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Effects of Inhaled Chlorotrifluoroethylene and Hexafluoropropene on the Rat Kidney[1,2]

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Effects of Inhaled Chlorotrifluoroethylene and Hexafluoropropene on the Rat Kidney. POTTER, C. L., GANDOLFI, A. J., NAGLE, R. M., AND CLAYTON, J. W. (1981). *Toxicol. Appl. Pharmacol.* **59**, 431–440. Male Fischer-344 rats were subjected to 4.0 hr inhalation exposure to chlorotrifluoroethylene (CTFE) concentrations ranging from 100 to 540 ppm, or hexafluoropropene (HFP) concentrations ranging from 380 to 1200 ppm. Within 2 days following exposure, the rats exhibited dose-related proximal tubular necrosis, diuresis, increases in urinary fluoride, urinary lactic dehydrogenase (LDH) activity, serum creatinine, and BUN. The toxicities of CTFE and HFP were similar except that (1) CTFE was the more potent renal toxin and (2) HFP produced necrosis of the pars recta and pars convoluta portions of the proximal tubule, while CTFE produced necrosis of only the pars recta. At the lowest exposure concentrations, diuresis was the most sensitive index of toxicity manifesting 50% increases in water intake and 25% decreases in urine osmolality. Increases in urinary LDH activity correlated with the degree of proximal renal tubular necrosis, with greater than 100-fold increases at the highest concentrations of CTFE and HFP. At 100 ppm, CTFE induced renal dysfunction (mild diuresis), but no significant increase in urinary LDH nor necrosis were apparent. All concentrations of HFP studied produced necrosis within 24 hr postexposure, with tubular cell regeneration apparent within 4 days.

Chlorotrifluoroethylene (CTFE) and hexafluoropropene (HFP) are gaseous compounds encountered in the industrial setting. CTFE serves as a monomer for industrial production of the fluoropolymer polychlorotrifluoroethylene (Krespan, 1965). Waritz and Kwon (1968) found HFP formed during pyrolysis of polytetrafluoroethylene.

Early studies describe the toxicity and metabolism of CTFE (Clayton, 1977) and HFP (Dilley *et al.*, 1974), when inhaled by rats. They found both CTFE and HFP could produce renal dysfunction and necrosis of the proximal renal tubules with no apparent injury to other organs, and therefore represent possible occupational hazards as potential renal toxins. This study was designed to provide information about the acute renal toxicities of CTFE and HFP in order to provide guidelines for future studies of chronic low-level exposures to these compounds. Rats were subjected to single 4.0-hr exposures to various concentrations of CTFE and HFP, and time courses of renal functional and morphological changes were examined.

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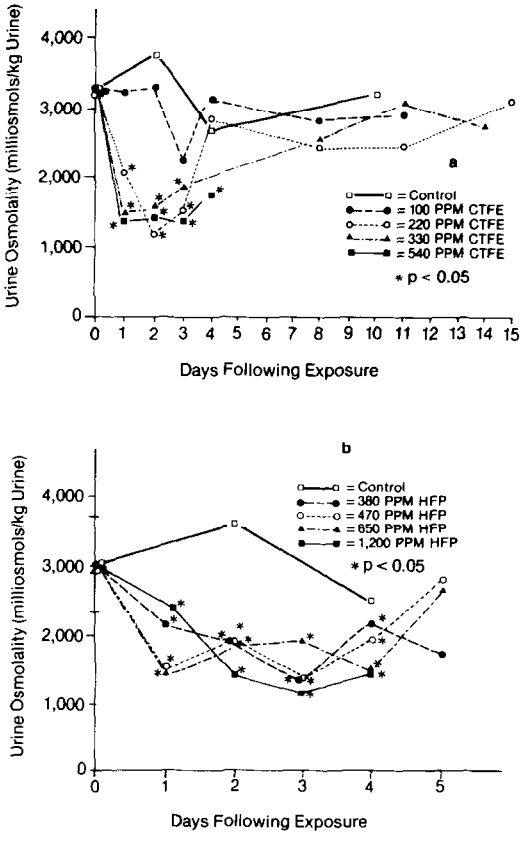

FIG. 1. Mean daily urine osmolalities following single 4.0-hr exposures of rats to CTFE (a) and HFP (b). ($N = 4$.)

METHODS

Animals. Male Fischer-344 rats (Charles River, Wilmington, Mass.) weighing 200–275 g were randomly divided into groups of 10 and placed in stainless-steel metabolism cages, two rats per cage. The rats were housed for at least 5 days prior to exposure so they could adapt to these surroundings. Food (Wayne Lab Blox) and tap water were allowed *ad libitum*. The tap water contained 1–2 μM fluoride.

Chemicals. CTFE (Matheson, Cucamonga, Calif.) and HFP (Peninsular Chemical Research, Gainsville, Fla.) were analyzed for purity using a Finnigan 9500 gas chromatograph with a Porapak Q stainless-steel column interfaced with a Finnigan 3300 mass spectrometer with attached data system 6110. HFP showed 100% purity with this detection system. CTFE was 99% pure; the only contaminant found was trifluoroethylene.

