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Determination of *s*-Triazine Herbicide Residues in Urine: Analytical Method Development

Mitchell D. Erickson,[1] Clyde W. Frank,* and Donald P. Morgan

Analytical methodology for the separation and characterization of *s*-triazine herbicide residues in urine was developed. In the sample preparation procedure developed, a urine sample at pH 12 was extracted with hexane three times, using sodium chloride as an emulsion inhibitor. The combined hexane extract was dried by passing it through a sodium sulfate column and concentrated by rotary evaporation. The sample was transferred to a graduated centrifuge tube and further concentrated to 0.5 mL under a stream of dry nitrogen. The sample was analyzed by gas chromatography using the Hall electrolytic conductivity detector (HECD) in the nitrogen-specific mode. The method is advantageous in that it is simple and fast, lending itself to use as a routine method by technical personnel.

Analysis of *s*-triazine herbicide residues has been reported using a number of techniques and procedures. Several colorimetric methods were developed for the determination of the *s*-triazines in soils and crops. At that time, colorimetric determination was the recommended method for ten different *s*-triazines (Knuesli, 1964). The detection limit was reported to be 2 μg (about 100 ppb in the sample), but the method is neither selective among the individual triazines, nor is it free from interference. In two previously reported studies of nonradiolabeled *s*-triazines in animals, atrizine (St. John et al., 1965) and simazine (St. John et al., 1965) were assayed in cow urine and milk using the colorimetric procedure.

Procedures using thin-layer chromatography for the identification of residue samples have been described (Lawrence and Laver, 1974; Balinova, 1973; Ebing, 1973; Huss and Adamovic, 1973; Mueller, 1973), with detection limits in two cases reported as 0.02 μg/spot and 0.1 μg/spot 5 ppb in the sample). The attainment of these detection limits required the use of special developing reagents for observing the compounds on the plate.

Gas chromatography has been used for the analysis of the *s*-triazine herbicides in soils, plants, and water (Beynon et al., 1972a,b; Beynon, 1972; Kahn and Purayastha, 1975; Greenhalgh and Kavacicova, 1975; Kahn et al., 1975; Ramsteiner et al., 1974; Purkayastha and Cochrane, 1973; Young and Chu, 1973; Schultz, 1970; Lawrence, 1974a,b; Westlake et al., 1973; McKone et al., 1972).

Usually the preparation of residue samples for chemical analysis involves a straightforward partition to remove the bulk of the sample matrix, an extract cleanup process, and final sample preparation. The extraction procedures reported for the isolation of the *s*-triazine herbicides are similar to those generally employed for other pesticide residues. Some of the procedures for soil samples employ a 2-h methanol–water tumbling (Beynon et al., 1972a; Beynon, 1972), a 2-h water–acetonitrile reflux (Mattson et al., 1970), a 16-h chloroform Goldfisch extraction (Tindle et al., 1968), or a 2-h methanol Soxhlet extraction (Hill and Stobbe, 1974). More recently, an ultrasonic procedure has been described (Hill and Stobbe, 1974) in which two 15-min ultrasonic extractions were found to be comparable to a 24-h Soxhlet extraction for atrazine. The extraction of the *s*-triazine herbicides from plant materials involves maceration of the sample in a food blender with solvent, followed by filtration and partitioning. A number of different solvent systems have been employed for this work and were compared (Lawrence, 1974a). In addition, a low-temperature precipitation method has been described for the cleanup of plant residue samples (Lawrence and McLeod, 1974). All of the reported methods for the extraction of *s*-triazine herbicides from water samples involve a simple partitioning with methylene chloride (McKone et al., 1972; Tindle et al., 1968; Kahn and Purkayastha, 1975; Purkayastha and Cochrane, 1973).

While some investigators have found them unnecessary under certain GC conditions, a large number of column cleanup procedures have been used in the determination of *s*-triazine residues. The methods all utilize the successive elution with solvents of increasing polarity to selectively elute the desired compounds. The supports most successfully used are alumina (Kahn and Purkayastha, 1975; Kahn et al., 1975; Ramsteiner et al., 1974; Purkayastha and Cochrane, 1973; Young and Chu, 1973; Lawrence, 1974a; Schroeder et al., 1972), Florisil (Mestres et al., 1973; Westlake et al., 1973) and sodium bisulfate (Delley et al., 1967). Ion-exchange resins were extensively

Institute of Agricultural Medicine, University of Iowa, Oakdale, Iowa 52319.
[1]Present address: Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, NC 27709.

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used to separate radiolabeled s-triazines and metabolites (Bakke et al., 1967, 1971; Larson and Bakke, 1971) until it was shown that the procedure can quantitatively convert chlorinated s-triazines to their hydroxy analogues (Bakke et al., 1972).

