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Vinylidene Fluoride: Acute Hepatotoxicity in Rats Pretreated with PCB or Phenobarbital (40638)[1]

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Vinylidene fluoride (VDF: 1,1-difluoroethylene) is an important plastics monomer. VDF is produced by dehydrochlorination of chlorodifluoroethane at 600°F. Its polymerization products include poly-VDF and the elastomers fluorinated ethylene–propylene and polychlorotrifluorethylene. In 1970 the total production of fluorocarbon resins in the USA, excluding polytetrafluoroethylene, was about 4×10^6 lbs. (1). Occupational exposure to VDF is not regulated by a specific threshold limit value (2), reflecting the fact that it is not regarded as a compound of significant toxicity.

Few reports have been published on the toxicology of VDF. Carpenter *et al.* (3) found that a 4-hr inhalation of 128,000 ppm VDF was acutely lethal to rats. However, Lester and Greenberg (4) observed only an unsteady gait in rats exposed to 80% (800,000 ppm) VDF + 20% O_2 for 19 hr. The postural reflex and consciousness were not affected. Jaeger *et al.* (5) confirmed this lack of obvious acute toxicity by exposing rats to 82,000 ppm VDF for 4 hr. No mutagenicity of VDF was detected in bacteria by Bartsch *et al.* (6). They also failed to find any evidence that VDF is metabolically activated to alkylating intermediates using a 4-nitro(4-benzyl)pyridine trapping system. Evidence that VDF is metabolized *in vivo* and that it can affect renal function in rats was published by Dilley *et al.* (7). They exposed rats to 2200 ppm VDF for 30 min and found significant increases in urinary excretion of fluoride and potassium ions. Urinary protein levels were also increased after this exposure.

Ethylene, vinyl fluoride, vinyl chloride, and vinyl bromide are plastics monomers that share with VDF both structural similarities and a lack of acute toxicity. We have previously shown, however, that ethylene and its monohalogenated analogs are acutely hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB) (8). PCB is a potent inducer of the hepatic mixed-function oxidase system (MFOS) (9). The similarities between VDF and these hepatotoxic monomers, as well as their lack of acute toxicity in normal (not pretreated) rats, led us to investigate the influence of the MFOS inducers PCB and phenobarbital (PB) on VDF hepatotoxicity. This toxicity was evaluated by measurement of liver weight, serum sorbital dehydrogenase (SDH) activity, and by light microscopic examination of the liver. SDH is a sensitive and specific index of acute hepatic injury (11).

Methods. Animals. Male Holtzman rats, 170–250 g, housed five or six per cage, were maintained on a 12-hr light–dark cycle. They were supplied with commercial rat chow (Purina Rat Chow, Ralston Purina Co., St. Louis, Mo.) and tap water *ad libitum.* The chow was removed 18 hr before VDF exposure. During exposure neither water nor chow were available. After exposure only water was allowed. Rats were sacrificed by cervical transection 24 hr after VDF exposure. Experience with compounds of similar structure and toxicity had suggested that the 24 hr interval was sufficient for injury to develop (10).

PCB and phenobarbital treatments. PCB (Aroclor 1254, Monsanto Chemical Co., St. Louis, Mo.) was solubilized in a vehicle of water containing 0.5% (w/v) methyl cellulose and 0.5% (v/v) Tween 80. Between 2:00 and 5:00 PM each day the rats were given 100 mg

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PCB/kg by gavage for 3 consecutive days. VDF exposure was on the day following the last PCB treatment. PB (0.1% w/v) was supplied in the drinking water for the 7 days prior to VDF exposure.

VDF exposure. VDF (99% minimum purity, Matheson Gas Company, Gloucester, Mass.) inhalation began between 9:00 and 11:00 AM and lasted 4 or 6 hr. The inhalation chambers and analytical methodology were as previously described (8).