Certified standard mixture of CTFE (326 and 524 ppm) and HFP (529 and 3102 ppm) were obtained from Matheson for calibration standards. All other chemicals were reagent grade.

Exposure system. The dynamic exposure system consisted of a 34.0-liter Pyrex cylinder capable of housing 10 rats. The delivery system consisted of an infusion pump (Harvard) with two 50-cc glass syringes. From these, fluoroalkene was injected into a hose carrying air released from a cylinder (3000 psi), prior to entry into the exposure chamber. The air flow rate was 10 liters/min in CTFE exposures and 15 liters/min in HFP exposures.

The exposure atmosphere of the chamber was analyzed by gas chromatography on a Porapak Q stainless-steel column using a Varian 3700 gas chromatograph equipped with a flame ionization detector and Varian CDS 111 integrator calibrated with certified standards described above.

Exposure. Exposures were performed between 10 AM and 2 PM to avoid diurnal variation. Rats in groups of 10 were exposed for 4.0 hr to CTFE or HFP as follows: 100 (102 ± 16.3), 200 (222 ± 36.9), 330 (330 ± 66.4), 540 (544 ± 24.4) ppm CTFE, or 380 (380 ± 27.0), 470 (467 ± 72.0), 660 (660 ± 191.0), and 1200 (1188 ± 60.0) ppm HFP. Control animals were handled the same as experimental animals except they were exposed for 4.0 hr to air only. Mean exposure concentrations were calculated by averaging chamber concentrations determined at half-hour intervals during the exposure. The temperature remained constant at 25°C throughout the exposure.

After 4.0 hr the rats were removed from the exposure chamber and immediately returned to metabolism cages. Control rats and rats exposed to CTFE were killed (cervical dislocation) on Days 1, 2, 3, 8, and 14. HFP exposed rats were killed on Days 1 through 5 following exposure.

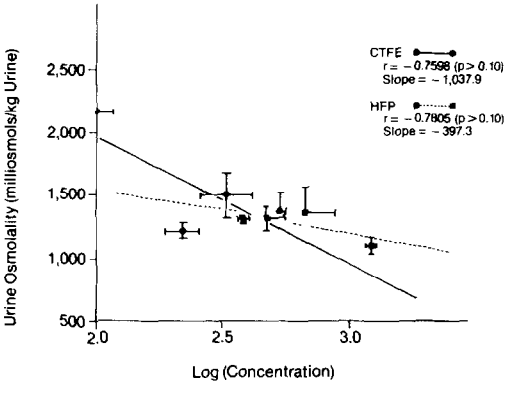

FIG. 2. Urine osmolality dose–response curves following single 4.0-hr exposures to CTFE or HFP. The values represent the lowest osmolalities that occurred following exposure to each concentration. The data are mean ± SD.

Urine analysis. Twenty-four-hour urine samples were collected in plastic cups for each pair of rats in the metabolism cages. Samples in the HFP study contained 50 μl of streptomycin (100 mg/ml) to prevent bacterial growth; samples in the CTFE study contained no antibiotics. Urine osmolalities were measured by freezing-point depression osmometry (Advanced Osmometer, Advanced Instruments, Inc., Newton Highlands, Mass.). Urinary fluoride levels were measured with a Corning Digital 110 Expanded Scale pH meter with an Orion Research Model 605 electrode switch. The determinations were made with a fluoride ion specific electrode as described by Fry and Taves (1970). Urinary LDH activities were determined on dialyzed urine using a modified Sigma LDH assay procedure, and Sigma LDH determination kit No. 340-UV.

Blood analysis. Serum creatinine and BUN (whole blood) assays, were performed with an automatic analyzer (Technicon AutoAnalyzer, Chauncey, N.Y.). The method employed for serum creatinine was a modification of the Folin and Wu procedure (1919).

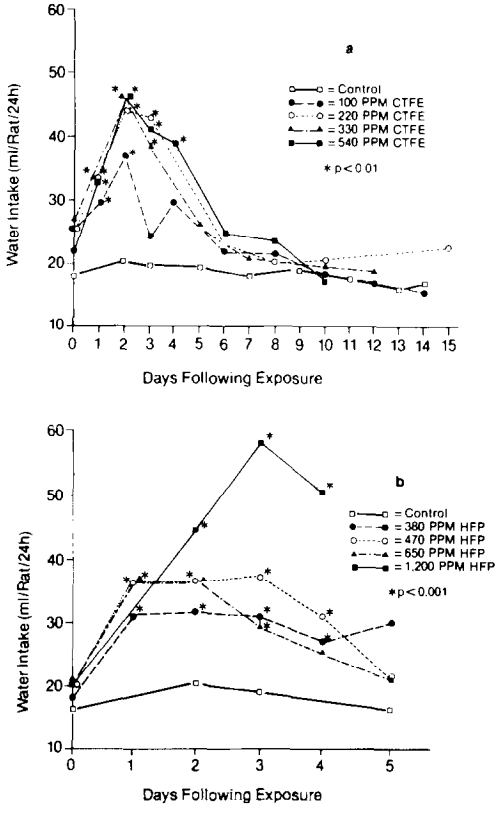

FIG. 3. Mean daily water intake by rats following single 4.0-hr exposure to CTFE (a) and HFP (b). ($N = 4$).

