UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

DTIC FILE COPY

REPORT DOCUMENTATION PAGE

AD-A216 820	1b. RESTRICTIVE MARKINGS
	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited.
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S): AFOSR-TR- 89-1859
6a. NAME OF PERFORMING ORGANIZATION: Southeastern Louisiana Univ Dept of Biological Sciences	6b. OFFICE SYMBOL (If applicable):
7a. NAME OF MONITORING ORGANIZATION: Air Force Office of Scientific Research/NL	
6c. ADDRESS (City, State and ZIP Code): Box 358 University Station Hammond LA 70402	7b. ADDRESS (City, State and ZIP Code): Building 410 Bolling AFB, DC 20332-6448
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: AFOSR	8b. OFFICE SYMBOL (If applicable): NL
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: AFOSR-84-0310	
8c. ADDRESS (City, State and ZIP Code): Building 410 Bolling AFB DC 20332-6448	10. SOURCE OF FUNDING NOS.

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT NO.
61102F	2312	A5	

11. TITLE (Include Security Classification): "Ultra-structural and Cytochemical Evaluation of the Cytotoxicities of Trimethypentane of Rat Renal and Hepatic Tissues"

12. PERSONAL AUTHOR(S): Dr William N Norton

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Yr., Mo., Day)	15. PAGE COUNT
recast Report	FROM 1 Sept 84 TO 31 Aug 85	29 April 1985	03

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB. GR.	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC ELECTE JAN 04 1990 S B D

20030205021

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS ☐	UNCLASSIFIED

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE NUMBER (Include Area Code)	22c. OFFICE SYMBOL
Dr Christopher Lind Lt Col	(202) 767-5021	NL

DD FORM 1473, 83 APR — EDITION OF 1 JAN 73 IS OBSOLETE. — UNCLASSIFIED SECURITY CLASSIFICATION OF THIS PAGE

90 01 04 190

Ultrastructural and Cytochemical Evaluation of the Cytotoxicities of Trimethylpentane on Rat Renal and Hepatic Tissues

Forcast and Progress Report

William N. Norton, Ph.D.

(1)

The primary objective of the present investigation is to determine the acute cytopathological effects of trimethylpentane on the hepatic and renal tissues of the sexually mature male rat. Specifically, the first year of the project is designed to evaluate, cytochemically, the activity of lysosomes associated with kidney cells of the proximal convoluted tubule, to determine by means of ferritin tracers whether the glomerular basement membrane has been compromised, and to analyze by scanning electron microscopy various regions of the kidney for manifestations of cellular toxicity. (KR)

Subsequent to a consultation with scientists from Wright-Patterson AFB who are knowledgeable in the area of hydrocarbon toxicity the protocol described in the submitted grant proposal was modified in 2 significant ways. The originally stated dose of 10 ml/kg body weight was considered to be too high for a critical evaluation of the initial subtle cellular changes associated with lesion development. Thus, a lower concentration of 1.5 ml/kg body weight was chosen. Additionally, the administration of trimethylpentane was altered from a single dose to exposure periods of twice weekly for 7, 14 or 28 days. The general consensus was that low consistent exposures of the experimental animals to the hydrocarbon would be more conducive to an analysis of the initial phases of lesion development. As a result of the modifications, sexually mature male rats were administered trimethylpentane by the gavage method twice weekly at a concentration of 1.5 ml/kg body weight. Control animals were given a comparable concentration of distilled water. Experimental and control rats were sacrificed at 7, 14 or 28 days subsequent to the initial exposure. All tissues to be examined during the investigation were processed at this time.

This progress report consists of a brief description of the major structural changes detected by a scanning electron microscopy analysis of control and experimental kidneys. All tissues projected for observation by scanning electron microscopy were fixed chemically by means of perfusion via the heart with a 4% solution of glutaraldehyde which was buffered with 0.1M sodium cacodylate (pH 7.4). The tissues were post-fixed with osmium tetroxide, dehydrated in a graded series of ethanol and cryofractured by submerging the material in liquid nitrogen and fracturing the latter with a chilled razor blade. The fractured tissue was critical point dried, sputter-coated with a layer of gold (90Å) and viewed on either an ETECH Autoscan or AMRAY 1200B scanning electron microscope.