In order to assess the environmental impact of pesticides, their usage, and their long-term health effects, a large number of monitoring studies have been undertaken in recent years. The development of rapid methodology for s-triazine analysis in metabolic fluids enables the expansion of monitoring studies into new areas to help in the assessment of the environmental impact and human effects of these compounds. Thus, the purpose of this study is to develop analytical methodology suitable for monitoring residues of the s-triazine herbicides and their metabolites in human urine and to illustrate the effectiveness of the method.

EXPERIMENTAL SECTION

Chemicals. Analytical reference standard cyprazine was obtained from Gulf Oil Chemicals Co., analytical reference cyanazine was obtained from Shell Chemical Co., and analytical standard dipropetryne was obtained from the Agricultural Division of CIBA-GEIGY Corp. Technical grade cyanazine, atrazine, and procyazine were obtained from the Botany Department of Iowa State University, Ames, IA, as the 80W commercial preparation and purified by recrystallization from acetone.

All other compounds were obtained as analytical reference standards from Quality Assurance Section, Environmental Toxicology Division, HERL, EPA, Research Triangle Park, NC. The purity of all samples was verified by gas chromatography using the Hall electrolytic conductivity detector and by mass spectrometry using a Hitachi-Perkin Elmer RMU-6E single-focusing, medium-resolution mass spectrometer. Two samples (propazine and prometone) were found to be contaminated with reaction byproducts. Pure samples of these compounds were subsequently obtained. The solvents used for all solutions were purchased, distilled in glass, from Burdick and Jackson Laboratories, Muskegon, MI.

Gas Chromatograph. A MT-220 gas chromatograph (Tracor, Inc., Austin, TX), fitted with a Model 310 Hall electrolytic conductivity detector (HECD) in the nitrogen mode. The liquid phases, Reoplex 400, OV-1, OV-101, and OV-210 and the solid support, Chromosorb W-HP (800–100 mesh), were purchased from Applied Science Laboratories, State College, PA. The liquid phase (0.60 g for a 3% column) was dissolved in 60 mL of chloroform and slurried with 19.40 g of Chromosorb W-HP and then rotated in a fluted round bottom flask for 0.5 h. The solvent was removed by rotary evaporation and the bulk packing material was dried at 275 °C (190 °C for the Reoplex 400 columns) for 48 h. After packing, columns were cured for at least 3 days or more at 250 °C (185 °C for Reoplex 400) in the column oven of the MT-220 under a flow of 20 mL/min of N_2.

Extraction Procedure. The procedure for extraction and workup of s-triazine residues from urine was as follows. Urine volume was measured and a 100-mL aliquot taken. The pH was adjusted to 12.0 with 10% NaOH and 5 g of NaCl added to retard emulsions. The sample was extracted thrice with 30 mL of hexane by shaking 30 min on a mechanical shaker. The combined extracts were dried on a 8 × 2 cm i.d. column of Na_2SO_4, and the solvent was removed by rotary evaporation, followed by evaporation under a nitrogen stream. All transfers were accompanied by appropriate washings. The rationale and validation of the method is discussed below.

Table I. Relative Retention Times of s-Triazine Herbicides on Different GLC Columns

compound	5% Reoplex 400 (60 cm)	3% OV-101 (150 cm)	3% OV-210 (150 cm)	5% OV-1 (150 cm)
atrazine	1.00	1.00	1.00	1.00
prometone	0.56	0.94	0.85	1.17
atratone	0.69	0.94	0.91	1.11
propazine	0.79	1.00	0.99	1.07
prometryne	1.08	1.69	1.41	1.92
terbutryne	1.17	1.86	1.56	2.09
simazine	1.26	0.92	1.01	0.93
ametryne	1.39	1.64	1.44	1.75
desmetryne	1.49	1.42	1.29	1.55
cyprazine	1.84	1.44	1.41	1.55
sumitol	0.84	1.17	1.13	1.50
cyanazine	9.2	1.97	4.61	2.43
procyazine	16	3.00	6.43	3.46

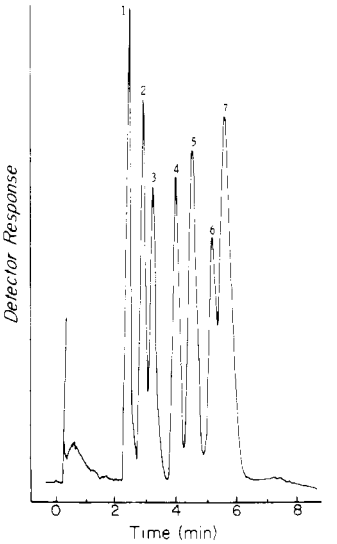

Figure 1. GLC separation and detection of s-triazine herbicides using the Hall electrolytic conductivity detector. The following conditions were used [compound (relative retention time), condition]: (1) prometone (0.56) 3% Reoplex 400 on Chromosorb on W-HP (150 cm); (2) atratone (0.69) T_{column} = 199 °C; (3) propazine (0.79) $T_{furnace}$ = 840 °C; (4) atrazine (1.00) $T_{transfer}$ = 225 °C; (5) terbutryne (1.17) T_{inlet} = 230 °C; (6) ametryne (1.39) He flow = 150 mL/min; (7) desmetryne (1.49) H_2 flow = 20 mL/min. Attenuator, ×2. Conductivity, ×3. About 100 ng of each component was injected.

