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Monochlorodiisobutylene Vapor: Acute and 9-Day Inhalation Studies in Fischer-344 Rats and B6C3F1 Mice[1]

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Monochlorodiisobutylene Vapor: Acute and 9-Day Inhalation Studies in Fischer-344 Rats and B6C3F1 Mice. SNELLINGS, W. M., DODD, D. E., GRICE, H. C., AND PHILLIPS, R. D. (1985) *Fundam. Appl. Toxicol.* 5, 506–514. Acute and 9-day repeated exposures to monochlorodiisobutylene (CDIB) were conducted in male and female Fischer-344 rats and B6C3F1 mice. The 4-hr LC50 values for these animals ranged between 1400 and 2100 ppm. Animals in the 9-day study were exposed at a mean concentration of 478, 97, or 25 ppm of CDIB for 6 hr per day. Treatment-related effects differed between species in this study. Body weight change was decreased in rats. Morphologic changes in the kidneys with accompanying polyuria and hematuria/hemoglobinuria were observed in male rats. The only effect observed at 25 ppm was a low incidence of hematuria/hemoglobinuria in male rats. Mice appeared unaffected by exposure to CDIB at levels as high as 478 ppm.

Monochlorodiisobutylene (CDIB) is a reaction product formed in the manufacturing of chlorobutyl rubber. Chlorobutyl rubber is a chlorinated isobutylene/isoprene copolymer. During the manufacture of rubber, not all monomers polymerize completely, and a small percentage of chlorinated monomers and oligomers remain in the process and in the rubber. CDIB is a chlorinated isobutylene dimer containing one chlorine atom. Since the toxicity of this material was unknown, rats and mice received single and 9-day repeated exposure to CDIB to determine its toxicity in acute and short-term repetitive exposures.

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METHODS

Test material. The composition and purity of the test compound (obtained from Exxon Research and Engineering Company, Linden, N.J.) was determined prior to and following the study. Two isomers, 3-chloro-2,4,4-trimethyl-1-pentene and 2-chloromethyl-4,4-dimethyl-1-pentene, were found to be 21 and 78%, respectively, of the test compound. The vapor concentrations of these two isomers in the inhalation chamber were calculated, combined, and reported as parts per million of CDIB.

Animals. Fischer-344 rats (Charles River Breeding Laboratories, Portage, Mich.) and B6C3F1 mice (Charles River Breeding Laboratories, Kingston, N.Y.) were acclimated for approximately 2 weeks prior to the initiation of exposures. Males and females of each species were approximately 7 weeks of age on the first day of exposure. At the time of random selection, the only animals accepted for study were those appearing in good health and whose body weights were within plus or minus two standard deviations of the mean weight of all animals of the same species and sex. Food (powdered NIH-07, Zeigler Brothers, Inc., Gardners, Pa.) and water were removed during the inhalation exposure period and were available *ad libitum* at all other times.

Exposure and analytical systems. The test material was metered using a syringe pump into a heated, spiral-grooved, glass evaporator similar in design to the one described by Carpenter *et al.* (1975). A minimum amount

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of heat was used to completely vaporize the liquid stream during dilution with a counter-current air stream entering the bottom of the tube. The resulting vapor and air mixture was then introduced into a 550-liter inhalation chamber where rats and mice were exposed at the same time. The concentration of CDIB in the chamber air was monitored by a gas chromatograph equipped with a flame ionization detector. The column was packed with SP-2100 stationary phase (20%) on Supelcoport (80/100 mesh) support material. The solvent used in making the standards was *n*-hexane. Approximately six hourly analyses were obtained from each chamber during each exposure period.

Experimental design for 4-hr LC50. Three groups of six animals per sex per species were exposed to CDIB for 4 hr. The target chamber concentrations were 3900, 1900, and 850 ppm. A fourth group of six animals per sex per species was handled in the same manner as the CDIB-treated groups but was exposed to air only and served as a negative control. All animals were observed for toxic effects during exposure and after exposure for a 14-day period. Body weights were recorded four times during the postexposure period. Gross pathologic examinations were performed on animals that died or survived the 14-day observation.

