other degradation pathways. Reduction of the γ-BHC level in cooked meat is not yet detoxification; from the toxicological aspect, CB is of similar toxicity to γ-BHC.

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Identification of *N*-Methylsaccharin as a Peak at the Retention Time of Methylated (2,4,5-Trichlorophenoxy)acetic Acid in Human Urinary Exposure Samples

Human exposure to (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) may be detected by analysis of a 24–48 h postexposure urine sample. Acid hydrolysis of the urine followed by extraction with benzene and methylation of the extract with diazomethane will yield a gas chromatographic peak when a 6 ft long by 4 mm i.d. 4% SE-30/6% OV-210 column is used that matches that of a similarly treated standard of 2,4,5-T. Evidence is shown that *N*-methylsaccharin has an almost identical retention time and can be confused with 2,4,5-T methyl ester. Saccharin in diet drinks provides adequate amounts of urine saccharin for detection. GC/MS confirmation analysis supports the identification of *N*-methylsaccharin at the same retention time. Alternate columns can be used to detect 2,4,5-T exposure by using the same hydrolysis and extraction procedure. These would include a 6 ft long × 4 mm i.d. 1.5% OV-17/1.95% OV-210 and a 15 ft long by 4 mm i.d. SE-30/OV-210 column, both of which resolve the 2,4,5-T and saccharin peaks. Human exposure to 2,4,5-T is frequently determined by use of a modified Bevenue procedure (Bevenue et al., 1968) as described by Rivers et al. (1970). The procedure requires the benzene extraction of an acid-hydrolyzed urine or blood sample, followed by methylation of the extract with diazomethane. Determination is by electron-capture GC. Of the several options for column selection in the GC determination, it is frequently a choice of the analyst to use 4% SE-30/6% OV-210 as the liquid phase because of its general applicability to pesticide chemistry (Watts, 1980). This paper will describe work wherein an analyst can easily report a false positive for 2,4,5-T when the compound may actually be *N*-methylsaccharin [2-methyl-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide]. Diet drinks commonly yield enough saccharin in the urine so that a strong indication of 2,4,5-T could easily be suggested if a 6 ft long SE-30/OV-210 column is used for GC separation of peaks. Obviously, other column selections should be made, and the present work describes some successful alternatives.

EXPERIMENTAL SECTION

Apparatus. A Tracor Microtek 220 gas–liquid chromatograph equipped with a tritium electron-capture detector was fitted with a borosilicate glass column $^1/_4$ in. o.d. × 4 mm i.d. × 6 ft long. The column was packed with 4% SE-30/6% OV-210 on Gas-Chrom Q (80–100 mesh) and conditioned as described in the manual of analytical methods for the analysis of pesticide residues in human and environmental samples (Watts, 1980). Conditions for operating the chromatograph include the following: nitrogen flow, 100 mL/min.; column temperature, 160 °C; inlet temperature, 235 °C; detector temperature, 210 °C. The chart speed was $^1/_4$ in./min. The mass spectra were recorded on two separate instruments. Samples from subjects D.G. and D.M. were run on a Hewlett-Packard 5985, while the sample from subject M.C. was run on a Finnegan 4023 GC/MS/data system.

Reagents. Solvents were obtained from Burdick and Jackson, Muskegon, MI. Saccharin was purchased from Pfaltz and Bauer, Stanford, CT. Diazomethane was prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine purchased from Aldrich Chemical Co., Milwaukee, WI. Sulfuric acid was reagent grade, and a 0.1 N solution was extracted with Burdick and Jackson benzene prior to use. 2,4,5-T was from the EPA repository at Research Triangle Park, NC. Standard solutions were prepared in benzene with suitable dilutions. The diet cola consumed was a commercial product.

Sample Handling. Two subjects, M.C. and D.G., submitted urine samples after having had an alleged exposure to 2,4,5-T. The urine samples were collected several days postexposure and examined by the Bevenue–Rivers method. In both instances when the sample was analyzed by using the 6 ft SE-30/OV-210 column, a significant peak resulted within 0.5 mm or less of a similarly treated standard of 2,4,5-T. Because of the importance of identifying the 2,4,5-T, each sample was subjected to electron-impact GC/MS analysis. In Figure 1 are shown the spectra of the compounds isolated from two urine samples at the principal retention time of the methyl ester of

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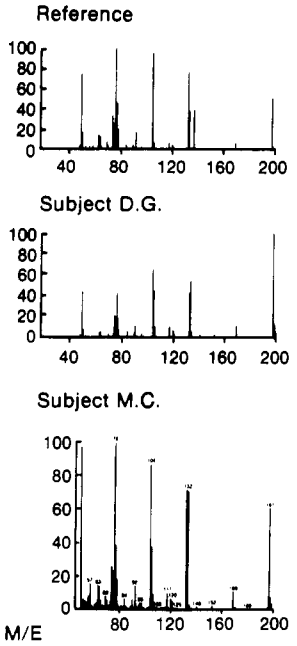


Figure 1. GC/MS of 2-methyl-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide(*N*-methylsaccharin) human urine extracts.