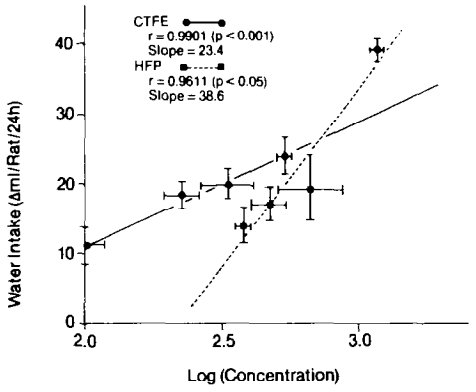

FIG. 4. Water intake dose–response curves following single 4.0-hr exposures to CTFE or HFP. The values represent the maximum water intake that occurred following exposure to each concentration. The data are mean ± SD.

The procedure for BUN was a modification of the carbamide–diacetyl reaction as applied to urea nitrogen (Skeggs, 1957; Marsh and Fingerhut, 1957).

Histology. Rats were sacrificed at intervals described and the kidneys immediately removed, sectioned longitudinally, and placed in 10% buffered formalin. Kidney samples from rats exposed to CTFE or HFP were embedded in paraffin and sections of 6.0 μm thickness were stained with hemotoxylin and eosin (H&E) or periodic acid–Schiff (PAS) stain, respectively.

Statistics. Tests of significance of difference between two means were calculated using Student's t test. Probability of 0.05 was applied to the data as the level for inferring a significant change from controls.

RESULTS

Urine osmolality and water intake. Urine osmolality decreased following exposures to CTFE and HFP at all concentrations except 100 ppm CTFE (Figs. 1, 2). Rats exposed to 220, 330, and 540 ppm CTFE exhibited significant decreases in urine osmolality for at least 3 days postexposure. All groups exposed to HFP exhibited significant decreases for 4 days. Significant increases in daily water intake levels were noted through Day 3 for all groups except the 100-ppm-CTFE group (Figs. 3, 4).

Urinary fluoride. Increases in urinary fluoride (F^-), a probable metabolite of CTFE

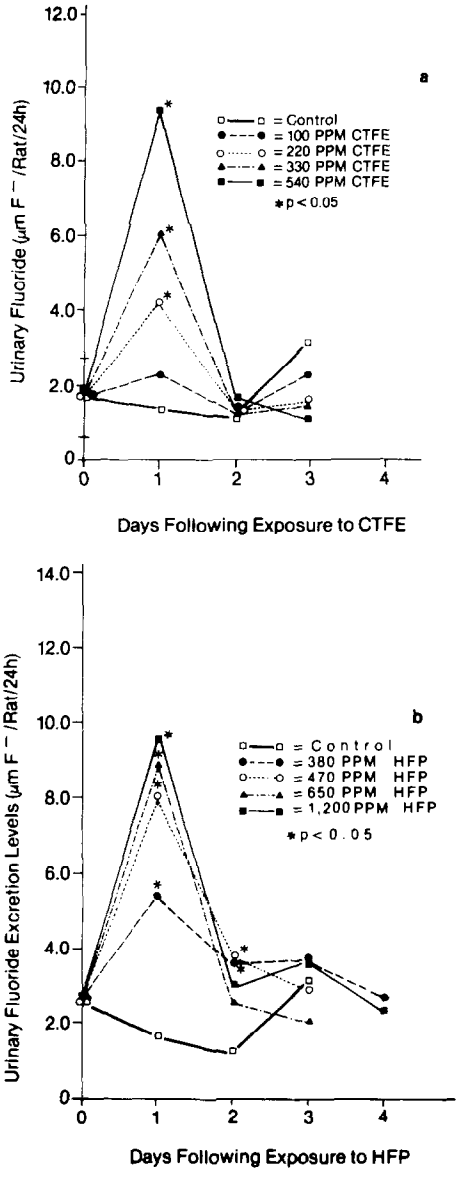

FIG. 5. Mean daily urinary fluoride excretions by rats following single 4.0-hr exposures to CTFE (a) and HFP (b). (N = 4).

and HFP, appeared within 24 hr following exposure to CTFE or HFP (Figs. 5, 6).

It appears that CTFE and HFP underwent metabolism such that similar amounts of F^- were excreted per unit concentration of CTFE or HFP to which the rats were exposed. The amounts of F^- excreted were proportional to the dose of fluoroalkene until the concentration of HFP exceeded 660 ppm. This apparent maximum urinary F^- excretion, after exposure to 660 ppm HFP, may reflect renal failure following exposure to higher concentrations.