Sorbitol dehydrogenase assay. Sorbitol dehydrogenase activity was measured with a method modified from that of Gerlach and Hiby (11). Serum (0.3 ml or an appropriate dilution thereof), triethanolamine buffer (2.3 ml, 0.3 *M*, pH 7.4), and NADH (0.1 ml, 12 m*M* in 0.1% NaHCO3) were mixed together and incubated at 30°C for 30 min. At the end of this incubation D(+)−fructose (0.3 ml., 4.0 *M*) was added. The disappearance of NADH absorbance was monitored at 366 nm by a Gilford 240 spectrophotometer equipped with a Gilford 410 digital absorbance meter.

Histological preparation. Slices of liver were fixed by immersion in 10% aqueous buffered formaldehyde. Paraffin-embedded sections (5–6 μm) were stained with hematoxylin and eosin.

Data presentation and analysis. Liver weight data are presented as g liver/100 g body wt to correct for differences in rat weights. Statistical evaluation of liver weight and SDH values are based on the logarithmic transformation of the raw data to assure similarity of variances. The data are presented as the geometric mean and standard error range. Differences between means were evaluated using the independent *t* test (12). A *P* value of less than 0.05 was considered to be significant.

Results. Gross inspection of rats exposed to VDF revealed hepatotoxic effects only in animals pretreated with PCB. In severe injury, intermingled areas of pallor and congestion were seen in a brittle liver. Light microscopic examination showed VDF-related hepatic damage (centrolobular to midzonal ballooning and necrosis) which was severe in rats pretreated with PCB and relatively mild in PB-pretreated rats. The ballooning of hepatocytes probably represents severe degeneration, i.e., endstage changes leading to cell death or necrosis. No gross or microscopic effects of VDF on other organs were seen regardless of pretreatment.

Hepatotoxicity and mortality after VDF exposure. No pretreatment: No effect was detectable on liver weight, SDH, and mortality in rats exposed to 25,000 ppm VDF for 6 hr with no preexposure treatment (Table I).

Phenobarbital pretreatment. Table I shows that 25,000 ppm VDF for 6 hr significantly increased liver weight in PB-pretreated rats. SDH and mortality were not affected.

Polychlorinated biphenyl pretreatment. Four-hour VDF exposure of rats pretreated with PCB caused increased liver weight at all of the concentrations tested. This effect was statistically significant at 15,000 and 25,000

TABLE I. ACUTE HEPATOTOXICITY OF VINYLIDENE FLUORIDE IN FASTED RATS PRETREATED WITH POLYCHLORINATED BIPHENYL[a] OR WITH PHENOBARBITAL[b]

Pretreatment	Vinylidene fluoride exposure (ppm)	Duration of exposure (hr)	Liver weight (g/100 g body wt)	SDH	Mortality
None	none	—	3.25 (3.16–3.33)	23.9 (19.9–28.7)	0/5
None	25,000	6	3.38 (3.34–3.43)	9.3 (8.7–9.8)	0/6
PB	None	—	4.27 (4.19–4.36)	20.6 (19.3–22.0)	0/6
PB	25,000	6	4.88 (4.76–5.01)[c]	14.6 (13.1–16.2)	0/5
Tween 80-methyl cellulose vehicle	None	—	3.21 (3.20–3.22)	11.7 (11.4–12.3)	0/4
PCB	None	—	6.59 (6.44–6.75)	16.1 (14.9–17.4)	0/6
PCB	5,000	4	7.70 (7.23–8.20)	63.6 (49.9–81.1)[d]	0/9
PCB	15,000	4	9.91 (9.54–10.29)[d]	137 (114–163)[d]	0/9
PCB	25,000	4	9.57 (9.05–10.12)[d]	281 (208–381)[d]	2/6

