N PAGE

Form Approved
OMB No. 0704-0188



AD-A252 192

1. AGENCY USE ONLY (Leave blank)

2. REPORT TYPE AND DATES COVERED
ANNUAL 01 Jun 91 TO 31 May 92

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
G - AFOSR-89-0396
PE - 61102F
PR - 2312
TA - A5

6. AUTHOR(S)
Dr 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- 92 0559

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr Jimmy Cornette
AFOSR/NL
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

13. ABSTRACT (Maximum 200 words)

Male Fischer 344 rats were dosed with the saturated branched chain hydrocarbon 3-methylheptane. Pathological examination of the kidneys of the dosed animals compared to the kidneys of control rats dosed with water indicated that there was a noticeable difference in the ability of the hydrocarbon to induce the classic nephrotoxicity produced by 2,2,4-trimethylpentane and other branched chain hydrocarbons. Hyaline droplet formation was used as the principal indicator of kidney damage. There was little indication of any cast formation in the corticomedullary junction area of the kidney. Identification of the rat urinary metabolites of 3-methylheptane yielded 3,5-diethyl-2,3-dihydrofuran, 3-ethyl-6-methyl-2,3-dihydropyran, 3-methyl-2-heptanol, 5-methyl-2-heptanol, 2-n-butyl-1,3-butanediol, 2-ethyl-1,3-hexanediol, B-methyl-8-enantholactone, 3-methyl-3,4-heptanediol, 3-methyl-2,3-heptanediol, 3-methyl-3,5-heptanediol, 2-ethyl-1,4-hexanediol, 8-methyl-8-enantholactone, 5-methyl-2,5-heptanediol, 2-ethyl-1,5-hexanediol and 2-ethyl-1-hexanoic acid.

14. SUBJECT TERMS

15. NUMBER OF PAGES

16. PRICE CODE

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| (U) | (U) | (U) | (U) |

# 1992 ANNUAL TECHNICAL REPORT FOR THE AIR FORCE GRANT AFOSR NO. 89-0396 ENTITLED "A STUDY OF THE EFFECT OF HYDROCARBON STRUCTURE ON THE INDUCTION OF MALE RAT NEPHROPATHY AND METABOLITE STRUCTURE."


SUMMARY:

Male Fischer 344 rats were dosed with the saturated branched chain hydrocarbon 3-methylheptane. Pathological examination of the kidneys of the dosed animals compared to the kidneys of control rats dosed with water indicated that there was a noticeable difference in the ability of the hydrocarbon to induce the classic nephrotoxicity produced by 2,2,4-trimethylpentane and other branched chain hydrocarbons. Hyaline droplet formation was used as the principal indicator of kidney damage. There was little indication of any cast formation in the corticomedullary junction area of the kidney. Identification of the rat urinary metabolites of 3-methylheptane yielded 3,5-diethyl-2,3-dihydrofuran, 3-ethyl-6-methyl-2,3-dihydropyran, 3-methyl-2-heptanol, 5-methyl-2-heptanol, 2-n-butyl-1,3-butanediol, 2-ethyl-1,3hexanediol, β-methyl-δ-enantholactone, 3-methyl-3,4-heptanediol, 3-methyl-2,3-heptanediol, 3-methyl-3,5-heptanediol, 2-ethyl-1,4-hexanediol, δ-methyl-δ-enantholactone, 5-methyl-2,5-heptanediol, 2-ethyl-1,5-hexanediol and 2-ethyl-1-hexanoic acid.


RESEARCH OBJECTIVES:

To examine the effects of alkyl branching on saturated acyclic hydrocarbons with respect to:

A. The ability of the hydrocarbon to induce in male rats a nephropathy characterized by hyaline droplet formation, cast production and cell necrosis.

B. The differences in structure of urinary metabolites produced by male and female rats exposed to the various hydrocarbons.

C. The presence of any hydrocarbon metabolites residing in the kidneys of rats exposed to the hydrocarbons which may reveal information regarding the metabolic route of mechanism(s) of the induced nephropathy.

D. The correlation between hydrocarbon structure, urinary metabolite structure and degree of nephrotoxic damage to gain an understanding fo the progression of the nephropathy.

For the purpose of brevity, hereafter, the term "branched-chain hydrocarbon" will refer to the following molecules; 2-methylheptane, 3-methylheptane, 4-methylheptane, and 2,5-dimethylhexane.

Since the chemicals to be studied have separate physical, chemical and biological properties, each of the chemicals will be individually treated as a potential nephrotoxic agent. The information on background and experimental details will be included in the final technical after all the compounds have been studied and the results compiled.

STATUS OF THE RESEARCH:

Male Fischer 344 rats were dosed with 3-methylheptane over a 14 day period. The urines were collected after 48 hours and analyzed for metabolites suing the experimental procedures outlined in the previous work of the investigators ["Metabolism and Nephrotoxicity of Tetralin in Male Fischer 344 Rats", M.P. Serve', B.M. Llewelyn, K.O. Yu, C.T. Olson and D.W. Hobson, J. Toxicol. and Environ. Health, 25, 267-275 (1989)]. One kidney from each of the dosed rats was set aside for determination of the presence of any metabolites of the hydrocarbons that the rat had been dosed with. The other kidney of each of the dosed animals was sent for pathological examination to determine the extent of nephrotoxic damage induced by the hydrocarbon.