Experimental design for 9-day study. Three groups of 10 per sex per species were exposed to CDIB. The target chamber concentrations were 500, 100, and 25 ppm of CDIB. A fourth group of 10 animals per sex per species was handled in the same manner as the CDIB-treated groups but was exposed to air only and served as a negative control. Animals were exposed 6 hr per day for 5 consecutive days. After 2 days without exposure, animals were exposed for an additional 4 consecutive days and then sacrificed. The chamber temperature (21 to 25°C) and relative humidity (40 to 60%) were maintained within similar ranges for all inhalation chambers. Chamber airflow was maintained at approximately 11 air changes per hour. To compensate for any possible, but undetected, variation in chamber exposure conditions, the positions of the animal cages were rotated on a daily basis. All animals were observed prior to, during and following exposure for any signs indicative of toxic effect. The body weights for all animals were determined on the morning preceding the first, second, fifth, sixth, and seventh exposure days and again preceding sacrifice. Following the fifth and ninth exposures, urine was collected for approximately 18 hr from all male and female rats. These rats were individually placed in metabolism cages with food and water available *ad libitum*. Urine samples were clinically evaluated using a dip stick (Chemstrip, BMC, Indianapolis, Ind.) method of analysis. Blood was obtained from the orbital sinuses of rats and mice anesthetized with methoxyflurane on the day of sacrifice. Hematologic tests included erythrocyte count, hemoglobin, hematocrit, leukocyte count, and differential leukocytes. All animals were then killed by severing the brachial blood vessels. Histologic evaluation was performed on paraffin-embedded sections of lungs and bronchi, heart, liver, gonads, and kidneys, which were stained with hematoxylin and eosin, of animals in the highest exposure and control groups. In addition, male rat kidneys of animals of the intermediate and low exposure groups were examined histologically. Kidneys of male rats from all exposure groups were stained with Mallory–Heidenhain's[4] to enhance detection of protein droplets. Liver, lungs, and kidneys of all animals and testes from males were weighed.

Statistical analyses. The fiducial limit of 0.05 (two-tailed) was selected as the critical level of significance. All data of each exposure group were compared statistically to the air-control group by using the following tests: continuous variable data were analyzed by Bartlett's test for homogeneity of variance (Sokal and Rohlf, 1981), analysis of variance, and Duncan's multiple range test (Duncan, 1955, 1957; Harter, 1960). Whenever the F value for analysis of variance was significant, Duncan's multiple range test was used to denote which groups differed significantly from the controls. If Bartlett's test indicated heterogenous variance, the F test (Sokal and Rohlf, 1981) was employed to compare each exposure group with the air-control group. The type of t test then used was selected according to the significance of the F value. The Student's t test (Sokal and Rohlf, 1981) was used when the F value was not significant. The Cochran t test (Cochran and Cox, 1957) was used when the F value was significant. Contingency data were analyzed by Fisher's exact test (Sokal and Rohlf, 1981). The LC50 values were calculated according to a modification of the probit analysis method of Finney (1971). The median and quartile deviations (Beyer, 1968) were reported for the nonparametric variables.

RESULTS

4-hr LC50

The actual mean chamber concentrations (±SD) recorded during the acute study were 3895 (±451), 1912 (±172), and 853 (±25) ppm. The incidence of mortality in each group is given in Fig. 1. Mortality in rats occurred during exposure; however, for mice, mortality occurred during exposure and up to 2 days following the exposure. The cause of death was unknown. The calculated LC50 values with 95% confidence limits are given in Table 1 for rats and mice. In all groups of surviving rats and mice (except female mice),

[4] *Animal Histology Procedures.* (1972). DHEW/NIH/NCI 72-275.

