of 254, 252 and is identified as the parent compound, procyazine. Peak D has a parent ion of 121. The compound does not contain chlorine and has not been identified.

CONCLUSIONS

Atrazine and procyazine excretion in swine urine is essentially complete within 24 h. The primary observable metabolite of atrazine is the deethyl derivative. The confirmation of metabolites by GC/MS and the lack of significant interference in the GC/HECD analysis of the urine extracts indicate that the use of GC/HECD for human monitoring studies should be reliable.

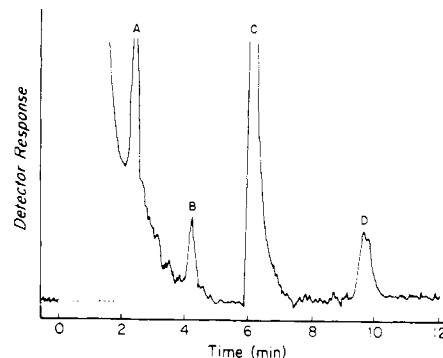

Figure 4. Total ion current gas chromatogram of GC/MS analysis of urine extract containing procyazine metabolites: (A) P = 177; (B) P = 225, 227; (C) procyazine; (D) P = 121. Conditions: Finnigan GC/mass spectrometer; column, 3% OV-101; injection, 5 μL.

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1-(4-Nitrobenzyl)-3-(4-tolyl)triazene as a Derivatizing Reagent for the Analysis of Urinary Dialkyl Phosphate Metabolites of Organophosphorus Pesticides by Gas Chromatography

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Human exposure to organophosphorus compounds may be monitored by the determination of urinary levels of dialkyl phosphate metabolites. A sensitive, reproducible method for the determination of the *O,O*-dimethyl and *O,O*-diethyl derivatives of phosphoric, phosphorothioic, and phosphorodithioic acids is reported. The methodology consists of lyophilization of the urine, derivatization of dialkyl phosphates with a triazene alkylating agent [1-(4-nitrobenzyl)-3-(4-tolyl)triazene], cleanup by adsorptive removal of certain contaminants with anhydrous nickel sulfate, separation of P=O and P=S compounds by silica gel chromatography into two fractions, and quantitation of individual dialkyl phosphates in each fraction by gas chromatography. The method is sufficiently sensitive such that each of six urinary dialkyl phosphate metabolites studied may be determined at levels as low as 0.01 ppm. The method was evaluated by the analysis of urine samples collected from pesticide formulators and from unexposed control workers.

The determination of dialkyl phosphates is becoming increasingly important in connection with the evaluation of both environmental and occupational hazards associated with the use of organophosphorus (OP) chemicals and pesticides. In human beings, dialkyl phosphates occur as metabolic products of OP compounds, and these metabolic derivatives are excreted in the urine of individuals exposed to OP pesticides. Accordingly, their determination in urine provides a convenient means for estimating exposure to such compounds. These urinary alkyl phosphate metabolites (UAPM) include the *O,O*-dialkylphosphoric, *O,O*-dialkylphosphorothioic, and *O,O*-dialkylphosphorodithioic acids (see Table I). Previously published methods for determining these metabolites involve alkylation to increase their volatility, thereby permitting quantitation by gas chromatographic (GC) analysis (St. John and Lisk, 1968; Shafik and Enos, 1969; Shafik et al., 1973; Blair and Roderick, 1976; Daughton et al., 1976; Lores and Bradway, 1977). In each of the published methods alkylation was achieved using a diazoalkane.

Although diazoalkanes are effective alkylating reagents, they have undesirable characteristics. These include the necessity for generation of the diazoalkane immediately prior to use as well as problems related to toxicity, mutagenicity, carcinogenicity, volatility, high reactivity, and explosiveness (deBoer and Backer, 1963; Osterman-Golkar, 1974). Other difficulties are also associated with the nature of the derivatives which are generated by the use of diazoalkanes. For example, the methyl and ethyl derivatives of alkyl phosphates are not as stable to hydrolysis and do not have as favorable GC characteristics as higher molecular weight derivatives (Shafik et al., 1973). Their gas chromatographic peaks are also easily obscured by alkylated inorganic phosphate unless special procedures are employed (Shafik and Enos, 1969; Daughton et al., 1976; Blair and Roderick, 1976). Each of these difficulties is avoided through the formation of the higher molecular weight pentyl or hexyl derivatives (Shafik et al., 1973). However, repeated experimental trials in our laboratory with these diazoalkanes resulted in the generation of several different phosphorus-containing products, presumably arising from rearrangement of the diazoalkane carbon backbone before or during derivatization. Such rearrangement is possible and has been reported previously (Zollinger, 1961). These problems indicate a need for a more suitable derivatizing reagent.

Aryl–alkyl triazenes have been recommended as substitutes for diazoalkanes as alkylating reagents (White et al., 1968). This document describes a method by which suitable esters of UAPM can be prepared for GC analysis

Table I. Organophosphate Compounds and Designated Abbreviations

compound	abbreviation
O,O-dimethylphosphoric acid	H-DMP
O,O-diethylphosphoric acid	H-DEP
potassium *O,O*-dimethyl phosphorothioate	K-DMPT
potassium *O,O*-diethyl phosphorothioate	K-DEPT
potassium *O,O*-dimethyl phosphorodithioate	K-DMPDT
potassium *O,O*-diethyl phosphorodithioate	K-DEPDT
O,O-dimethyl *O*-(4-nitrobenzyl) phosphate	*O*N-DMP
O,O-diethyl *O*-(4-nitrobenzyl) phosphate	*O*N-DEP
O,O-dimethyl *O*-(4-nitrobenzyl) phosphorothionate	*O*N-DMPT
O,O-diethyl *O*-(4-nitrobenzyl) phosphorothionate	*O*N-DEPT
O,O-dimethyl *S*-(4-nitrobenzyl) phosphorothiolate	*S*N-DMPT
O,O-diethyl *S*-(4-nitrobenzyl) phosphorothiolate	*S*N-DEPT
O,O-dimethyl *S*-(4-nitrobenzyl) phosphorodithioate	*S*N-DMPDT
O,O-diethyl *S*-(4-nitrobenzyl) phosphorodithioate	*S*N-DEPDT

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