Urinary LDH. Rats exhibited dose-related increases in urinary LDH excretions within 24 hr following exposure to CTFE or HFP (Fig. 7). All groups showed elevated urinary LDH activities except the group exposed to 100 ppm CTFE. From dose–response curves, CTFE appears the more potent toxin in that it produced larger responses than HFP at comparable exposure concentrations (Fig. 8).

BUN and serum creatinine. Following exposure to CTFE and HFP, rats exhibited dose-related increases in serum creatinine and BUN (Figs. 9–11). Rats exposed to CTFE had elevated levels of serum creatinine on Days 1 and 2 at all doses above 100 ppm. HFP produced dose-related increases in serum creatinine on Days 1 through 5. Dose–response curves show that rats exposed to CTFE responded with higher levels of serum creatinine and BUN than

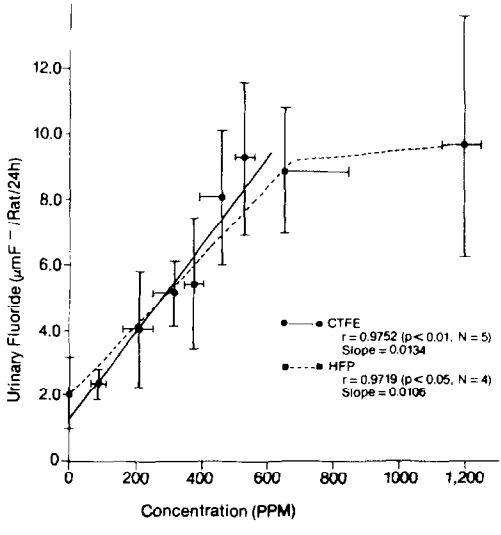

FIG. 6. Urinary fluoride dose–response curves following single 4.0-hr exposures to CTFE or HFP. The values represent maximum urinary fluoride excretion levels that occurred following exposure to each concentration. The data are mean ± SD.

rats exposed to similar concentrations of HFP, which agrees with other data indicating CTFE to be the more potent renal toxin (Figs. 10, 11).

Histology. Rats exhibited cellular necrosis of the proximal renal tubules within 24 hr following exposure to each concentration of HFP and to concentrations of CTFE greater than 100 ppm. Tubular necrosis was noted in the pars convoluta (segments P_1 and P_2) as well as the pars recta (segment P_3) of the proximal convoluted tubule in HFP-exposed rats (Figs. 12a, b), but was localized to the pars recta in rats exposed to CTFE (Figs. 12c, d).

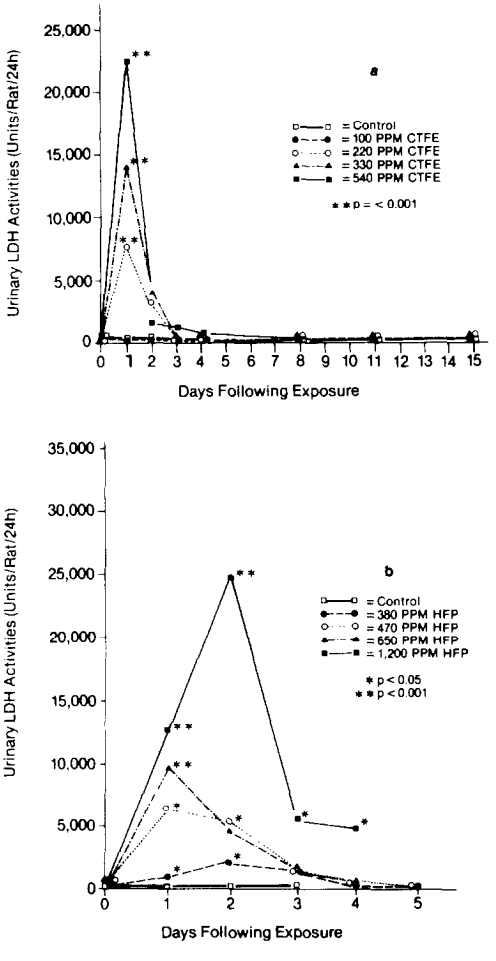

FIG. 7. Mean daily levels of urinary LDH excretions by rats following single 4.0-hr exposures to CTFE (a) and HFP (b). (N = 4).

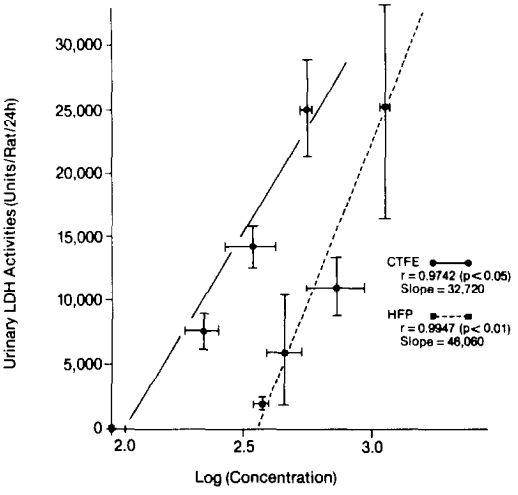

FIG. 8. Urinary LDH dose–response curves following single 4.0-hr exposures to CTFE or HFP. The values represent the maximum urinary LDH activities that occurred following exposure to each concentration. The data are mean ± SD.