[a] Polychlorinated biphenyl (PCB), 100 mg/kg once a day for 3 consecutive days by gavage.
[b] Phenobarbital (PB), 0.1% (w/v) in drinking water for 7 days prior to exposure.
[c] Significantly different ($P < 0.01$ by the independent *t* test) from PB treated, not exposed.
[d] Significantly different ($P < 0.01$ by the independent *t* test) from PCB treated, not exposed.

ppm but not at 5000 ppm. The increase in liver weight was dose dependent from 5000 to 15,000 ppm VDF, but not from 15,000 to 25,000 VDF. SDH values were elevated significantly and in a dose-dependent fashion at all the VDF concentrations tested. No signs of necrosis nor of other gross abnormalities were noted at the end of VDF exposure. However, in the group of PCB-pretreated rats exposed to 25,000 ppm VDF, two out of six rats were found dead the following morning, 22–24 hr after the beginning of VDF exposure (Table I).

Histologic changes. Phenobarbital treated, not exposed. Phenobarbital did not alter the light microscopic appearance of the liver.

PCB treated, not exposed. The methyl cellulose–Tween 80 vehicle itself had no effect on the histology of the liver. PCB treatment caused cytoplasmic vacuolization of centrolobular and midzonal areas (Fig. 6) (8). In addition, there were a few foci of subcapsular hepatocyte ballooning and necrosis. The balloon cells had pyknotic nuclei peripherally displaced by an unstained vacuole which almost completely replaced the normal cytoplasm. Necrotic areas were interspersed with cellular debris and a few polymorphonuclear leukocytes.

VDF (25,000 ppm), not pretreated. No appreciable effect was noted on the histologic appearance of liver in rats which received 25,000 ppm VDF for 6 hr without pretreatment (Figs. 1 and 5).

Phenobarbital + VDF (25,000 ppm). In subcapsular areas hepatocytes with pale cytoplasm showed grouping of chromatin along the nuclear membrane. In other peripheral regions, ballooning liver cells surrounded rare foci of inflammatory reaction with necrosis (Fig. 2). The balloon cells were identical in appearance to those occasionally seen in the group treated only with PCB. Mild congestion was seen in the deep hepatic parenchyma.

PCB + VDF (5000 ppm). The PCB-related vacuolization of hepatocytes was accompanied by extensive centrolobular ballooning often involving half of the lobules (Fig. 7). The empty-looking hepatocytes had scanty cytoplasm with pyknotic nuclei, nuclear debris, or no visible nuclear material. Subcapsular areas contained balloon cells and foci of coagulative necrosis (Fig. 3). A variable degree of congestion was also noticed.

