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Identification of a Major Human Urinary Metabolite of Metolachlor by LC-MS/MS

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Metolachlor is a preemergence herbicide commonly used on soybeans, corn, and other crops. Herbicides are the most used class of pesticides, and metolachlor is one of the top three herbicides, with 40 - 60 million pounds used annually (EPA 21T-1005, 1991). Recently, we have been involved in studies of herbicide exposure that required us to develop procedures to measure metolachlor metabolites, alachlor metabolites, and other pesticide metabolites in urine. When we began the study, we found that neither alachlor nor metolachlor metabolism in humans was well understood although alachlor metabolism had been studied in monkeys (Carr et al. 1986). Therefore, to understand the metabolism of alachlor in humans, we analyzed for alachlor metabolites in the urine of occupationally exposed subjects. We were able to identify a major human metabolite of alachlor, alachlor mercapturate (Driskell et a1.,1996). Metolachlor metabolism has not been studied in humans, and only a few studies of its metabolism in animals have been done. In this study, we tested the hypothesis that metolachlor, which is structurally similar to alachlor, is metabolized to metolachlor mercapturate in humans as alachlor is metabolized to alachor mercapturate. We synthesized metolachlor mercapturate to use as a standard to determine whether the urine samples from subjects who had been occupationally exposed to metolachlor contained metolachlor mercapturate.

MATERIALS AND METHODS

To test the hypothesis that metolachlor, like alachlor, is metabolized to a mercapturate, we followed this regimen: 1) We synthesized metolachlor mercapturate to use as a standard 2) developed an LC-MS/MS method for metolachlor mercapturate 3) developed a solid phase urinary extraction method for metolachlor mercapturate and 4) analyzed for metolachlor mercapturate in urine

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samples from subjects exposed to metolachlor.

The urine samples were collected for the pilot Agricultural Health Study, an EPA-sponsored study of pesticide exposure of agricultural workers (Alvanga et al., 1994). Many of the workers in the study had been exposed to metolachlor.

Using a synthesis method similar to that used for alachlor mercapturate (Driskell et al,, 19961, we synthesized metolachlor mercapturate as follows: We added 35 mg metolachlor and 20 mg N-acetylcysteine to 5 mL pyridine in a 25 ml round-bottom flask, sealed the flask, and incubated for 24 hr at room temperature. We evaporated the pyridine in the Savant vacuum concentrator, dissolved the oily residue in 5 mL of a solution of acetonitrile:water:acetic acid (10:89.8:0.2), and applied the solution to the same type of Cl8 solid-phase extraction cartridge as was described previously. We washed the cartridge with 5 mL of a solution of acetonitrile:water:acetic acid (55:44.8:0.2). We eluted the metolachlor mercapturate with a solution of acetonitrile:water:acetic acid (80:19.8:0.2), and dried the eluate in the Savant centrifugal evaporator. We corroborated the synthesis of metolachlormercapturate on the basis of the LC-MS/MS spectrum (Fig. 1) using the isocratic chromatographic method described below. We estimated the product to be at least 98% pure on the basis of analysis by LC-MS using a scan range from 50 m/z to 1000 m/z; the same column as described below was used with a gradient from 10% methanol in water with 0.1% acetic acid to 95% methanol in water containing 0.1% acetic acid over 10 min with a 5 min 95% methanol isocratic phase.

We developed a solid-phase extraction method for metolachlor mercapturate using control urine spiked with metolachlor mercapturate. We passed the urine sample (5 mL) through a solid-phase cartridge containing 500 mg Cl8 packing (Analytichem, Harbor City, CA), which had been washed with 2 mL of methanol, followed by 8 mL deionized water. The cartridge was washed with 5 mL of deionized water and the eluate discarded. Metolachlor mercapturate was eluted with 3 mL of methanol. We concentrated the eluate to 200 uL in a Savant vacuum concentrator (Farmingdale, NY). Recovery of metolachlor mercapturate from urine ranged between 60% and 75% in three analyses of 25 ppb spiked urine.