The following descriptions represent a general preliminary analysis. Critical statistical data have not yet been computed. The glomeruli, proximal convoluted tubules and corticomedullary junctions represent the 3 general zones of the kidney which were investigated by scanning electron microscopy. Within the region of the glomeruli 2 structural aberrations were noted. Initial observations indicated a proliferation of microprojections associated with the primary, secondary and tertiary branches which extended from the nucleated portion of the podocyte cell body (Figs. 1,2). Such an increase of tubular extensions would be

very difficult to detect with conventional transmission electron microscopy. Preliminary observations also indicated a significant increase in the number of membranous blebs which protruded from the secondary and tertiary branches.

An analysis of freeze-fractured proximal convoluted tubules revealed distinct regions along the inner lumen where a loss of cilia was evident (Figs. 3,4). At several sites the reduction of cilia appeared to have occurred concomitant with cellular degradation. The latter was manifested by an extrusion of cellular components into the tubular lumen. A general lysis of the plasma membrane was not evident. Cellular necrosis was noted along the length of the proximal tubule. Within the epithelial cells comprising the tubule hyaline droplets of various dimensions form as a result of exposure to the hydrocarbon. The lumina of several tubules at the corticomedullary junction contained substantial amounts of what appeared to be cellular debris.

The results obtained thus far indicate that trimethylpentane is capable of inducing consistent cellular lesions throughout the specific time points selected for observation. Several of the cellular alterations are similar to those reported for other tissues exposed to specific aromatic hydrocarbons such as benzene and toluene. However, certain structural abnormalities detected in the present study have not yet been reported for hydrocarbon cytotoxicity.

The remainder of the first year will be devoted to analyzing the cytochemical phase of the project. In addition, a statistical analysis will be conducted on the information obtained from the scanning electron micrographs. The second year of the study will be concerned with an investigation of the developmental phases of the cellular lesions associated with the kidney, and a morphometric analysis (quantification) of the endoplasmic reticulum system of hepatocytes. Transmission electron microscopy related techniques will be employed during this phase.

There is a need to acquire basic biochemical and cytological information regarding cellular responses to trimethylpentane since the hydrocarbon is capable of inducing the formation of renal tumors. The multi-facet analysis of this project should provide pertinent information concerning initial and progressive cellular changes which result from acute exposure to a neoplastic agent such as trimethylpentane.