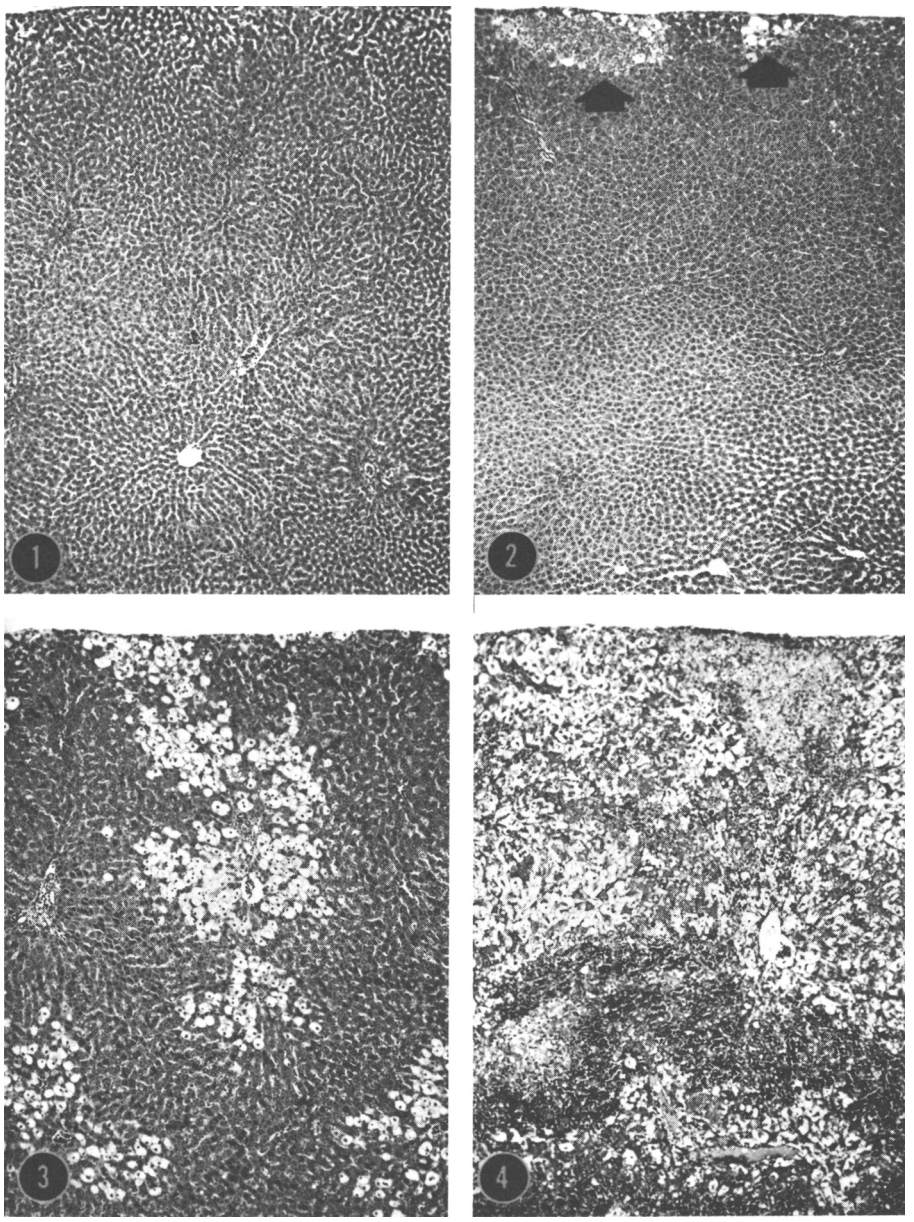
PCB + VDF (15,000 ppm). Animals in this group suffered extensive liver damage. Derangement of the hepatic architecture was complete, extending from the central vein to the portal triad. Ballooning of hepatocytes was detected even around the portal triad. Pale-staining necrotic tissue and large tracts of hemorrhage occurred in midzonal and centrolobular areas. The necrosis often involved the subcapsular regions (Fig. 4). Inflammatory cells were present in the necrotic tissue.

PCB + VDF (25,000 ppm). Livers of rats in this group were badly damaged, but not always clearly worse than those of the PCB + 15,000 VDF group. Massive areas of pale-staining necrosis involved the centrolobular and midzonal areas, frequently reaching the subcapsular spaces. Foci of inflammatory cells and hemorrhage bordered these necrotic areas. The periportal areas were filled with balloon cells (Fig. 8).

Discussion. Acute hepatic effects of exposure to VDF developed only in rats pretreated with PB or PCB (Table I), both of which induce the MFOS (9, 13). This dependence of acute VDF toxicity on pretreatment with MFOS inducers implies that metabolism of VDF to tissue-damaging metabolites is responsible for the toxic reaction. Metabolic activation of chlorinated ethylenes is generally believed to occur by epoxidation (14). The structural similarities of VDF with the family of chlorinated ethylenes suggest that an epoxide of VDF may also be formed. Such a reactive intermediate could be responsible for the acute hepatotoxicity reported here.