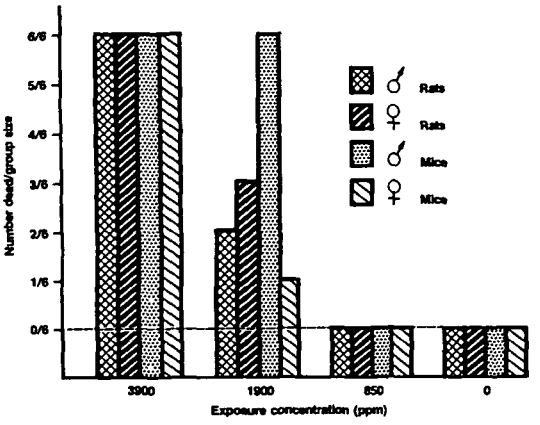

FIG. 1. Incidence of mortality inclusive of 4-hr exposure and 14-day postexposure period.

there was a transient loss in body weight following the exposure; however, body weight had recovered by the end of the 14-day observation period. The body weights of the female mice in all CDIB-treated groups were similar to the control values throughout the postexposure observation period.

9-Day Inhalation

Mean chamber concentration values (and coefficient of variation) for the CDIB exposure groups were 478 (9%), 97 (7%), and 25 (8%) ppm. The mean analytical to nominal concentration ratios ranged from 1.0 to 1.1 for three exposure chambers. The time to reach target chamber concentration was less than 30 min for each chamber.

No treatment-related adverse effects on general demeanor were observed in the rats or mice, except that rats exposed to 478 ppm of CDIB kept their eyes closed during most of the 6-hr exposures. At the end of the 9 days of exposure, no gross ophthalmologic lesions were found.

The change in body weight values, using Day 0 of exposure as a reference point, are presented in Fig. 2 for rats. A statistically significant depression in body weight gain was noted in the male and female rats of the 478-ppm exposure group. A transient depression in body weight gain was observed during the first week of exposure for only the female rats in the 97-ppm group. No treatment-related effects on body weight were noted in the mice (data not shown).

A significant increase in urine volume (Fig. 3) occurred in male rats of both the 478- and 97-ppm exposure groups after 5 and 9 exposure days. For the female rats, urine volume was significantly increased in only the 478-ppm group after 5 exposure days. Blood or hemoglobin was detected in the urine of only the exposed male rats (Table 2). Blood in the urine was noted in seven males of the 478-ppm exposure group after the fifth exposure and was observed in all male rats after the ninth exposure. At this time, blood was also detected in the urine of several males from the 97- and 25-ppm exposure groups (Table 2). No other differences were observed in urinalysis of exposed groups compared to controls.

There were minimal, but statistically significant, differences in some hematologic parameters between the female rats of the 478-ppm group and the controls with respect to red blood cell count (decrease), hematocrit (decrease), mean corpuscular hemoglobin (increase), and mean corpuscular hemoglobin concentration (increase). However, the biological importance of these minimal (<10% in all instances) changes is questionable. The hematologic values for the CDIB-exposed male rats were similar to the control values. No effects were noted in the differential

TABLE 1

LC50 VALUES FOR MALE AND FEMALE FISCHER-344 RATS AND B6C3F1 MICE EXPOSED TO MONOCHLORODIISOBUTYLENE

	Rat	Mouse
	Male	
4-hr LC50	2000 ppm	1390 ppm
(95% confidence limits)	(1390–2389)	(1030–1890)
	Female	
4-hr LC50	1850 ppm	2170 ppm
(95% confidence limits)	(1280–2670)	(1510–3110)

