

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE 8/89	3. REPORT TYPE AND DATES COVERED Technical Report - 1989	
4. TITLE AND SUBTITLE The Effect of 2,3,4-Trimethylpentane on the Ultrastructure of Proximal Tubular Cells in Primary Cell Culture			5. FUNDING NUMBERS PE: 63 PA: 02 TA: 08 WU: 08	
6. AUTHOR(S) D. R. Mattie, J. J. Maslanka, N. J. Del Raso, and M. R. Chase				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armstrong Laboratory HSD/AFSC Wright-Patterson AFB, Oh 45433-6573			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSORING/MONITORING AGENCY REPORT NUMBER AAMRL-TR-89-040	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) To aid in the assessment of the risk of Air Force personnel working with hydrocarbon fuels and compounds, an attempt was made to further characterize the nephropathy that results from exposure to hydrocarbons. The purpose of this study was to isolate and establish purified primary cultures of male rat proximal tubular cells suitable for experimental exposure to sublethal concentrations of solubilized 2,3, 4-trimethylpentane (TMP), a model hydrocarbon. Experiments were conducted to evaluate the cytotoxicity and metabolism of solubilized TMP in media containing or lacking the protein albumin.				
14. SUBJECT TERMS Hydrocarbon Albumin Metabolism Cytotoxicity			15. NUMBER OF PAGES 2	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

G. W. Bailey, Ed., *Proceedings of the 47th Annual Meeting of the Electron Microscopy Society of America*
Copyright © 1989 by San Francisco Press, Inc., Box 6800, San Francisco, CA 94101-6800, USA

THE EFFECT OF 2,3,4-TRIMETHYLPENTANE ON THE ULTRASTRUCTURE OF PROXIMAL TUBULAR CELLS IN PRIMARY CELL CULTURE

D.R. Mattie, J.J. Maslanka, N.J. Del Raso*, and M.R. Chase

AAMRL/THT, Wright-Patterson AFB, OH 45433-6573 and * NSI THRU, Dayton, OH 45431

To aid in the assessment of the risk of Air Force personnel working with hydrocarbon fuels and compounds, an attempt was made to further characterize the nephropathy that results from exposure to hydrocarbons. The purpose of this study was to isolate and establish purified primary cultures of male rat proximal tubular cells suitable for experimental exposure to sublethal concentrations of solubilized 2,3,4-trimethylpentane (TMP), a model hydrocarbon. Experiments were conducted to evaluate the cytotoxicity and metabolism of solubilized TMP in media containing or lacking the protein albumin.

Proximal tubule cells in primary suspension culture were exposed to one of the following levels of TMP: 7.9, 12.0, 15.7, 19.1 or 25.5 mM. After 4 hours of exposure, pelleted cells were fixed for transmission electron microscopy by resuspension in 2% glutaraldehyde and 2.5% paraformaldehyde in 0.1M cacodylate buffer at pH 7.4. After a minimum fixation of at least 24 hours, the cells were post-fixed with 2% osmium tetroxide in 0.1M cacodylate buffer at pH 7.4. Cells were processed into Polybed 812 plastic capsules. Sections one micron thick were cut in order to verify that cells were intact and suitable for thin sectioning. Thin sections (60-90 nm) were cut on an ultramicrotome using a diamond knife. Thin sections, stained with uranyl acetate and lead citrate, were examined with a transmission electron microscope at 60 kV. Photographs of representative proximal tubule cells were taken at three levels of magnification.

All control proximal tubular (PT) cells contained vacuoles to varying degrees with a minimal degree of mitochondrial swelling. The outer compartment of many mitochondria appeared to be slightly swollen as evidenced by intracrystal swelling. There were no significant differences between controls with albumin and controls without albumin (FIG 1 and 2). As PT cells were exposed to higher doses of TMP, the number of viable and intact cells decreased. Cell viability was not quantitated at the ultrastructural level. Albumin in the media did not confer a protective effect on PT cells as previously seen with hepatocytes in culture.¹ However, albumin did allow slightly greater changes to occur in rough endoplasmic reticulum and mitochondria (FIG 3). No apparent differences were seen between albumin and non-albumin groups for nuclei, lipid, smooth endoplasmic reticulum, vacuoles, and microvilli (FIG 4).

Primary cultures of rat kidney proximal tubular cells can be exposed to a chemical such as TMP. Albumin in the media does not appear to confer a protective effect as seen with hepatocytes in primary culture. Media was analyzed for the presence of TMP metabolites. Although metabolites had previously been isolated in urine of dosed rats² and more recently in the culture media of primary hepatocytes exposed to TMP³, no metabolites were detected from the kidney cells. This confirms that primary metabolism of TMP occurs in the liver, not in the kidney, even though the kidney is the target organ for toxicity.