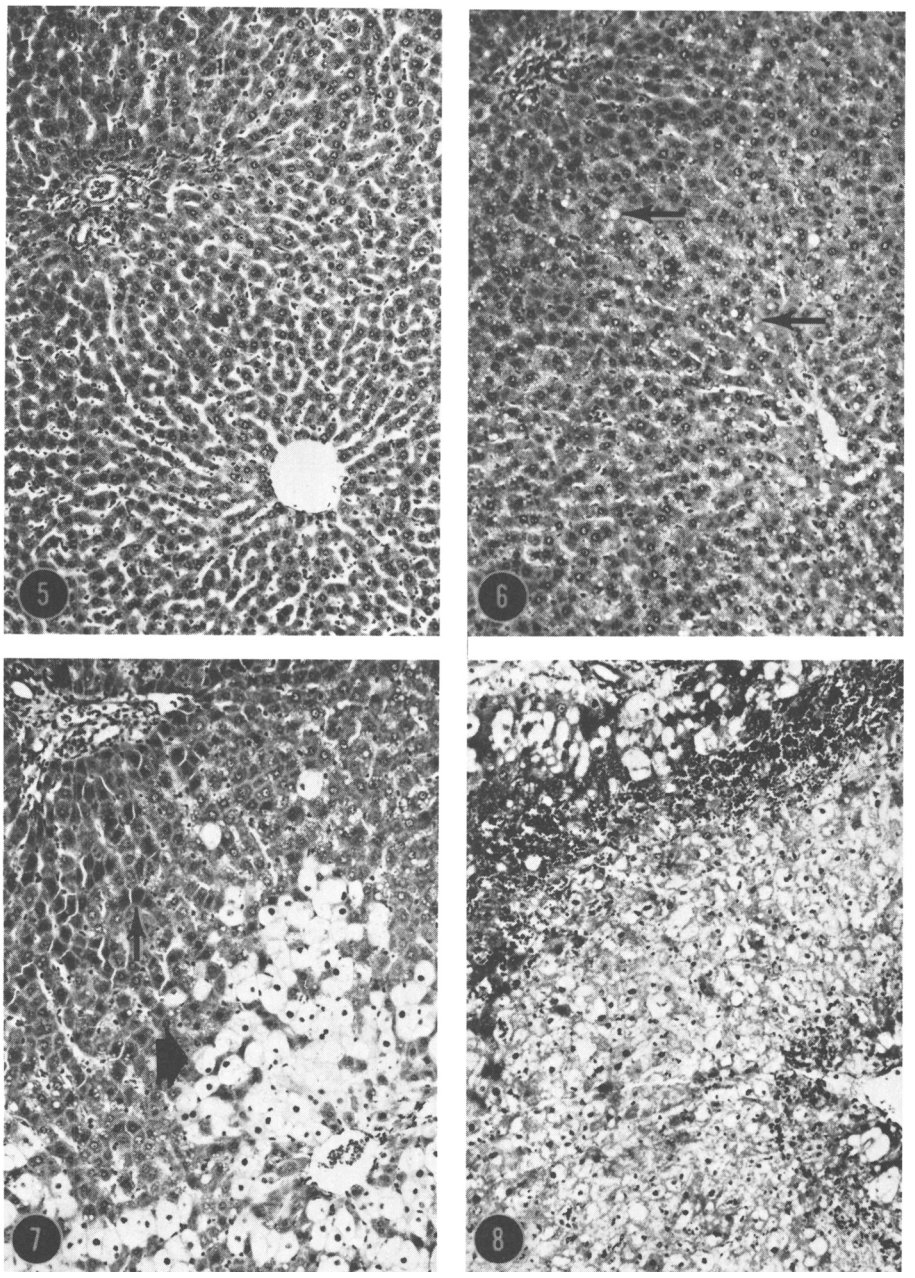
The potency of VDF as an acute hepatotoxin is roughly the same as that of a series of structurally related compounds—ethylene, vinyl fluoride, vinyl chloride, vinyl bromide—which are also acutely hepatotoxic only in rats treated with a MFOS inducer (8). In addition, the spectra of hepatic lesions, both gross and microscopic, caused by these compounds are very similar.