Pathological examination of the kidneys from the dosed rats compared to the kidneys of control rats dosed only with water showed that the rats dosed with the hydrocarbon showed mild to moderate damage. The nephrotoxic damage induced by the 3-methylheptane appeared to approach the extent of kidney damage induced by the 2,2,4- and 2,3,4-trimethylpentanes (previously determined to be the most nephrotoxic hydrocarbons examined). The pathological findings seem to lend support to the hypothesis that not only alkyl branching of the hydrocarbon structure is necessary, but also the extent of alkyl branching is important.

The identification of the structure of the urinary metabolites of 3-methylheptane has been completed with the following metabolites having been identified:

## METABOLITES ISOLATED FROM THE URINES OF MALE FISCHER 344 RATS DOSED WITH 3-METHYLHEPTANE

| | METABOLITE | RELATIVE ABUNDANCE |
|---|---|---|
| 1. | 3,5-DIETHYL-2,3-DIHYDROFURAN | 4.3 ± 1.3 |
| 2. | 3-ETHYL-6-METHYL-2,3-DIHYDROPYRAN | 9.9 ± 2.5 |
| 3. | 3-METHYL-3-HEPTANOL | 1.5 ± 0.6 |
| 4. | 3-METHYL-2-HEPTANOL | 3.9 ± 1.5 |
| 5. | 5-METHYL-2-HEPTANOL | 7.4 ± 2.6 |
| 6. | 2-n-BUTYL-1,3-BUTANEDIOL | 2.8 ± 0.8 |
| 7. | 2-ETHYL-1,3-HEXANEDIOL | 5.5 ± 1.7 |
| 8. | β-METHYL-δ-ENANTHOLACTONE | 1.5 ± 0.7 |
| 9. | 3-METHYL-3,4-HEPTANEDIOL | 8.0 ± 2.6 |
| 10. | 3-METHYL-2,3-HEPTANEDIOL | 7.9 ± 2.9 |
| 11. | 3-METHYL-3,5-HEPTANEDIOL | 1.3 ± 0.5 |
| 12. | 2-ETHYL-1,4-HEXANEDIOL | 1.6 ± 0.7 |
| 13. | δ-METHYL-δ-ENANTHOLACTONE | 2.6 ± 1.1 |
| 14. | 5-METHYL-2,5-HEPTANEDIOL | 3.0 ± 1.4 |
| 15. | 2-ETHYL-1,5-HEXANEDIOL | 1.0 ± 0.5 |
| 16. | 2-ETHYLHEXANOIC ACID (2-EHA) | 2.5 ± 1.1 |

The structures of the urinary metabolites of 3-methylheptane were much more complex than those found for 2,5-dimethylhexane and 2-methylheptane. The metabolite structures of 2,5-dimethylhexane and 2-methylheptane consisted of diols in which either the ω or the ω-1 carbons were oxidized to alcohols as well as δ-hydroxy-1-hexanoic acid. In the case of 3-methylheptane the urinary metabolite structures consisted of monoalcohols, diols which were substituted at the 1,3-, 2,5- 3,4- and 3,5-, positions, and δ-hydroxy acids. Since the 3-methylheptane was more nephrotoxic than the 2-methylheptane, it is possible that one or more of the addtional metabolites was resposible for the kidney damage.

ARTICLES PLANNED FOR PUBLICATION

The work on the metabolism is currently being written up for publication in one of the toxicology journals. (Journal of Toxicology and Environmental Health or Chemosphere). A copy of the proposed paper is enclosed It is also intended that different portions of the work will be presented at national meetings of the Society of Toxicology and the American Chemical Society.

PARTICIPATING PROFESSIONALS

The work on the 3-methylheptane was performed primarily by J. Matthew Clemmens who received his Masters Degree from Wright State University in December 1991. During the past year work on the 4-methylheptane has commenced with the assistance of the following students Kevin McChord, a second year medical student and Theresa Rezek, a senior and new first year graduate student at Wright State University.

PUBLICATIONS AND PRESENTATIONS

1. "The Metabolism of 2-Methylheptane in Male Fischer 344 Rats", M.P> Serve', D.D. Bombick, J.M. Clemmens, G.A. McDonald and D.R. Mattie, The Toxicologist 12, 381 (1992).
2. "Isolation and Identification of the Metabolites of 2-Methylheptane in Male Fischer 344 Rats", M.P. Serve', D.D. Bombick, J.M. Clemmens, G.A. McDonald and D.RE. Mattie, 203rd National American Chemical Society Meeting, San Francisco, CA, April 1992.
3. "The Metabolism of 2-Methylheptane in Male Fischer 344 Rats", M.P. Serve', D.D. Bombick, J,M. Clemmens, G.A. McDonald and D.R. Mattie, Chemosphere (1992).