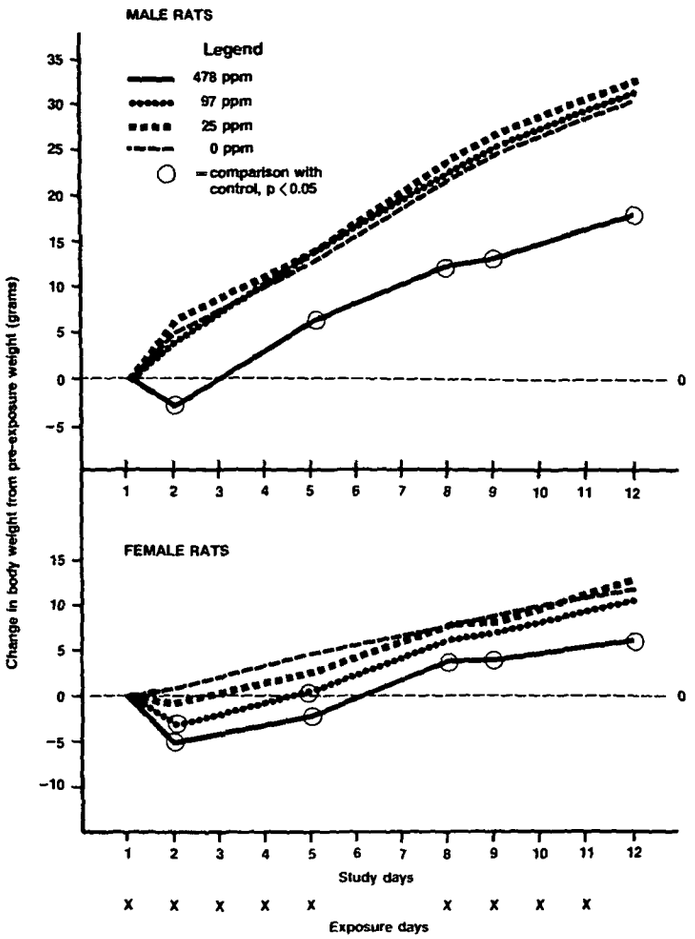

FIG. 2. Body weight gain for rats during the 9-day study.

leukocyte count of the CDIB-exposed rats when compared to the controls. Furthermore, there were no biologically significant hematologic alterations for male and female mice which could be attributed to treatment.

At necropsy no significant gross lesions were noted in rats or mice of either sex. Statistically significant increases in liver and kidney weights occurred for both sexes of rats in the 478-ppm exposure group. When the organ weights were expressed as a percentage of body weight, the liver weights were approximately 16% greater than those of the controls for both sexes, and the kidney weights were 10% (males) or 6% (females) greater than those of the controls.

Following histologic evaluation, the only lesions of statistical significance were found in kidneys of male rats (Figs. 4 and 5). Renal tubule epithelial cell degeneration and regeneration and tubular proteinosis occurred in male rats of the 478-ppm group, and tubular hyperplasia was observed in the 478- and 97-ppm exposure groups (Table 3). No statistically significant increase in incidence of these particular lesions was noted in the 25-ppm group. Other histologic findings in rats treated with 478 ppm of CDIB were not statistically

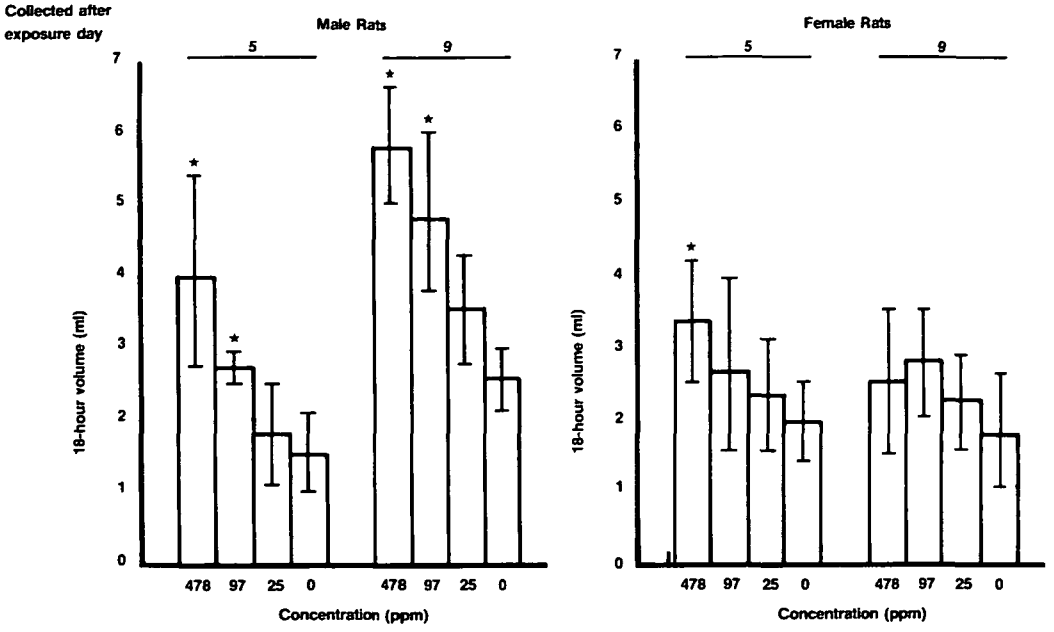

FIG. 3. Urine volume determinations in rats made after 5 and 9 days of exposure. * Value is significantly different from control, $p < 0.05$.