RESULTS AND DISCUSSION

Development of Analytical Conditions. Four liquid phases, Reoplex 400, OV-1, OV-101, and OV-210, were selected for use in separation of the s-triazine herbicides on the basis of literature evidence (Fishbein, 1970; Cochrane and Purkayatha, 1973). The relative retention times (RRT) for a number of s-triazine herbicides using these columns are listed in Table I. Reoplex 400 gave the greatest efficiency of separation of the compounds, as shown in Figure 1, but the retention times for cyanazine and procyazine were so long that the use of this column for analysis of these compounds was impractical. For general work, either OV-101 or OV-210 proved satisfactory. The OV-101 column gave a slightly wider distribution of retention times than did the OV-210.

Sensitivity. The limit of detection of the HECD to atrazine was about 0.2 ng. The sensitivity of the overall method was limited by the volume to which the urine extract could be concentrated (0.3 mL) and the maximum injection volume (10 mL). Using these limits, the method was sensitive to about 6 ng atrazine/sample or 60 pg/mL

Table II. Recovery of Atrazine Using Hexane and Benzene

	% recovery of atrazine	
replicate	hexane extraction	benzene extraction
1	90.4	77.2
2	84.7	57.3
3	89.6	57.8
av	88.2	64.1
RSD, %	3.5	17.7

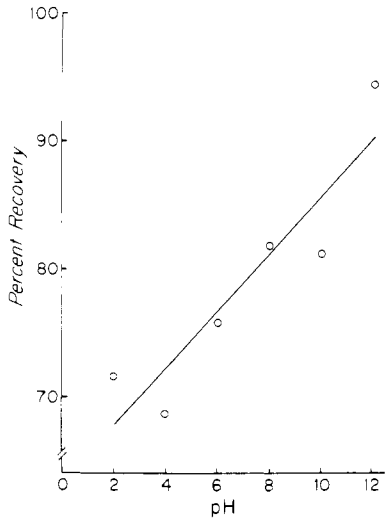

Figure 2. Effect of urine pH on extraction efficiency using hexane. Human urine fortified with 10.2 μg/mL (10.2 ppm) of atrazine. Correlation coefficient = 0.995.

(60 pptr) based on a 100-mL aliquot.

Development of Sample Preparation Techniques. Only a limited number of solvents are suitable for use with the HECD in the nitrogen mode: these solvents include aliphatic and aromatic hydrocarbons and alcohols (Erickson et al., 1978). Since alcohols are miscible with water, they are not suitable as extraction solvents. Benzene and hexane were therefore chosen as the most logical representatives of the two remaining solvent classes.

To assess which solvent was most applicable, 600 mL of urine was fortified with atrazine. The mixture was titrated to pH 12.0 with NaOH and divided into six equal portions, each containing 10.16 μg of atrazine. Three of the samples were extracted with hexane, and three with benzene, following the procedure outlined above. The percent recovery of each sample (analyzed by GC/HECD) is shown in Table II. A fourth extraction of each sample with either solvent did not yield a detectable amount of atrazine. Several experiments were conducted to determine if a mixture of hexane and benzene would more efficiently extract atrazine from urine. The recoveries from these experiments indicated that there was no improvement in the extraction efficiency with a mixed extraction solvent over that of hexane.

The effects of urine pH on the extraction efficiency were studied and are summarized for hexane in Figure 2. Similar results were obtained for benzene, although the percent extracted was lower throughout. For both extraction solvents, atrazine was most efficiently extracted at high pH.

Application of the Method. A graduate student (R.H.) in the Botany Department, Iowa State University, was exposed while spraying test plots to a wide variety of herbicides of up to 60 different formulations in 1 day, including atrazine, cyanazine, cyprazine, and procyazine. Between April 15, 1975, and May 19, 1975, 17 urine samples were collected and analyzed. Procyazine (~1 ppb) was detected in one sample while atrazine was found in three urine samples at levels ranging from 0.1–10 ppb. The records show that he was applying atrazine several times during this period.

A commercial pesticide applicator who had sprayed an atrazine–alachor mixture and other herbicides for 10 days contracted a suspected poisoning from this work. Analysis of a 24-h urine sample (1380 mL) obtained immediately after the onset of the symptoms contained no atrazine.

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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

Mitchell D. Erickson,[1] C. W. Frank,* and Donald P. Morgan

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

Institute of Agricultural Medicine and Environmental Health, University of Iowa, Oakdale, Iowa 52319.

[1]Present address: Research Triangle Institute, Chemistry and Life Sciences Division, Research Triangle Park, NC 27709.

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