

AD-A237 848

ATION PAGE

Form Approved  
OMB No. 0704-0188Pu  
ga  
col  
Da

Average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering the collection of information. Send comments regarding this burden estimate or any other aspect of this form to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 17 June 1991	3. REPORT TYPE AND DATES COVERED ANNUAL 01 Jun 90 to 31 May 91	
4. TITLE AND SUBTITLE A Study of the Effect of Hydrocarbon Structure on the Induction of Male Rat Nephropathy and Metabolite Structure			5. FUNDING NUMBERS PE - 61102f PR - 2312 TA - A5 GR - AFOSR-89-0396	
6. AUTHOR(S) M. Paul Serve'				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Wright State University Department of Chemistry Dayton, OH 45431			8. PERFORMING ORGANIZATION REPORT NUMBER AFOSR-TR- 91 0622	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Office of Scientific Research Life Sciences Directorate Building 410 Bolling AFB DC 20332-6448			10. SPONSORING/MONITORING AGENCY REPORT NUMBER ✓	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE A-1	
13. ABSTRACT (Maximum 200 words) The identification of the urinary metabolites have been identified using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Histopathologic examination of the kidneys revealed minimal hyaline droplet formation (α <sub>2</sub> u-globulin nephropathy) in the proximal tubule area from 2-methylheptane (2-MH). While 2,5-dimethylhexane (2,5-DMH) revealed moderate hyaline droplet formation in the renal proximal tubule. Work is currently progressing on the metabolism of 3-methylheptane. There are 16 separate metabolites which have been found, but only 3 have been identified. This molecule's metabolism is very complex. Pathological results indicate that it is more nephrotoxic than 2-methylheptane or n-octane, two structural isomers.				
14. SUBJECT TERMS			15. NUMBER OF PAGES 9	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT (U)	18. SECURITY CLASSIFICATION OF THIS PAGE (U)	19. SECURITY CLASSIFICATION OF ABSTRACT (U)	20. LIMITATION OF ABSTRACT (UNLIMITED)	

## THE METABOLISM OF 2-METHYLHEPTANE IN MALE FISCHER 344 RATS

M. Paul Serve<sup>a</sup>, D.D. Bombick<sup>a</sup>, J. Matthew Clemens<sup>a</sup>,  
Gayle A. McDonald<sup>a</sup> and David R. Mattie<sup>b</sup>

<sup>a</sup> Department of Chemistry, Wright State University, Dayton, OH 45435

<sup>b</sup> Harry G. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH 45433.

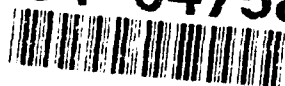
### ABSTRACT

The urinary metabolites of 2-methylheptane (2-MH) in male Fischer 344 rats, administered the hydrocarbon by gavage, included 2-methyl-1,2-heptanediol, 2-methyl-1,5-heptanediol, 2-methyl-2,5-heptanediol, 2-methylheptanoic acid and 2-methyl-5-hydroxy-1-heptanoic acid. Metabolism strongly favored the formation of diols. The metabolites were identified using gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Histopathologic examination of the kidneys revealed minimal hyaline droplet formation ( $\alpha_2$ -globulin nephropathy) in the proximal tubule area.

### INTRODUCTION

2-Methylheptane (2-MH), an isomer of octane is a component of crude oil and a product of gasoline combustion engines [1,2]. It is not surprising, therefore, that 2-MH has been found as a component of the air pollution of major cities [3,4]. As a constituent of tobacco smoke, 2-MH is frequently passively taken into the body [5,6]. 2-MH has been identified as a volatile chemical emitted from plastic building materials [7] and as a trace compound in landfill gas [8]. Isomers of octane, when injected into animals, have been shown to produce hemorrhage, edema and polymorphonuclear leukocytic reactions such as angitis, abscess formation, thrombosis and fibrosis [9].