Regeneration of tubule epithelial cells usually appeared by Day 3, and was characterized by flattened squamoid cells with basophilic cytoplasm. Occasional mitotic figures were seen. In rats exposed to CTFE, full regeneration was apparent within 2 weeks.

DISCUSSION

CTFE and HFP have been shown to disrupt renal function and cause focal necrosis of the proximal tubules of the kidneys of rats. Since there is potential for human exposure, via accidental industrial exposure, the effects of chronic low-level exposure to these fluoroalkenes is of interest. The evaluation of long-term carcinogenic potential as well as any irreversible renal effects resulting from chronic exposure may provide information instrumental in establishing proper hygienic guidelines for safe handling of these compounds in the work place. It is anticipated that the acute toxicity data presented here will aid in the design of chronic toxicity studies of CTFE and HFP.

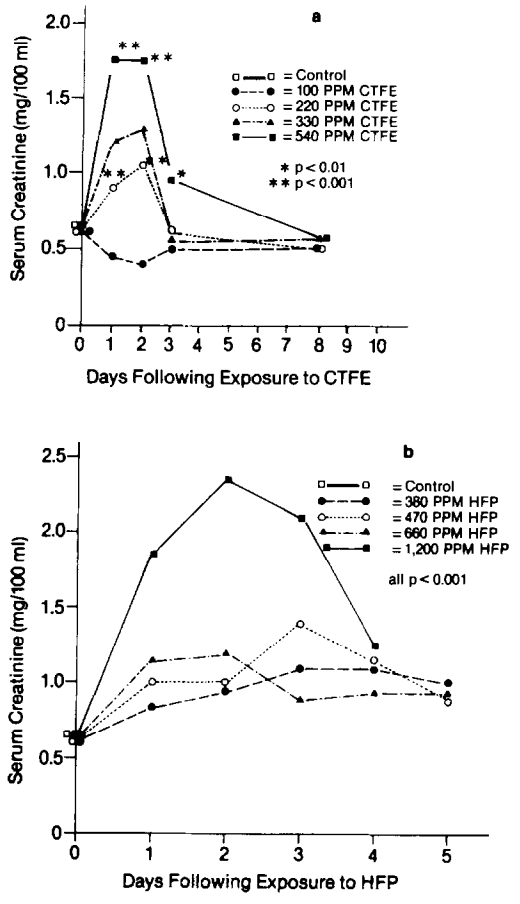

FIG. 9. Mean daily serum creatinine levels in rats following single 4.0-hr exposures to CTFE (a) and HFP (b). (N = 4).

Though HFP produced a water intake dose–response curve with a greater slope, CTFE produced larger responses than HFP at lower exposure concentrations. This is consistent with dose–response curves for serum creatinine, BUN, and urinary LDH, which show CTFE to produce renal toxicity at lower concentrations than HFP.

The dose-related increases in urinary LDH activity were indicative of dose-related necrosis of the proximal tubule occurring within 24 hr following exposure to CTFE or HFP. Examination of the kidneys by light microscopy showed damage occurring to the pars recta by 1 day post-exposure. These fluoroalkenes caused destruction of epithelial cells, but not of the basement membrane of the tubules. Thus rapid regeneration of epithelium followed the damage and often began within 3 days following exposure. The CTFE-exposed rats exhibited complete recovery of renal function and regeneration of damaged cells within 2 weeks suggesting that the damage inflicted was largely reversible at the concentrations to which rats were exposed.

CTFE and HFP are fluoroalkenes of slight to moderate toxicity relative to *cis*- and *trans*-2,3-dichlorohexafluorobutene-2 and perfluoroisobutylene which have been designated "highly" toxic fluoroalkenes (Clayton, 1977). The approximate lethal concentrations of these compounds in rats are 61 and 179 ppm for 1.0-hr single-inhalation exposures to *cis*- and *trans*-dichlorohexafluorobutene, and 0.76 ppm for 4.0 hr exposure to perfluoroisobutylene, respectively (Clayton, 1967). The reported LC_{50} values in rats for CTFE and HFP are 1000 and 3000 ppm, respectively, for a 4.0-hr single-inhalation exposure (Clayton, 1967). Histology in rats exposed to dichlorohexafluorobutene and perfluoroisobutylene has revealed pulmonary edema and what was