In contrast, the acute toxicity of VDF is very different from that of its halogenated analog, vinylidene chloride (VDC: 1,1-dichloroethylene). VDC is a potent acute hepatotoxin, having a 24-hr LC_{50} (the concentration which kills 50% of the exposed rats in



FIGS. 1–4 Hepatic capsule at top, hematoxylin and eosin. ×53.2. (1) Essentially normal architecture of liver of rats which received only VDF (25,000 ppm) exposure. (2) Two foci (arrows) of ballooned and necrotic liver cells in the subcapsular region (PB + VDF 25,000 ppm). (3) Ballooning of hepatocytes extends from the surface into the deep parenchyma (PCB + VDF 5000 ppm). Note that the liver damage is more severe than with higher concentration of VDF (25,000 ppm) in PB-pretreated rats. (4) Almost uniform derangement of hepatic architecture; generalized ballooning, region of hemorrhagic necrosis (PCB + VDF 15,000 ppm).

24 hr) of 600 ppm in rats exposed for 4 hr (15). Pretreatment with MFOS inducers protects against, rather than enhances, the acute effects of VDC and the hepatic lesion which develops after VDC exposure is morphologically distinct from that caused by VDF (16). Jaeger *et al.* (17) have proposed that the VDC metabolite chloroacetate undergoes a "lethal



FIGS. 5–8 Central vein at lower right, portal triad upper left, hematoxylin and eosin. ×133. (5) The liver of rats exposed only to VDF (25,000 ppm) appears normal at higher magnification as well. (6) PCB treatment caused fine vacuolization (arrows) of hepatocytes in the centrolobular and midzonal areas. (7) Ballooning of hepatocytes (horizontal arrow) in centrolobular space involving about half of the lobule. In other regions hypereosinophylic (dark) hepatocytes with pyknotic nuclei are visible (vertical arrow) (PCB + VDF 5000 ppm). (8) Panlobular hepatic injury with hemorrhage around the pale, necrotic part and ballooning of hepatocytes around the portal triad (PCB + VDF 25,000 ppm).

synthesis" in the citric acid cycle in a manner similar to that of fluoroacetate. This metabolic sequence for VDC involves a chloride shift as VDC epoxide rearranges to the corresponding chloroacyl chloride (18, 19). F–C bonds are stronger than Cl–C bonds and F migration during metabolism is less likely than is Cl migration (20). If the epoxides of both VDF and VDC are formed, the strikingly different toxicities of these two compounds may, therefore, be due in part to the differing potentials of the two epoxides to undergo rearrangements involving halogen migration.

PCB was much more effective than PB in sensitizing rats to the acute hepatotoxicity of VDF (Table I). This parallels the reports of Moslen *et al.* (21), Jaeger *et al.* (22), and of unpublished data from our laboratory which show PCB to be significantly more effective and potent than PB in sensitizing rats to the acute hepatic effects of ethylene, vinyl fluoride, and vinyl chloride. Alvares and Kappas (9) have shown PCB (Aroclor 1254) to be a "mixed type" inducer, having both PB and 3-methylcholanthrene-like effects on the MFOS. These data suggest that the toxic metabolite(s) of VDF is (are) generated by a specific enzyme or group of enzymes preferentially induced by PCB.

It is clear that VDF, ethylene, vinyl fluoride, vinyl chloride, and vinyl bromide share a number of characteristics: They are structurally similar; none is normally acutely toxic in humans or rats; their potencies as hepatotoxins are roughly similar; both the gross and microscopic appearances of the hepatic lesions they cause are similar; and their toxic effects in the rat are enhanced to a greater extent by PCB than by PB. These similarities suggest that a common mechanism may underlie the acute hepatic injury which results from PCB treatment with subsequent exposure to one of these compounds.