91-04758



91

11

052

Certain branched chain isomers of octane e.g. 2,2,4-trimethylpentane (2,2,4-TMP) and 2,3,4-trimethylpentane (2,3,4-TMP) have been reported to induce a proximal tubular nephropathy in male rats [10,11]. This nephropathy is characterized by the inability of the rat to breakdown the testosterone controlled protein  $\alpha_2$ -globulin through the normal lysosomal enzyme processes [12]. 2-MH, similar to other hydrocarbons, is a totally non-polar molecule. Hydrocarbons once in the animal body, are normally gotten rid of by either exhalation or excretion via the urine. The latter process requires metabolic conversion of the hydrocarbon into a water soluble derivative. It is the production of metabolites with certain functionalities, which, by tying up the  $\alpha_2$ -globulin and inhibiting its degradation, is generally considered to be responsible for the hydrocarbon-induced nephropathy cited above.

Because of its ubiquitous nature in the environment and since many people will be exposed to various concentrations of 2-MH, it was decided to examine the metabolic fate of 2-MH in male rats. Male rats are frequently used to study the metabolism of xenobiotic materials because of the similarity of biotransformation reactions that exist between man and rat. Secondly, a comparison of the ability of 2-MH to 2,2,4- and 2,3,4-TMP to induce the male rat nephropathy would yield interesting information regarding the extent and positioning of structural branching necessary to induce the nephrotoxicity.

## MATERIALS

2-Methyl-1-heptene, 6-methyl-1-heptene, 6-methyl-6-hepten-3-ol, and 2-ethylcyclopentanone were purchased from Wiley Organics, Coshcoton, OH. 2-Methyl-1,2-heptanediol and 6-methyl-1,2-heptanediol were prepared by the reaction of 2-methyl-1-heptene and 6-methyl-1-heptene, respectively with osmium tetroxide [13]. 2-Methyl-1,5-heptanediol was synthesized by the hydroboration of 6-methyl-6-hepten-3-ol [14]. 2-Methyl-2,5-heptanediol was obtained via the mercuric acetate-sodium borohydride reaction of 6-methyl-6-hepten-3-ol [15]. 2-Methylheptanoic acid was prepared using literature [16]. Treatment of 2-ethyl-5-methylcyclopentanone, obtained from the alkylation of the pyrrolidine enamine of 2-ethylcyclopentanone, with trifluoroacetic acid gave 2-methyl-5-heptanolactone [17].

## METHODS

Twelve Fischer 344 male rats weighing  $237 \pm 16$  grams were randomly divided into two groups (8 treated, 4 control). Dose (0.8 g/kg) of 2-MH were administered by gavage on an every other day regimen for two weeks. Feed (Purina Rat Chow, Ralston Purina Co., St. Louis, MO) and water were provided ad libitum and animals were weighed daily.

Following the 14 day exposure period, the rats were sacrificed by halothane overdose and the kidneys were excised 24 hours following the final dose. Histopathologic examination was performed on paraffin embedded kidney sections stained with hematoxylin and eosin. Tissues from treated rats were compared to controls for characteristic lesions of hydrocarbon-induced nephropathy, including hyaline droplet formation, tubular cysts and papillary calcification. Lesions were graded by pathologists for degree of severity.

During the first 48 hours of the initial dosing period, the rats were placed in metabolism cages and the urine collected. A 5.0 mL aliquot of each urine sample was adjusted to a pH of 4.0 and 0.2 mL glucuronidase/sulfatase (Calbiochem, La Jolla, CA) was added. The sample was shaken for 16 hours at  $37^{\circ}\text{C}$ , then cooled to room temperature and filtered through a diatomaceous earth column (Clin Elut, Analytichem International Harbor, CA) using methylene chloride as the eluent.