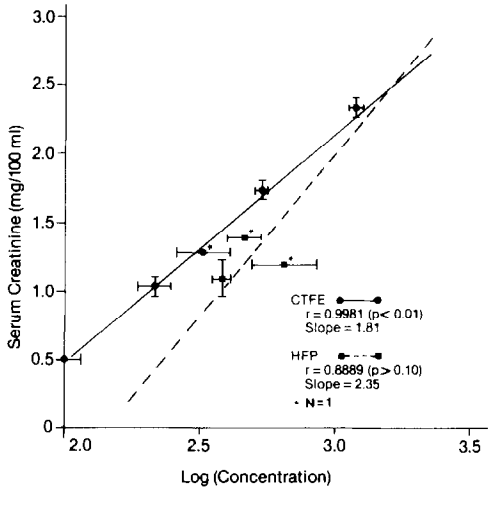

FIG. 10. Serum creatinine dose–response curves following single 4.0-hr exposures to CTFE of HFP. Values represent the maximum serum creatinine levels that occurred following exposure to each concentration. The data are mean ± SD except where small samples were pooled. N = 1.

described as degenerative changes in the liver and kidney (Clayton, 1967, 1977; Danishevskii and Kochanov, 1961; Cohen *et al.*, 1965).

A fluoroalkene of relatively low toxicity is tetrafluoroethylene with a reported LC_{50} value of 40,000 ppm for a 4.0-hr single-inhalation exposure in rats (Clayton, 1967). In a comparative study, Dilley *et al.* (1974) showed that tetrafluoroethylene induced renal dysfunction, as did HFP, but did not cause renal necrosis in rats exposed to 3500 ppm for 30 min; a 30-min exposure to 2600 ppm HFP produced some necrosis of the proximal renal tubules in rats. It is interesting that displacement of one of the fluorines of tetrafluoroethylene with a chlorine, to yield CTFE, lowers the 4.0-hr LC_{50} value from 40,000 to 1000 ppm, a 40-fold increase in toxicity.

Though no liver or lung pathology was performed on the rats we exposed to HFP, Dilley (1974) reported no changes in organs other than the kidneys following exposures to concentrations of HFP as high as 2600 ppm for 4.0 hr. Conventional screening methods for hepatic toxicity (histology, SGPT, SGOT) revealed no pathologic changes in the livers of rats exposed to CTFE in our study (data not shown).

The distribution of renal injury, produced by CTFE and HFP, resembles that of mercury poisoning (Rodin and Crawson, 1962; Ganote *et al.*, 1975; Taylor, 1965). Low doses of mercury and fluoroalkene produce necrosis of the pars recta portion of the proximal tubule, while higher doses produce necrosis also extending to more proximal portions. This type of injury has also been noted following administration of acetominophen (McMurtry *et al.*, 1978) and cephaloridine (Atkinson *et al.*, 1966), and following sustained renal hypotension (Kriesberg *et al.*, 1976; Thurau *et al.*, 1972).

Simonds (1942) suggested that injury distribution of this type may result from a gradient concentration of toxin in the tubule. After entering the tubule, in dilute glomerular filtrate, the toxin attains toxic

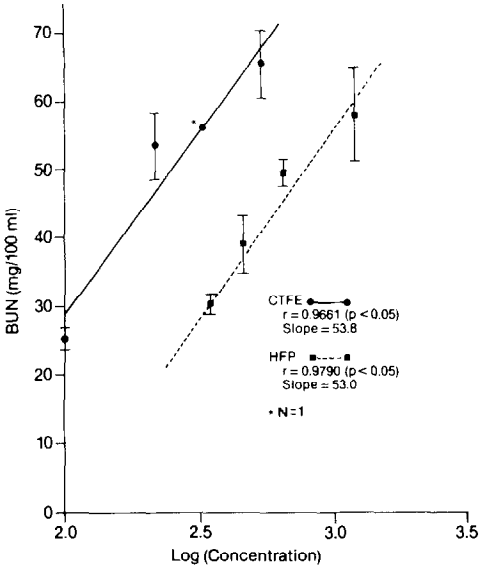

FIG. 11. BUN dose–response curves for rats following single 4.0-hr exposures to CTFE of HFP. Values represent the maximum BUN levels that occurred following exposure to each concentration. The data are mean ± SD except where small samples had to be pooled, $N = 1$.

threshold concentration subsequent to reabsorption of water. At low doses, toxic concentrations develop only in the terminal portions of the tubule. But at higher concentrations, toxicity results in more proximal regions as well as the terminal portion. Alternatively, structural and functional differences between cells of various segments of the proximal tubule may render different cells susceptible to varying degrees and types of injury. This may involve different or special types of metabolism, different enzymes within different renal segments, or varying degrees of transport of the toxin into cells of different parts of the kidney.

