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Determination and Metabolism of Ethylene Dibromide in Minipigs

Kenneth W. Kirby*, Helen Tremmel*, and Jeffrey E. Keiser†

Institute of Agricultural Medicine, Department of Preventive Medicine and Environmental Health, University of Iowa, Oakdale Iowa 52319 †Department of Chemistry, Coe College, Cedar Rapids, Iowa 52402

Ethylene dibromide (EDB, 1,2-dibromoethane) is used as a gasoline additive and as a fumigant, particularly for stored grains. Over 300 million pounds are sold in the United States annually. WARD (1974) and OLSON (1973) have reported that EDB appears to induce squamous cell carcinomas in the stomachs of rats when administered by gastric intubation. Oral ingestion of EDB has led to liver necrosis and kidney tubular damage (OLMSTEAD 1960, INTERNATIONAL 1972).

To begin an investigation of the potential risk to humans from EDB exposure, we have developed a convenient gas chromatographic method for ppb analysis for EDB in biological fluids, conducted a feeding study with Pitman-Moore minipigs, and determined the serum levels of bromide ion resulting from metabolism of EDB.

MATERIALS AND METHODS

Analysis. EDB was obtained from Eastman Kodak. Hexane was from Burdick and Jackson distilled in glass. All other chemicals were reagent grade materials from commercial suppliers. Gas chromatography was performed using a ^{3}H electron capture detector and a 1.2 m X 4 mm i.d. glass column packed with 0.2% Carbowax 1500 on 80/100 mesh Carbopack C.(Supelco). Inlet, column, and detector temperatures were 180, 100, and 200° C, respectively; nitrogen carrier gas flow was 90 mL/min. EDB exhibited a retention time of 4 min and 40 pg gave 20% fsd. Visible spectroscopy was conducted at 470 nm.

Both urine and serum were analyzed for EDB by extracting them in 15-mL Teflon-lined screw cap centrifuge tubes for 1 h on a Fisher-Roto Rack Mixer, centrifuging in a table top centrifuge for 30 min followed by injection of 2 µL aliquots of the hexane layer without further preparation.

Urine and serum were analyzed for inorganic bromide using the method of DAVIDSON (1969). The method is sensitive to levels of about 10 mg percent (100 ppm).

Feeding Studies. Three types of feeding studies were conducted with 4 month-old Pitman-Moore minipigs. In all cases the EDB was administered by dissolving it in corn oil and mixing the solution with a small quantity of pulverized feed. The treated feed was fed to the pigs each morning, followed by the remainder of a regular ration. In this manner the entire dose was consumed. The three feeding

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studies were:

1. Two 18 kg pigs were each fed 1, 5, 20, 100 mg/day dosages on 4 consecutive days.

2. One 18 kg female was fed 1 g/day for 14 days.

3. One 25 kg male pig was fed 1 g/day for 9 weeks.

Blood and urine samples were taken regularly for all tests. Studies #2 and #3 represent feeding at about one-third and one-fourth of the LD_{50} for an adult male rat respectively.

Direct Injection of EDB. A 1 g sample of EDB was dissolved in sufficient ethanol to make a total volume of 1 mL and injected into a catheter which extended into the vena cava of a 22 kg male minipig.

RESULTS AND DISCUSSION

Chemical Analysis. Various analyses have been reported for EDB, most of them utilizing gas chromatography. An English PANEL (1974) has suggested that either Apiezon L or polypropylene glycol columns might be used with electron capture detection for analysis of EDB extracts from grain. RUSSELL (1975) has used various columns and flame ionization detection to analyze EDB in adsorbed air pollution samples. SAITO (1978) has used a mixed phase of SF-96 and polyethylene glycol to analyze EDB in water and bottom mud.

We found that non-polar columns such as OV-1 did not retain the EDB long enough for our purposes and polar columns gave excessively broad peaks. The Carbowax 1500 on Carbopack packing, which we eventually utilized, is the same one recommended by the U.S. Environmental Protection Agency for the analysis of priority pollutants. Use of this column with a tritium electron capture detector offers several advantages. The EDB peak is very sharp, free of interferences, and concentrations as low as 50 ppb may be quantitated without concentration. Quantities as low as 40 pg were readily detected. Extracts from predose urine and serum were free of peaks in the EDB region. Recoveries from fortified samples averaged 80 %.

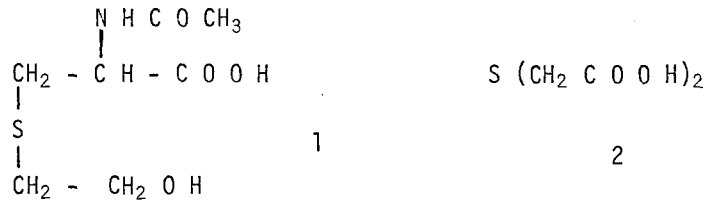
Feeding Studies. Analysis of serum from pigs fed 100 mg or less failed to show any quantity of EDB at 0.05 ppm, the detection limit. Analysis of pig serum 15 min after feeding of 1 g of EDB revealed a serum level of 1 ppm (about 0.1 % of the dose based on blood volume). Examination of the urine failed to detect EDB at 0.1 ppm, the detection limit.

These data are consistent with very rapid metabolism of EDB. Other workers have found evidence for rapid metabolism. JONES & EDWARDS (1968) reported that EDB is rapidly metabolized by the rat. PLOTNICK & CONNER (1976) fed radiolabeled EDB to guinea pigs and found 66 % of the dose excreted within 72 h. HILL et al. (1978) found enzymatic metabolism of EDB by rat liver transferase *in vitro* to be very rapid.

To gain insight into the rate of metabolism, a 1 g sample of EDB was mixed with ethanol in sufficient quantity to yield a 1 mL volume and injected into the vena cava of a male minipig and samples withdrawn for analysis every few minutes. Results show that the level of EDB in the serum decreased from a high of about 130 ppm in 10 min to 5 ppm in 120 min indicating a very rapid metabolism of an exceedingly high dosage. Direct injection bypasses the liver and the metabolic transformation normally effected during oral ingestion is avoided. The animal survived the treatment although an immediate effect on the heart was evident.

No bromide was found in the serum of the injected minipig. Analysis for bromide in the serum of orally fed minipigs showed a detectable amount 2 h after feeding 1 g of EDB. Continued feeding up to 9 weeks showed a gradual increase up to 7 weeks after which the level began to decrease. These observations could suggest a slow adaptation of the animal to the constant level of dosage or a gradual loss of capability to form bromide ion as a means of removing the toxicant from its system. The need for long-term studies is strongly indicated.

Metabolic products of EDB have been studied. NACHTOMI (1970) and NACHTOMI et al.(1966) found S-(β-hydroxyethyl)-N-acetylcysteine (1) and the related sulfoxide. They have proposed initial conjugation with glutathione to account for these products. HILL et al. (1978) have confirmed that EDB is a substrate for rat liver glutathione-S-transferase.



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WATANBE (1979) found these substances plus a third compound, tentatively identified as thiodiglycolic acid (2). Efforts are currently underway in these laboratories to identify the metabolites produced by minipigs.

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