significantly different when compared to controls, and therefore, additional histology on the intermediate and low exposure groups was not performed. None of the histologic findings among the male and female mice indicated a toxic effect from CDIB exposure.

TABLE 2

INCIDENCE OF HEMATURIA/HEMOGLOBINURIA IN MALE FISCHER-344 RATS AFTER 5 AND 9 DAYS OF EXPOSURE TO MONOCHLORODIISOBUTYLENE

	Exposure concentration (ppm)			
Severity[a]	478	97	25	0
		After five exposures		
0	3/10[b]	10/10	10/10	10/10
1+	7/10	—	—	—
2+	—	—	—	—
		After nine exposures		
0	—	6/10	3/10	10/10
1+	—	4/10	7/10	—
2+	10/10	—	—	—

[a] Severity = the qualitative reading with the Chemstrip.
[b] Values represent the incidence of the hematuria/hemoglobinuria at the respective degree of severity.

DISCUSSION

The approximate 4-hr LC50 values were 1400 ppm for the male mouse and 2000 ppm for the female mouse, and also the male and female rat. These LC50 values for CDIB were within the range of other monochlorinated hydrocarbons (Smyth *et al.*, 1951, 1954, 1962, 1969; Clary *et al.*, 1978). The unsaturated monochlorinated hydrocarbon 2-chloro-1,3-butadiene was reported to have an approximate lethal concentration of 2280 ppm in rats (Clary *et al.*, 1978).

In the 9-day repeated exposure study with CDIB, the primary target organ appeared to be the kidney in male rats. Haloalkanes and haloalkenes are noted for their toxic effects on the kidney. For the most part, halogenated hydrocarbons producing kidney injury have been limited to short chain hydrocarbons with one or more chlorine or bromine sub-