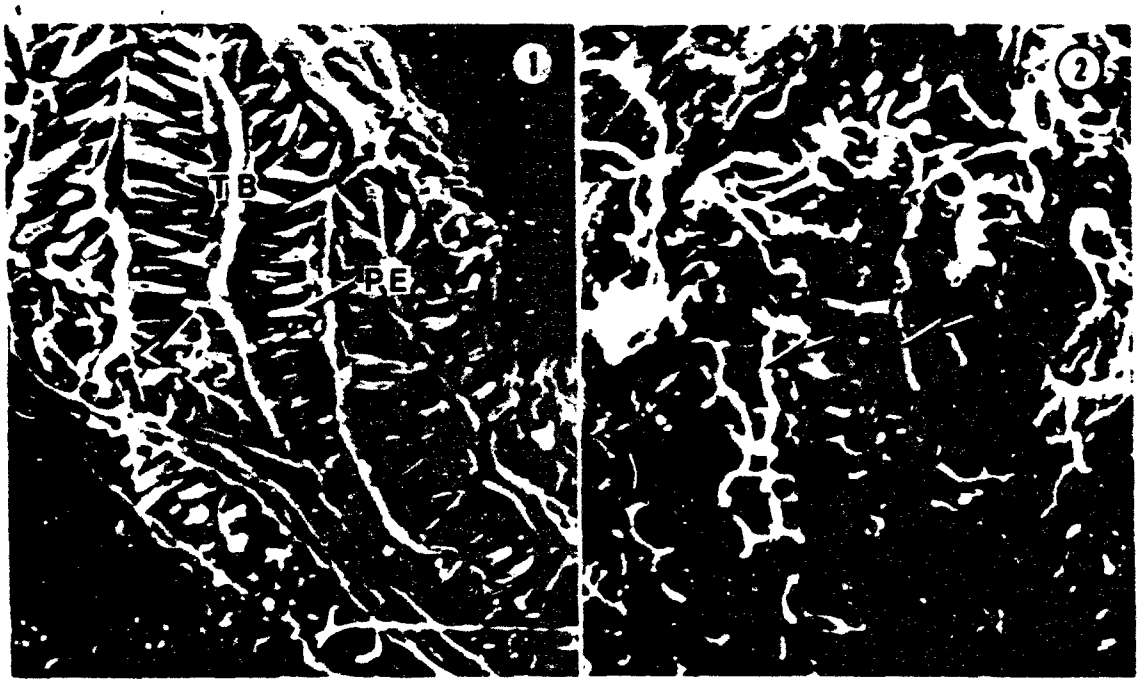

Figure 1. Scanning electron micrograph depicts a region of the glomerulus with orderly arranged pedicels (PE) and tertiary branches (TB) of podocytes. Note sparsity of microprojections (arrow). Control tissue. X 10,000.

Figure 2. Microprojections (arrows) are evident in high concentrations along the length of the glomerular capillary. Experimental tissue - 14 days of exposure. X 10,000.

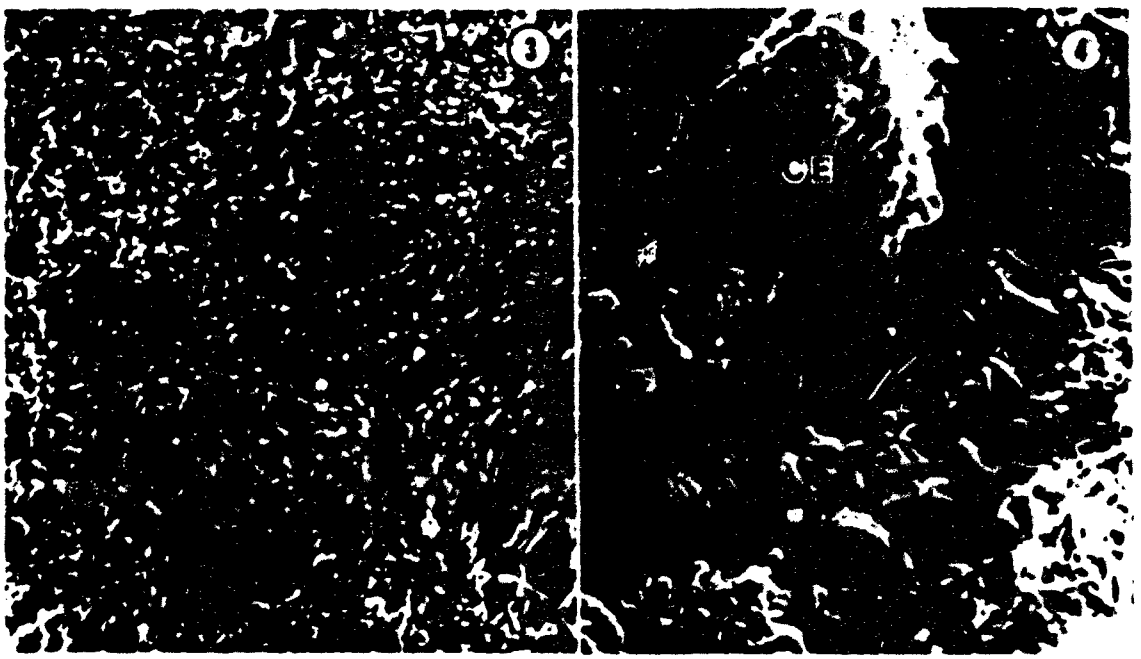

Figure 3. Compact arrays of cilia are evident along the lumen of the proximal convoluted tubule. Control tissue. X 10,000.

Figure 4. Regions of cilia reduction (arrow) occur at various sites along the proximal convoluted tubule. Note the presence of a necrotic cell (CE) within the lumen. Experimental tissue - 28 days of exposure. X 10,000.