The methylene chloride extracts of the hydrolyzed rat urine were analyzed on a gas-liquid chromatograph (GC) equipped with a flame ionization detector (model 3500, Varian Corp., Walnut Creek CA). A 30 m x 0.32 mm I.D. Carbowax 20M on polyethylene glycol column (Alltech Associates Inc., Deerfield IL) with injection port and detection temperatures of  $250^{\circ}\text{C}$ . The oven temperature was programmed to rise from  $100^{\circ}\text{C}$  to  $200^{\circ}\text{C}$  at a rate of  $50^{\circ}\text{C}/\text{min}$  and helium was used as the carrier gas. Additional metabolite identification was accomplished using a Finnegan MAT INCOS 50 -Varian 3400 gas chromatography/mass spectrometer (GC/MS) system (Varian Corp., Walnut Creek, CA). The GC was equipped with identical column as the gas chromatograph above while the injection port and the oven temperatures were the same as previously reported. Helium was the carrier gas. The MS was a quadrupole instrument operated in the electron impact mode with a voltage of 70eV and an ion source of  $200^{\circ}\text{C}$ .

The difficulty in totally separating all the products (including control metabolites) in the rat urine made quantitation of the metabolites impossible. However, relative areas under the peaks, listed in Table 1 were obtained from the GC program.

## RESULTS

The rat urine samples were hydrolyzed with glucuronidase/sulfatase and elutants were individually analyzed by GC and GC/MS for the identification of the volatile urinary metabolites. A representative GC chromatogram is shown in Figure 1. GC analysis of rat urines not treated with glucuronidase/sulfatase showed no trace of 2-MH metabolites.

Histopathologic results indicated that 2-MH graded out on a level of 1 (based on a scale of 0 to 4 where 0 indicated no damage and 4 indicated severe damage). A grade of 1 suggests minimal damage to the proximal tubule. n-Octane graded out at a level of 0 whereas 2,5-dimethylhexane (2,5-DMH), another isomer of octane produced nephrotoxic damage rated at a level of 3 [18]. 2,2,4- and 2,3,4-TMP produced severe damage to the male rat proximal tubule which graded out to a level of 4. In none of the rats dosed with 2-MH was there any indication of cast formation. There was no trace of any 2-MH metabolites extracted from the homogenized kidneys of the dosed rats. No hyaline droplet formation was noted in the control animals.

The GC tracings of the animals dosed with 2-MH established the presence of the following urinary metabolites (relative abundancies) 2-methyl-1,5-heptanediol (1.0), 2-methyl-1,2-heptanediol (1.25), 2-methylheptanoic acid (1.4) 2-methyl-5-heptanolactone (1.6) and 2-methyl-2,5-heptanediol (1.8). The relative abundancies were determined by integrating the areas of GC tracings, using tridecane as an internal reference standard, and assuming relative equal detection efficiencies. A representative GC tracing of the urine metabolites of 2-MH is shown in Figure 1. The metabolite 2-methyl-5-heptanolactone was not a true metabolite, but was the result of cyclization of 2-methyl-5-hydroxy-1-heptanoic acid under the elevated injection port temperature conditions of the GC. It has been previously shown that lactone formation of hydroxy acids, in which the hydroxy group and the acid function are separated by 4 or 5 carbons, readily occurs. The identification of the lactone structure was accomplished by

examining the MS fragmentation pattern of the lactone. A major MS fragmentation peak of the lactone corresponded to the molecular ion -  $C_2H_5$ . A characteristic fragmentation pattern of  $\delta$ -lactones is the loss of the alkyl group attached to the  $\delta$ -carbon [19]. In addition, MS fragmentation peaks at  $m/z=56$  and 70 were also indicative of a  $\delta$ -lactone. To confirm the presence of the 2-methyl-5-ethylcyclopentanone was oxidized to a mixture of 2-methyl-5-heptanolactone and 2-ethyl-5-hexanolactone via the Baeyer-Villager reaction. Known samples of the hydroxy acid, the lactone and the urine were subjected to thin layer chromatography (tlc) using ethyl acetate as the eluent. The  $R_f$  of the known hydroxy acid matched the  $R_f$  of a metabolite in the urine sample. No tlc spot in the urine sample matched the  $R_f$  of the lactone.