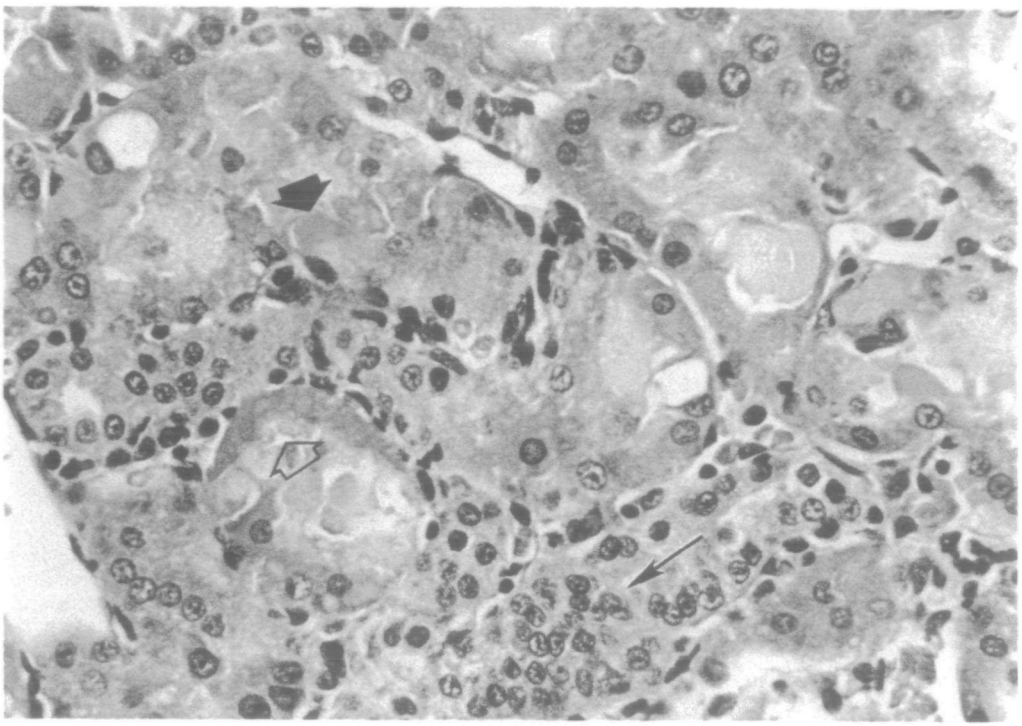

FIG. 4. High-power photomicrograph of kidney from 478-ppm group male rat. Tubular degeneration (open arrow), cytoplasmic protein in tubular cell (wide solid arrow), and tubular cell hyperplasia (thin arrow) are evident. (H & E, ×460)

stituents (Kluwe, 1983). The cytotoxicity produced by halogenated hydrocarbons is characterized morphologically by a vacuolar degeneration and/or necrosis (depending on the severity of intoxication) of the proximal tubular epithelium. Glomerular and distal tubular lesions are relatively infrequent.

The kidney injury observed in male rats following exposure to CDIB of up to 478 ppm for 9 days was similar to that observed with other short chain halocarbon-induced injury in that it was limited to the proximal tubules. However, CDIB-induced kidney injury was not as severe as that reported with other short chain halocarbons (e.g., vacuolization and necrosis). CDIB-induced kidney changes were limited to protein droplet accumulation (or hyaline droplets), tubular proteinosis, tubular hyperplasia, and epithelial degeneration and regeneration. These are nonspecific kidney changes which have been observed in untreated male rats (Coleman *et al.*, 1977), as evidenced in the present study where control male rats had a low incidence of these changes. Although an increase in the incidence of these changes following exposure suggests a treatment-related response, the extent of CDIB-induced kidney injury was not of the magnitude or severity of lesions produced by other short chain halocarbons.

The CDIB-induced kidney changes were similar to those described by several investigators in male rats following exposure to various nonhalogenated hydrocarbons (Phillips and Egan, 1984a,b; Gaworski *et al.*, 1982; Parker *et al.*, 1981). This phenomenon is termed light hydrocarbon nephropathy (LHN) and has been observed only in male rats following repeated exposure to various light hydrocarbons (Phillips and Cockrell, 1983). The lesions observed in LHN are described as an increase in hyaline droplets,