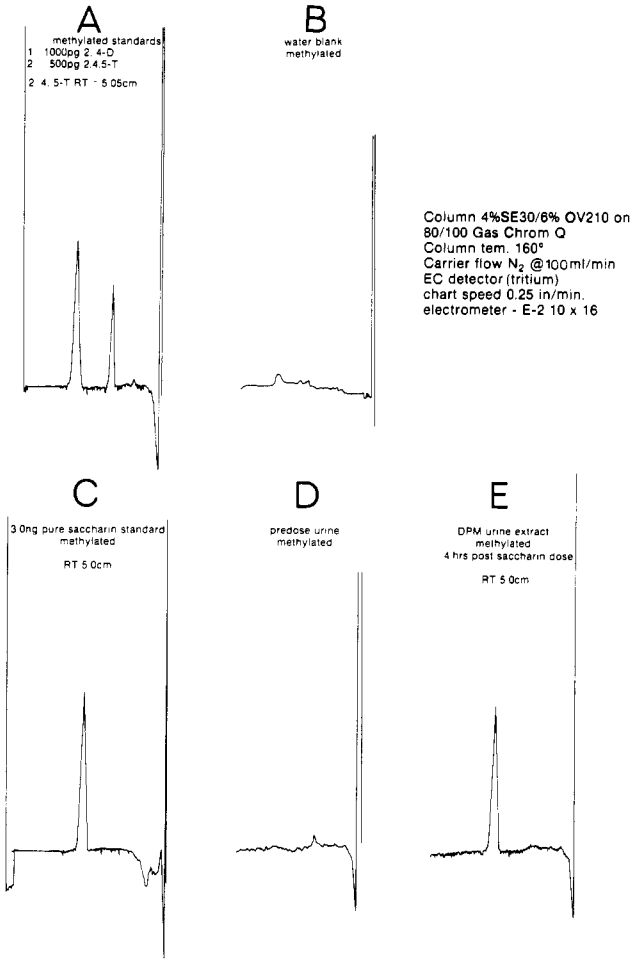
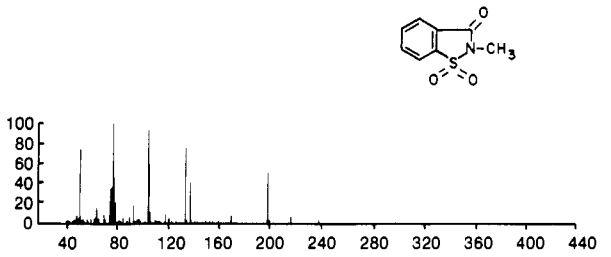


Figure 2. Gas chromatographic separation of 2,4,5-T and *N*-methylsaccharin on a 4% SE/6% OV-210, 1.8 m × 4 mm. i.d. column.

2,4,5-T. These show that the two samples have matching mass spectra. A search of the library of compounds with existing spectra indicated that only *N*-methylsaccharin matched these spectra. Thus, a follow-up experiment was conducted by having subject D.M. consume a single can



Subject: DPM, 4 hr post dose urine extract, Diet Pepsi

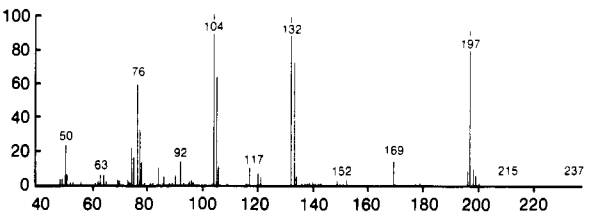


Figure 3. GC/MS of 2-methyl-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (*N*-methylsaccharin) postdose urine extracts.

of diet cola. A urine sample was collected 4-h postdosage and analyzed by the same Bevenue–Rivers procedure. There was no exposure to 2,4,5-T nor had subject D.M. consumed any saccharin-containing product immediately prior to the feeding.

Gas Chromatographic Analysis. Subjecting samples to the same procedure (Bevenue–Rivers), a group of gas chromatographic comparisons were developed to demonstrate the problem an analyst could face by selection of the conditions suggested in the procedure. In Figure 2 A-E, are shown comparative peaks obtained from (A) a methylated standard of (2,4-dichlorophenoxy)acetic acid (2,4-D) and 2,4,5-T, (B) a water blank extracted and methylated as one would extract a urine sample, (C) a 3-ng injection of a standard of methylated saccharin, (D) a predose urine sample, and (E) a urine sample from a subject, D.P.M., consuming a diet cola taken 4-h postdose. These chromatograms show the peaks obtained from the various substances. Confirmation of *N*-methylsaccharin was obtained by isolation of the peak in Figure 2e at the same retention time as methylated 2,4,5-T and subjecting it to electron-impact GC/MS analysis. The spectrum is shown in Figure 3.