Comparing the metabolic pathways of 2-MH to those of other branched chain acyclic hydrocarbons reveals similarities and differences in the structure and abundancies of the various types of metabolites isolated. 2-MH akin to 2,5-dimethylhexane (2,5-DMH) was metabolized to diols, which were positioned at the 1,2- 1,5- and the 2,5-sites [19]. In addition both 2-MH and 2,5-DMH yielded the corresponding 2-alkyl alkanolic acids and the 2-alkyl-5-hydroxy-alkanoic acids. In neither case was there any trace of an alcohol. A difference in the metabolism of 2-MH and 2,5-DMH was in the relative abundancies of the urinary metabolites found. In the instance of 2-MH the metabolites were isolated in almost equal amounts, while for 2,5-DMH, there was a wide range in the quantities of metabolites produced. The 2,5-dimethyl-2,5-hexanediol and the 2,5-dimethyl-5-hydroxy-1-hexanoic acid were found in abundancies 38.7 and 8.3 times the amount of 2,5-dimethyl-1-hexanoic acid, the least abundantly found metabolite. The highly branched chain hydrocarbons 2,2,4- and 2,3,4-TMP were metabolized to monoalcohols, carboxylic acids and 5-hydroxy-carboxylic acids; there was no trace of diol formation.

Unlike n-octane, which yielded urinary metabolites containing less than 8 carbons, there was no vestige of any urinary metabolites of 2-MH in which carbon atoms had been lost [20].

In conclusion, limiting the substitution of an octane isomer to a methyl group at the 2-position, changes the metabolism from that of n-octane. The structure of the urinary metabolites of 2-MH appear to resemble the metabolites of 2,5-DMH, the only difference being in the relative amounts of the metabolites formed. In the ability to induce renal proximal tubular damage to male rats, 2-MH more closely resembles n-octane than 2,5-DMH. This would lead to the

conclusion that the more closely related in structure an octane molecule is to n-octane, the less the ability of the metabolites to interfere with  $\alpha_2\mu$ -globulin degradation and produce the hydrocarbon nephrotoxicity.

#### REFERENCES

1. Ali, M.F. and Saleem, M. 1986. Composition and properties of straight run naphtha cuts from Saudia Arabian crude oils. Arabian J. Sci. Eng. 11, 251-258.
2. Siegert, H., Oelert, H.H. and Zajontz, J. 1976. Gaseous hydrocarbons from engine combustion. Erdol Kohle, Erdgas, Petrochem. 29, 169.
3. Sexton, K. 1983. Evidence of an additive effect for ozone plumes. Environ. Sci. Technol. 17, 402-407.
4. Sexton, K. and Westberg, H. 1980. Elevated ozone concentrations measured downwind of the Chicago-Gary urban complex. J. Air Pollut. Control Assoc. 30, 911-914.
5. Krotoszynski, B.K. and O'Neil, H.J. 1982. Involuntary bioaccumulation of environmental pollutants in non-smoking heterogeneous human populations. J. Environ. Sci. Health, Part A. A17, 855-883.
6. Dmitriev, M.T., Rastyannikov, E.G. and Malysheva, A.G.. 1983. Hygienic evaluation of organic substances in tobacco smoke. Gig. Sanit. 7-10.
7. Kiselev, A.V., Mal'tsev, V.V. Saada, B. and Valovi, V.A. 1983. Emission from plastic building materials. Chromatographia 17, 539-544.
8. Rettenberger, G. 1984. Trace compounds in landfill gas. Consequences for gas utilization. 4th Int. Recycl. Congr. 217-221.
9. Sandmeyer, E.E. 1981. Aliphatic Hydrocarbons. In Patty's Industrial Hygiene and Toxicology, 3rd editions, John Wiley Interscience, New York, N.Y., eds. G.D. Clayton and F.E. Clayton, pp 3190-3191.
10. Olson, C.T., Yu, K.O, Hobson, D.W. and Serve', M.P. 1985. Identification of urinary metabolites of the nephrotoxic hydrocarbon 2,2,4-trimethylpentane in male rats. Biochem. Biophys. Res. Comm. 130, 311-316.
11. Olson, C.T., Hobson, D.W., Yu, K.O. and Serve', M.P. 1987. The metabolism of 2,3,4-trimethylpentane in male Fischer 344 rats. Toxicol Lett. 37, 199-202.