Summary. In rats pretreated with either phenobarbital (PB) or with polychlorinated biphenyl (PCB), an acute hepatotoxic reaction developed within 24 hr of inhalation exposure to vinylidene fluoride (VDF:1,1-difluoroethylene). PCB is much more effective than PB in this sensitization, which was evaluated by measurement of liver weight, serum sorbitol dehydrogenase activity, and by light-microscopic examination of the liver. PCB-treated rats received 5000, 15,000, or 25,000 ppm VDF for 4 hr while rats treated with PB inhaled 25,000 ppm VDF for 6 hr. The toxic effects of VDF in PCB-pretreated rats were dose responsive. These findings indicate that hepatotoxicity after VDF exposure can occur in rats and that these reactions (e.g., metabolism) can be stimulated by enzyme inducers. In light of the known activation of chlorinated ethylenes to epoxides, the analogous structures of VDF and the chlorinated ethylenes suggest that the epoxide of VDF may be part of the observed toxicity.

1. Dean, J. C., *in* "Chemical Economics Handbook, Report 580 0721A." Stanford Research Institute, Menlo Park, Calif. (1971).
2. Anonymous, "TLVs Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1977." American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio (1977).
3. Carpenter, C. P., Smyth, H. F., Jr., and Pozzani, U. C., J. Ind. Hyg. **31,** 3–43 (1949).
4. Lester, D., and Greenberg, L. A., Arch. Ind. Hyg. Occup. Med. **3,** 335 (1950).
5. Jaeger, R. J., Conolly, R. B., and Murphy, S. D., Arch. Environ. Health **30,** 26 (1975).
6. Bartsch, H., Malaveille, C., Barbin, A., Planche, G., and Montesano, R., Proc. Amer. Ass. Cancer Res. **17,** 17 (1976).
7. Dilley, J. V., Carter, V. L., Jr., and Harris, E. S., Toxicol. Appl. Pharmacol. **27,** 582 (1974).
8. Conolly, R. B., Jaeger, R. J., and Szabo, S., Exp. Mol. Pathol. **28,** 25 (1978).
9. Alvares, A. P., and Kappas, A., J. Biol. Chem. **252,** 6373 (1977).
10. Conolly, R. B., and Jaeger, R. J., Environ. Health Perspect. **21,** 131 (1977).
11. Gerlach, H., and Hiby, W., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), Vol. 2. Academic Press, New York (1974).
12. Snedecor, G. W., and Cochran, W. G., "Statistical Methods." Iowa State Univ. Press, Ames, Iowa (1967).
13. Mannering, G. J., *in* "Selected Pharmacological Testing Methods" (A. Burger, ed.), Vol. 3, p. 51. Marcel Dekker, New York (1968).
14. Greim, H., Bonse, G., Radwan, Z., Reichert, D., and Henschler, D., Biochem. Pharmacol. **24,** 2013 (1975).
15. Jaeger, R. J., Conolly, R. B., and Murphy, S. D., Exp. Mol. Pathol. **20,** 187 (1974).
16. Reynolds, E. S., Moslen, M. T., Szabo, S., Jaeger, R. J., and Murphy, S. D., Amer. J. Pathol. **81,** 219 (1975).

17. Jaeger, R. J., Szabo, S., and Coffman, L. J., J. Toxicol. Environ. Health, **3,** 545 (1977).
18. Henschler, D., Environ. Health Perspect. **21,** 61 (1977).
19. Jones, B. K., and Hathway, D. E., Chem. Biol. Interact. **20,** 27 (1978).
20. Clayton, J. W., Fluorine Chem. Rev. **1,** 197 (1967).
21. Moslen, M. T., Jaeger, R. J., Szabo, S., and Reynolds, E. S., Toxicol. Appl. Pharmacol. **33,** 133 (1975).
22. Jaeger, R. J., Murphy, S. D., Reynolds, E. S., Szabo, S., and Moslen, M. T., Toxicol. Appl. Pharmacol. **41,** 597 (1977).

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