It is unclear whether the primary toxic agent involved in the renal damage was the parent compound, a bioactivated intermediate, or a toxic conjugate formed during phase II metabolism. Apparently CTFE and HFP underwent rapid metabolism as increases in urinary inorganic F^- occurred within 24 hr after exposure, and returned to normal by Day 2. Though inorganic F^- has been shown to induce diuresis in rats,

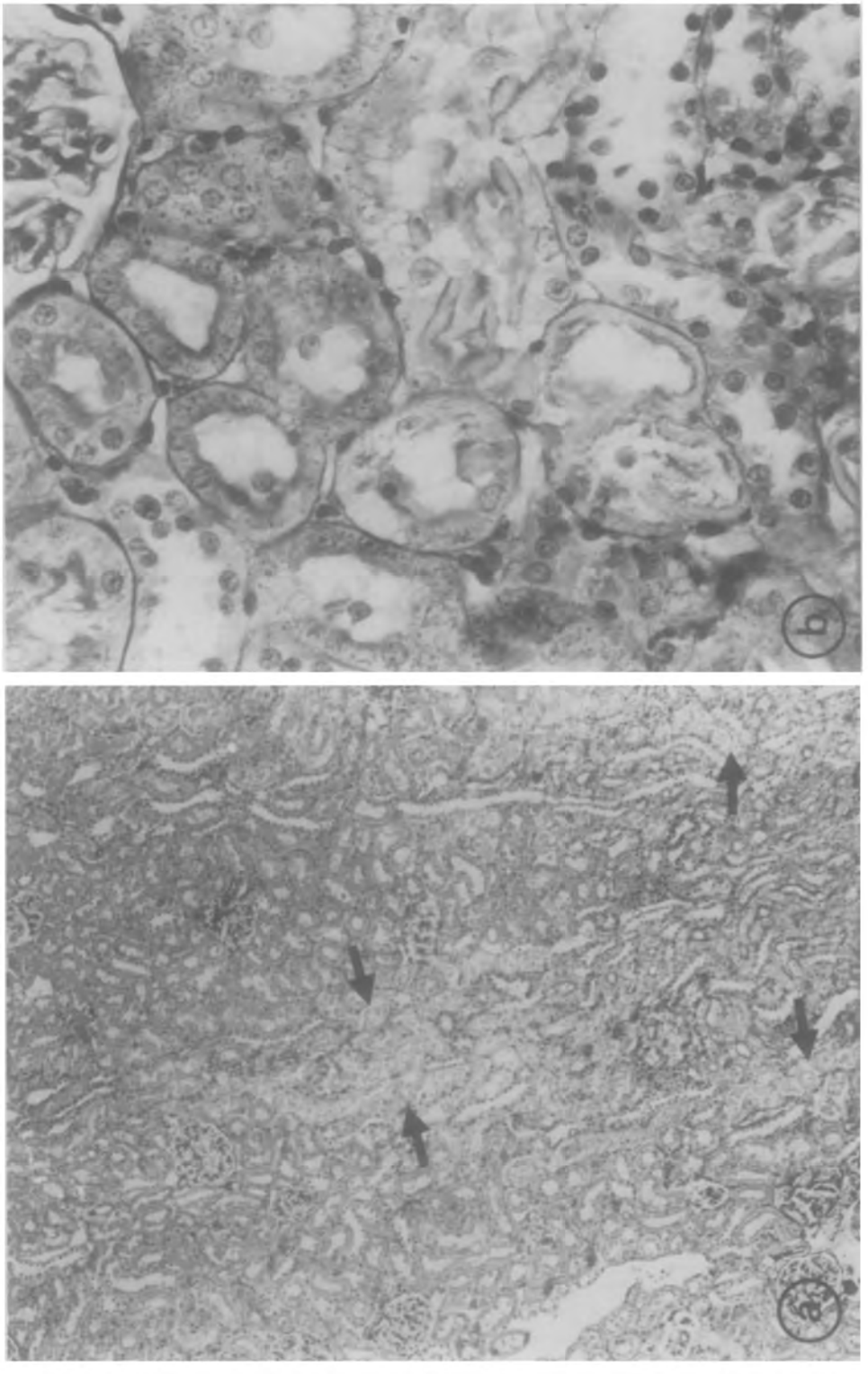

FIG. 12a. This light micrograph depicts the focal tubular necrosis seen in a kidney of an animal sacrificed 24 hr after exposure to HFP (470 ppm for 4.0 hr). Note necrosis involving pars recta (lower arrows), but also extending into cortical portions of the proximal tubule (two upper arrows). PAS, ×70.

FIG. 12b. This light micrograph shows higher magnification of the tubular necrosis involving pars convoluta in a rat sacrificed 24 hr after exposure to HFP (470 ppm for 4.0 hr). PAS, ×500.