Recommended Gas Chromatographic Analysis. Urine samples from human exposure situations may be extracted and prepared according to the Bevenue–Rivers procedure. However, gas chromatography would best be done by using alternate columns or a column of length sufficient to effect separation of *N*-methylsaccharin from methylated 2,4,5-T. A 6 ft long × 4 mm i.d. column of 1.5% OV-17/1.95% OV-210 effects a good separation (5.5 cm for *N*-methylsaccharin vs. 6.3 cm for methylated 2,4,5-T). Likewise, a 15 ft long × 4 mm i.d. SE-30/OV-210 column operated at 200 °C makes a satisfactory separation of the two substances, 7.0 vs. 8.75 cm, respectively, other conditions being equal.

RESULTS AND DISCUSSION

A number of procedures are available to the analyst for determination of 2,4,5-T residues in human urine, and the one described here is recommended [see Watts (1980, Section 5,A,4,C)]. The data in Figure 2 demonstrate that

a false positive may result if a 6 ft long × 4 mm i.d. SE-30/OV-210 column operated at 165 °C is used to compare the retention time with that of a methylated 2,4,5-T standard.

Because of the data found, another usual method for determining human 2,4,5-T exposure through urine analysis was investigated. The so-called multiphenol method (Shafik et al., 1973) has been used in our laboratory for analysis of about 5000 human urine samples. One of the substituents determined is 2,4,5-T. Extraction of the acid-hydrolyzed sample into diethyl ether could solubilize some saccharin. However, the acid hydrolysis step in the multiphenol method is adequate to convert most of the saccharin to the ammonium *o*-carboxybenzenesulfonate (DeGarmo et al., 1952). Subsequent columning on silica gel retains saccharin and the ammonium *o*-carboxybenzenesulfonate. Thus, ethylation of an 80% benzene–20% hexane eluate does not contain *N*-ethylsaccharin and would not possibly be confused with ethylated 2,4,5-T.

We have noticed in chromatograms where *N*-methylsaccharin was observed that a second, late-emerging peak appears to be characteristic of urine excreted following saccharin exposure. The relative retention time to *N*-methylsaccharin is about 3.5–3.6. No attempt has been made to identify this peak.

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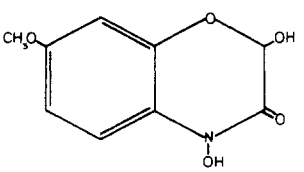
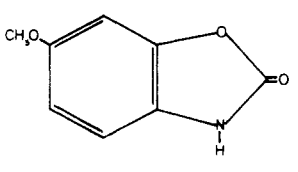
A High-Performance Liquid Chromatographic Method for Quantitation of DIMBOA and MBOA in Maize Plant Extract

A high-pressure liquid chromatographic (LC) method for quantitation of DIMBOA [2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxacin-3(4*H*)-one] and its main degradation product MBOA [6-methoxy-2-(3*H*)-benzoxazolinone] in aqueous extract from corn (*Zea mays* L.) is described. After recovering DIMBOA and MBOA from the aqueous extract with ethyl acetate, underivatized samples were chromatographed on LiChrosorb SI 100 by using a hexane–ethanol gradient and monitored at 288 nm. A typical LC pattern shows five main peaks, two of them eluting respectively at the same position as the MBOA and DIMBOA standards and enhanced with the addition to the sample of these compounds. Variation coefficients of 1.11% and 1.07% were respectively obtained for the molar responses of MBOA and DIMBOA standards. For the retention times coefficients of variation of 0.011% for MBOA and 0.019% for DIMBOA were determined. The samples recoveries ranged from 104 ± 2% for DIMBOA to 106 ± 3% for MBOA.

DIMBOA (1) is a cyclic hydroxamic acid first isolated from wheat and corn as a β-D-glucopyranoside (Wahlroos and Virtanen, 1959). The glucoside is enzymatically transformed into the aglycon by the action of β-glucosidases upon crushing the plant tissue (Wahlroos and Virtanen, 1959). The aglycon is converted into MBOA (2), its corresponding benzoxazolinone, by heating in aqueous solutions (Virtanen and Wahlroos, 1963), although a 100% conversion rate varies with temperature, pH, and some unknown working conditions (Woodward et al., 1978).



1 DIMBOA



2 MBOA

The biological role played by DIMBOA is as yet little understood. Nevertheless, this compound has been considered by several authors to be directly implicated in resistance to some pests and diseases as well as in detoxification of triazine herbicides as an iron carrier [for a

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