12. Ridder, G.M., VonBargen, E.C., Alder, C.A. and Parker, R.D. 1990. Increased hyaline droplet formation in male rats exposed to decalin is dependent on the presence of  $\alpha_2$ -globulin. Fund. Appl. Toxicol. 15, 732-743.
13. Akashi, K., Palermo, R.E. and Sharpless, K.B. 1978. A major improvement in the osmium catalyzed vicinal hydroxylation of olefins by t-butyl hydroperoxide. J. Org. Chem. 43, 2063-2065.
14. Utimoto, K., Uchida, K. and Nozaki, H. 1973. Reaction of Lithium trialkylvinylborates and oxiranes. Novel synthesis of 1,4-diols. Tetrahedron Letters 45, 4527-4528.
15. Brown, H.C. and Lynch, G.J. 1981. Solvomercuration-demercuration. 8. Oxymercuration-demercuration of methoxy-hydroxy- and acetoxy-substituted alkenes. J. Org. Chem. 46, 531-538.
16. Karrer, P., Shibata, B., Wettstein, A., and Jacobowicz, L. 1930. Sparteine. Helv. Chim. Acta. 13, 1292-1308.
17. Linstead, R.P. and Rydon, H.N. 1934. Olefinic Acids XIV. Preparation and additive reactions of  $\delta$ -hexenoic acid. An unusual isomeric change in the three-carbon system. J. Chem. Soc. 1995-2001.
18. Olson, C.T., Yu, K.O., Hobson, D.W. and Serve', M.P. 1986. The metabolism of n-octane in Fischer 344 rats. Toxicol. Lett. 31, 147-150.
19. McFadden, W.H., Day, E.A. and Diamond, M.J. 1965. Correlations and anomalies in mass spectra. Lactones. Anal. Chem. 37, 89-92.
20. Swenberg, J.A., Short, B., Borghoff, S., Strasser, J. and Charbonneau, M. 1989. The comparative pathobiology of  $\alpha_2$ -globulin nephropathy. Toxicol. Appl. Pharmacol. 97, 35-46.