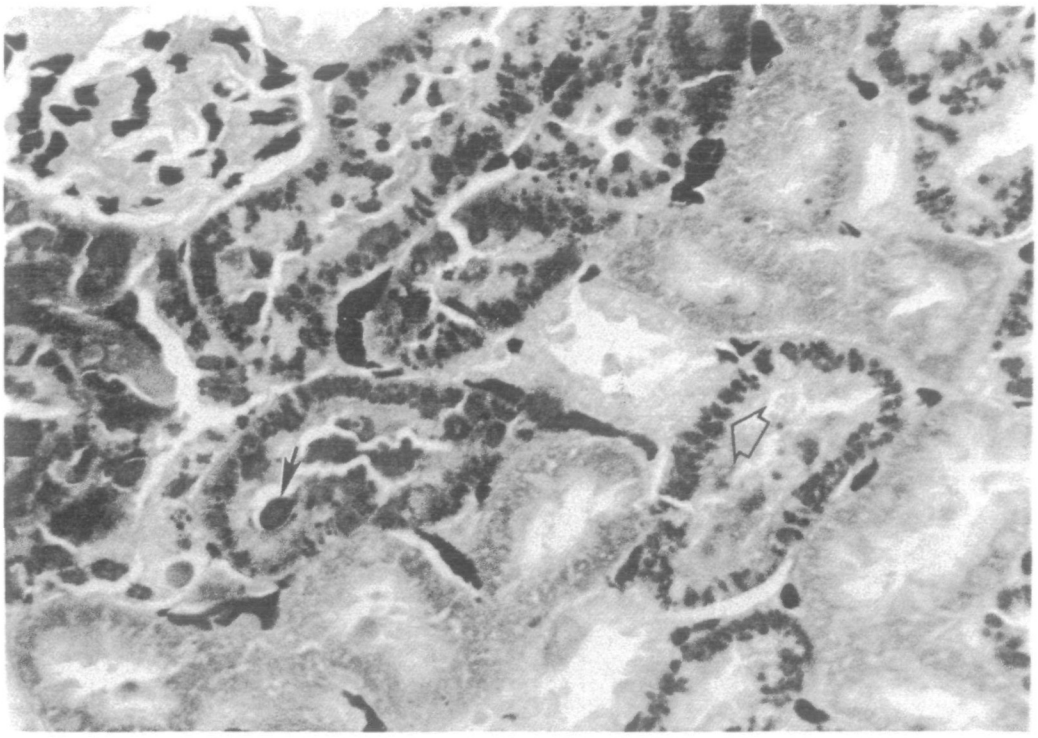

FIG. 5. High-power photomicrograph of kidney from 478-ppm group male rat. Darkly stained areas within tubular cell cytoplasm (e.g., open arrow) represent protein droplets. Protein is also seen within the tubular lumens (solid arrow). (Mallory–Heidenhain, ×460)

increased incidence of tubular regeneration, and dilated tubules at the cortico-medullary junction containing granular protein casts.

TABLE 3

HISTOLOGIC FINDINGS IN THE KIDNEYS OF MALE RATS AFTER 9 DAYS OF EXPOSURE TO MONOCHLORODIISOBUTYLENE

Finding	Exposure concentration (ppm)			
	478	97	25	0
Protein droplet accumulation	70[a]	60	60	50
Tubular proteinosis	60	10	0	10
Epithelial degeneration and regeneration	90[b]	60	50	20
Tubular hyperplasia	100[b]	100[b]	70	20

[a] Values are reported as percentages.
[b] $p < 0.01$ for comparison to control.

The only difference between LHN and CDIB-induced male rat kidney damage was the absence of granular protein casts at the cortico-medullary junction in CDIB-exposed animals. This could either be a valid distinction or exposure to CDIB may not have been of sufficient duration for this lesion to appear. In previous studies with light hydrocarbons, granular protein casts at the cortico-medullary junction have not been observed until exposures for 1 month (Phillips and Egan, 1984a,b).

The CDIB-induced kidney damage in male rats was not associated with major alterations in kidney function. An increase in urine volume was observed in CDIB-exposed male rats; however, effects on urine protein and glucose, or clinical chemistry indices indicative of kidney damage, were not observed. Similar minor effects on kidney function

have been observed in LHN (Phillips and Egan, 1984b). A decrease in urine-concentrating ability and an increase in urine protein and glucose excretion in male rats were observed following exposure to C_{10}–C_{11} isoparaffin. However, analysis for urinary constituents in the present CDIB study were by dipstick rather than quantitative analysis and may not have been sensitive enough to detect minimal changes.

A biologically significant effect was the treatment-related increase in hematuria/hemoglobinuria in male rats exposed to CDIB. A positive response to the dipstick analysis for hematuria/hemoglobinuria could result from either a lesion somewhere in the urinary tract beginning with the glomerulus (Bradley *et al.*, 1979) or with an effect on the integrity of red blood cells (Nelson, 1979). The kidney injury observed in the present CDIB study did not appear histologically to be severe enough to result in hematuria/hemoglobinuria. Also, hematology in male rats did not reveal any significant effects on blood indices. Therefore, the etiology of hematuria/hemoglobinuria induced by CDIB was not determined in this study.

In conclusion, exposure of rats and mice to CDIB for 9 days was associated with minor kidney damage in male rats. The damage was similar but less severe than that reported for other short chain halocarbons. There was also some similarity to the kidney changes produced by various light hydrocarbons. CDIB produced hematuria/hemoglobinuria of undetermined origin in male rats following nine exposures to concentrations as low as 25 ppm.

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