We used a Finnigan TSQ-7000 triple quadrupole mass spectrometer (San Jose, CA) in the atmospheric pressure chemical ionization (APCI) LC-MS/MS mode. The liquid chromatography column was a Whatman ODS-3 (4.6 mm x 25 cm) (Clifton, NJ). The mobile phase was water:methanol

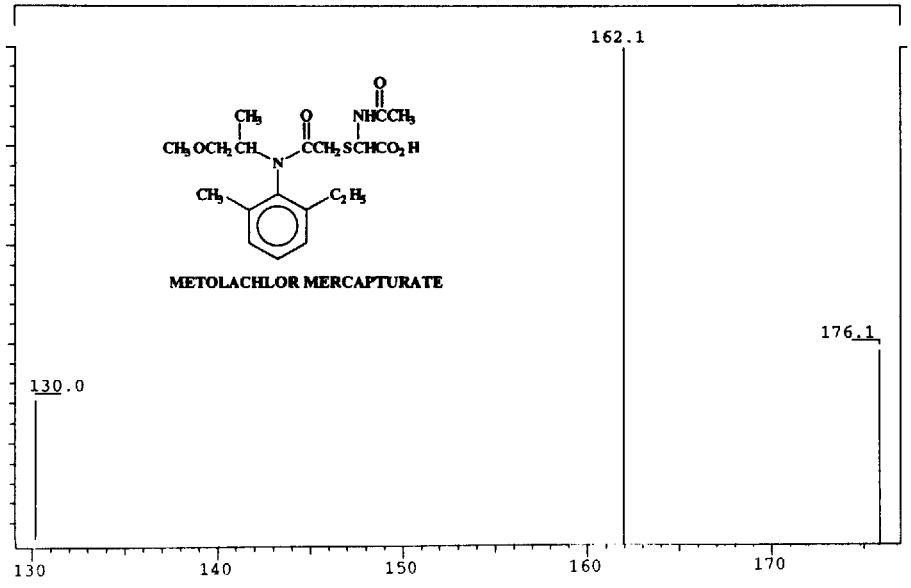

Figure 1. Structure and MS/MS spectrum (411 m/z parent) of metolachlor mercapturate; mass vs. relative intensity

(3:7 v/v) with 0.1% acetic acid; at a flow rate of 1 mL/min, the elution time was about 7.5 min. The mass spectrometer was set in the positive ionization MS/MS mode with the collision offset at -20 V. The 411 m/z MH^+ parent ion fragmented to three major daughters: 1) 130 m/z, the product of an N-acetylcysteine moiety in which the sulfur-carbon bond is cleaved 2) 162 m/z, the loss of the amine side-chain that includes N-acetylcysteine and the loss of CH20CH3 from the other amine side-chain 3) 176 m/z, the loss of the amine side-chain that includes N-acetylcysteine and the loss of OCH3 from the other amine side-chain. We set up the mass spectrometer to analyze each of the three daughters in the single reaction monitoring mode (SRM). A standard curve using spiked urine samples with concentrations of metolachlor mercapturate of 10 ppb, 50 ppb, and 250 ppb was linear. The limit of detection was 3 ppb with a 5 mL urine sample volume.

RESULTS AND DISCUSSION

In the light of the absence of information about the metabolism and excretion of metoachlor, we hypothesized that metolachlor mercapturate was a urinary metabolite in humans exposed to metolachlor. We synthesized the metolachlor mercapturate standard (Figure 1) and screened 86 urine samples for metolachlor mercapturate from agricultural workers and their families participating in the Agricultural Health Study. Nine of these samples had

serum levels of metolachlor above 200 ppt (personal communication, John Barr, CDC, 1996). We found metolachlor mercapturate in 10 of the 86 samples and in 7 of 9 of the samples from subjects whose serum had metolachlor concentrations above 200 ppt. The urine metolachlor mercapturate concentrations ranged between 5 and 300 ppb.