**Figure 1. GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE  
FISCHER 344 RATS DOSED WITH 2-METHYLHEPTANE**

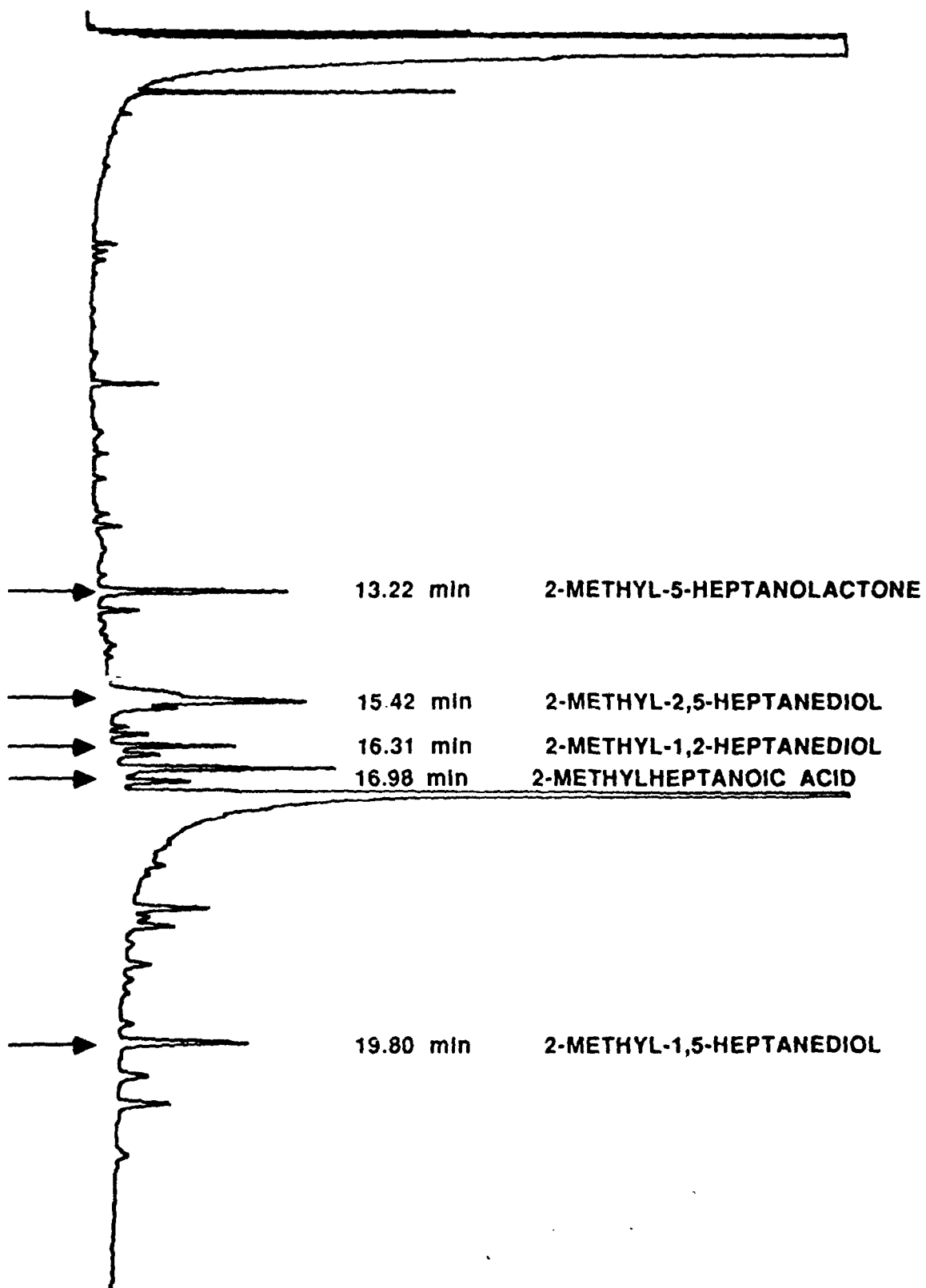
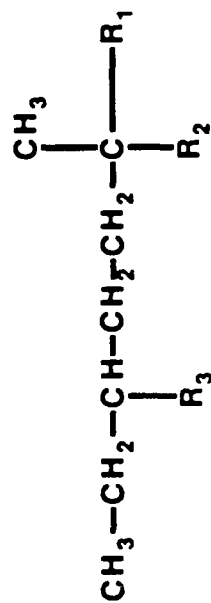


TABLE 1. 2-METHYLHEPTANE METABOLITES ISOLATED FROM THE URINE OF  
FISCHER 344 MALE RATS



<u>METABOLITE</u>	<u>R<sub>1</sub></u>	<u>R<sub>2</sub></u>	<u>R<sub>3</sub></u>	<u>GC TRACING ABUNDANCE</u>
2-Methyl-1,5-heptanediol	CH <sub>2</sub> OH	H	OH	1.0
2-Methyl-1,2-heptanediol	CH <sub>2</sub> OH	OH	H	1.3
2-Methyl-1-heptanoic Acid	CO <sub>2</sub> H	H	H	1.4
2-Methyl-5-hydroxy-1-heptanoic Acid	CO <sub>2</sub> H	H	OH	1.6
2-Methyl-2,5-heptanediol	H	OH	OH	1.8