1. Del Raso, N.J. and Mattie D.M. *The Toxicologist* 8(1):217, 1988
2. Olson, C.T., Hobson, D.W., Yu, K.O. and Serve, M.P. *Toxicology Letters* 37:199-202, 1987
3. Del Raso, N.J. Personal communication, 1989
4. Acknowledgment: Jeannie K. Freeman, assistance in preparing this abstract

1990218086

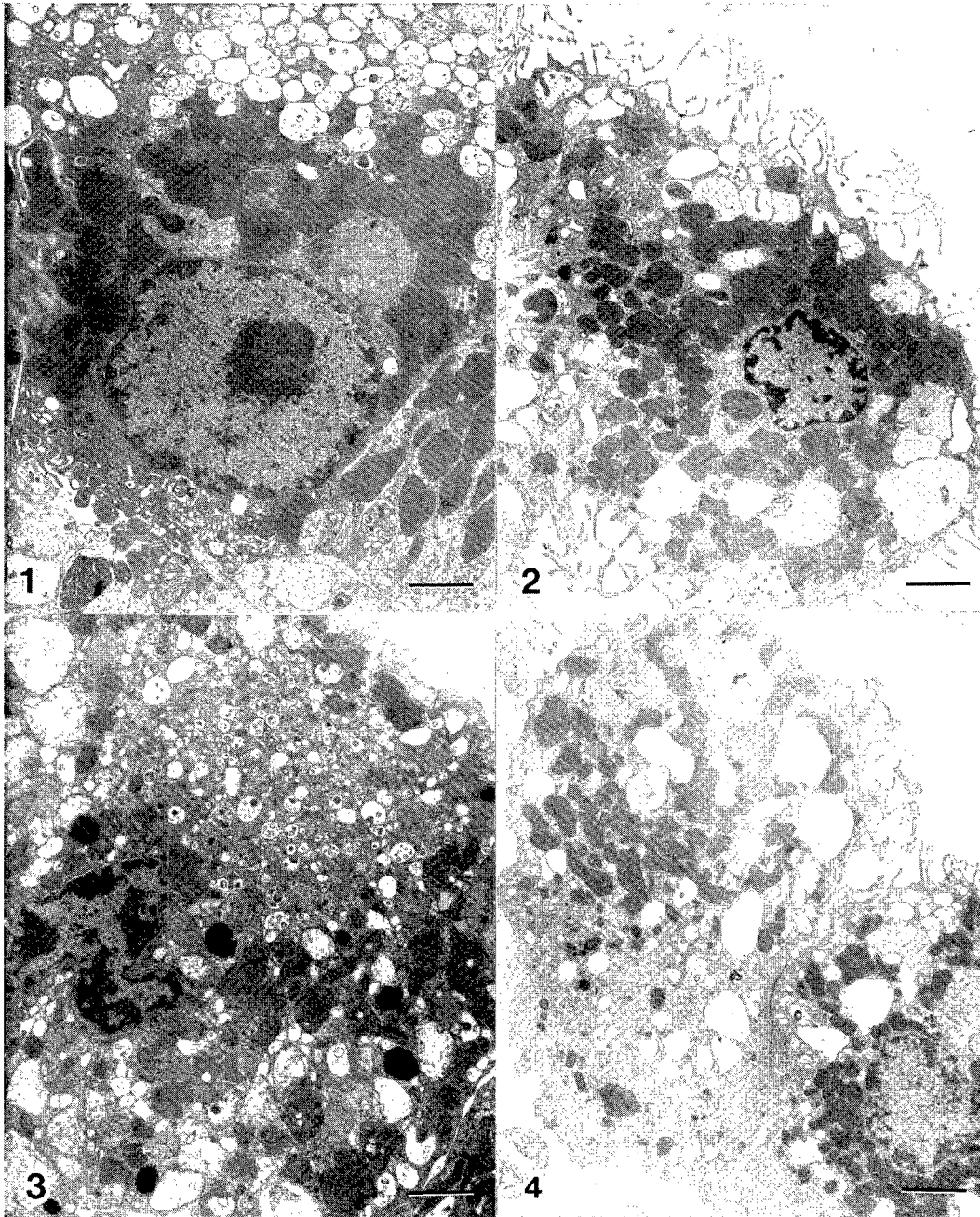


FIG. 1.--Control proximal tubule cell with albumin. Bar = 1 μ m.
FIG. 2.--Acetone control proximal tubule cell without albumin. Bar = 1 μ m.
FIG. 3.--Proximal tubule cell with albumin exposed to 12.0 mM TMP for 4 hours.
Bar = 1 μ m.
FIG. 4.--Proximal tubule cell without albumin exposed to 7.9 mM TMP for 4 hours.
Bar = 1 μ m.