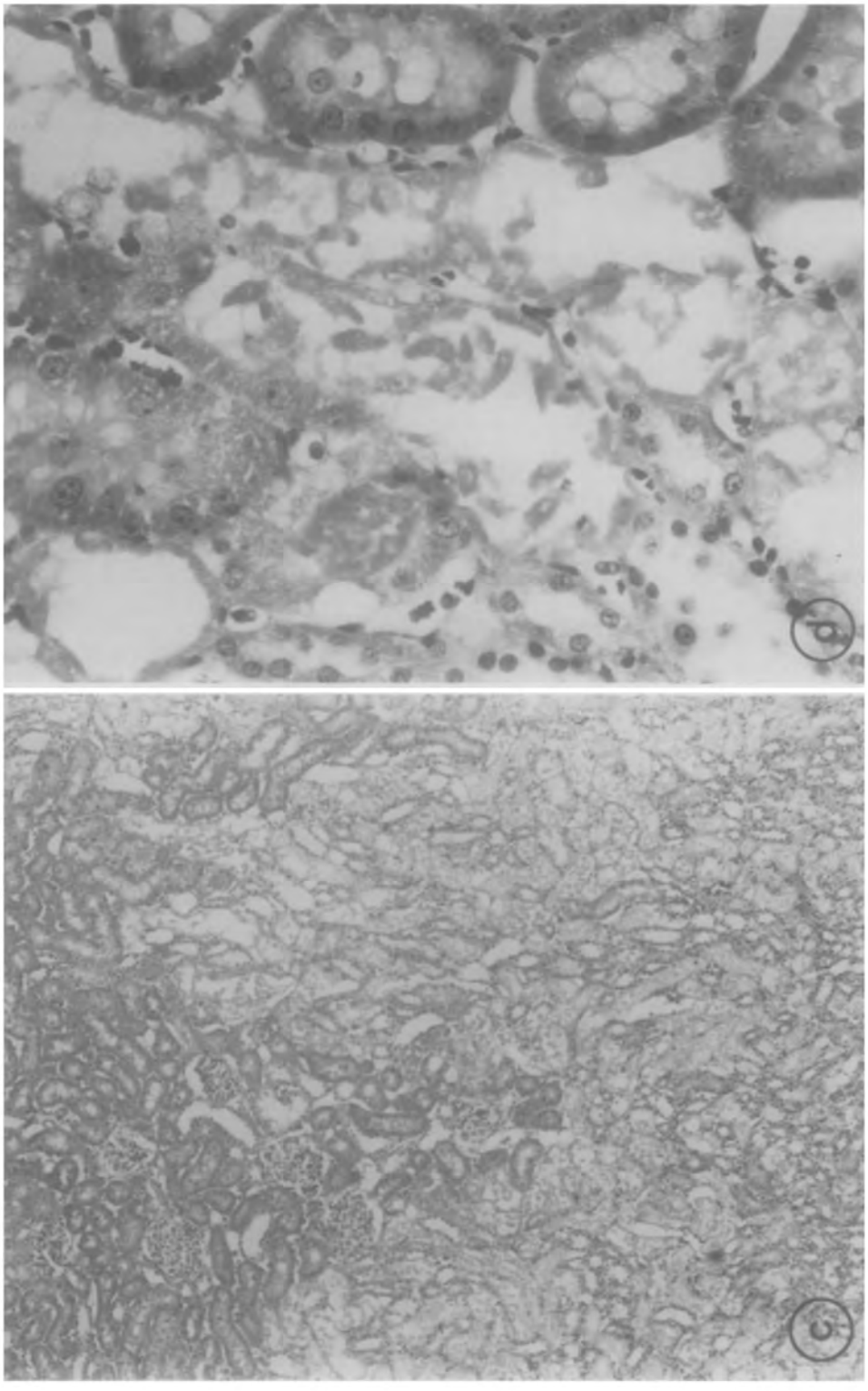

FIG. 12c. This light micrograph depicts the zonal necrosis involving only the pars recta portion of the proximal tubule in a rat sacrificed 24 hr after exposure to CTFE (330 ppm for 4.0 hr). Note the zonal nature of the necrosis with sparing of the cortical portions of the proximal tubule. H & E, ×70.

FIG. 12d. This light micrograph shows the complete coagulative necrosis of the pars recta within the medullary ray of a rat sacrificed 24 hr after exposure to CTFE (330 ppm for 4.0 hr). Note the sparing of the adjacent pars convoluta. H & E, ×500.

it seems unlikely that the inorganic F^- generated during metabolism was the primary toxic agent responsible for the diuresis noted following exposure to CTFE or HFP.

Cousins *et al.* (1974) performed a study in male Fischer rats in which diuresis was induced by the fluorinated anesthestic methoxyflurane. They determined the inorganic F^- generated during metabolism to be the primary toxic agent. However, for their rats to exhibit decreased urine concentration comparable to that of our rats, sufficient body levels of inorganic F^- were required such that the rats excreted nearly 40 μmol F^-/24-hr period, four times the amount excreted by our rats. This casts some doubt as to whether the inorganic F^- generated during metabolism, in this study, was the primary toxic agent involved in the fluoroalkene-induced diuresis. Furthermore, in this study, the amount of inorganic F^- excreted correlated with the exposure concentration of fluoroalkene; that is, the same amount of F^- was excreted per 24-hr period at equal parts per million exposure concentrations of CTFE and HFP. If inorganic F^- were the primary toxic agent, we would then expect the same dose–response relationships for CTFE as for HFP. This was not the case, however, since CTFE produced renal toxicity at lower concentrations than HFP. Thus it is suggested that a toxic metabolite other than inorganic F^- may be involved in the renal toxicity of fluoroalkenes.

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