We have shown that metolachlor, like alachlor, is metabolized to the mercapturate. It may be that there are other analogous metabolites. Five alachlor metabolites, alachlor mercapturate, alachlor cysteine, alachlor mercaptoacetate, secondary amide mercapturate, and alachlor O-glucuronide (Carr et al., 1986) were identified in monkeys by following the fate of radioactively labelled alachlor. In another study using radioactively dosed monkeys, alachlor mercapturate was found to be the major metabolite, accounting for about 40% of the metabolite concentration (Feng et al., 1994). We (Driskell et al., 1996) found that alachlor mercapturate made up between 25% and 62% of the total alachlor metabolite concentration in the human urine samples that we analyzed.

We analyzed the same collection of samples as described above for N-(l'-methyl-2'-hydroxyethyl)-2-ethyl-6-methylaniline and 4-(2-ethyl-6-methylphenyl)-2-hydroxy-5-methyl-3-morpholinone. On the basis of information about metolachlor furnished by the manufacturer of metolachlor (Ciba-Geigy, Greensboro, NC), we had selected these two metabolites as possible human markers for metolachlor exposure. We were unable to detect either of these compounds in any of the urine samples with limits of detection of about 1 ppb.

The mechanism for the metabolism of pesticides, drugs, and xenobiotics to thioether conjugates has been elucidated (Stanek et al., 1993). These compounds react with the tripeptide glutathione (GSH = g-Glu-Cys-Gly) . The products are then metabolized by the sequential loss of the glutamic acid residue, the glycine residue, and N-acetylation of the remaining S-substituted cysteine (Stanek et al. 1993). In addition to alachlor, lindane, cyanatryn (Aizawa 1982), and atrazine (Lucas et al., 1993), and xenobiotics, including acrolein, benzene, vinyl chloride, and various allylic and benzylic halides (Stanek et al., 1993), are metabolized through the glutathione pathway.

In this study, we have identified a major human metabolite of metolachlor, developed an LC-MS/MS method to measure it in urine, and shown that it is present in the urine of exposed individuals. We believe this metabolite will be useful as a marker for metolachlor

exposure, and we will investigate its usefulness by including it in future studies of pesticide exposure.

REFERENCES

Aizawa H (1982) Metabolic maps of pesticides. Academic Press Inc., New York. p 76 and p 216.

Alavanja MCR, Akland G, Baird D, Blair A, Bond A, Dosemeci M, Kamel F, Lewis R, Lubin J, Lynch C, McMaster SB, Moore M, Pennybacker M, Ritz L, Rothman N, Rowland A, Samdler DP, Sinha R, Swanson C, Tarone R, Weinberg C, Zahm SH (1994) Cancer and noncancer risk to women in agriculture and pest control: the Agricultural Health Study. J Occup Med 36:1247-1250.

Carr KH, Chott RC, Howe RK, Malik JM (1986) Metabolism of alachlor in the rhesus monkey. The Sixth International Congress of Pesticide Chemistry, IUPAC, Ottawa, Canada, Abstract 7c-06.

Driskell WJ, Hill RH, Shealy DB, Hull RD, Hines CJ (1996) Identification of a major human urinary metabolite of alachlor by LC-MS/MS. Bull Environ Contam To x i c o l 156:853-859.

Feng PCC, Sharp CR, Horton SR (1994) Quantitation of alachlor residues in monkey urine by ELISA. J Agric Food Chem 42:316-319.

Lucas AD, Jones AD, Goodrow MH, Saiz SG, Blewett C, Seiber JN, Hammock BD (1993) Determination of atrazine metabolites in human urine: development of a biomarker of exposure. Chem Res Toxicol 6:107-116.

Stanek W, Krenmayr P, Scherer G, Schmid ER (1993) Quantitative Determination of N-acetyl(-L-)cysteine derivatives in human urine by tandem mass spectrometry. Biol Mass Spec 22:133-142.

U.S. Environmental Protection Agency, (1991) EPA's Pesticide Programs, 21T-1005. Office of Pesticides and Toxic Substances, Washington DC. pp 132-139.