1,1-Dichloroethylene-Induced Pulmonary Injury*

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ABSTRACT: *Oral administration of 1,1-dichloroethylene (1,1-DCE) produces acute injury to the lungs of C57B1/6 mice. The bronchiolar epithelium is most severely affected with damage selective for Clara cells. After a 100 mg kg/dose of 1,1-DCE, Clara cells show extensive dilatation of cisternae and degeneration of the endoplasmic reticulum. At 6 hr after administration of 200 mg 1,1-DCE/kg, both ciliated and Clara cells are necrotic, and bronchiolar epithelial lining exfoliates. By 24 hr, pulmonary edema, hemorrhage, and focal atelectasis are also present. Pulmonary injury, at 24 hr after the high dose, is associated with a significant hypoxia as demonstrated through a decrease in the partial pressure of oxygen in arterial blood. In spite of the severe injury, recovery occurs and airways display an intact epithelial lining with normal parenchymal elements by 7 days.*

INTRODUCTION

The ②chloroethylene, 1,1-dichloroethylene (1,1-DCE), or ①vinylidine chloride is an important industrial compound which is widely used in the plastics industry. In 1976, the United States and Japan together produced approximately 150 million kilograms [1]. 1,1-DCE is most commonly used as a copolymer in the manufacture of flexible plastic films for food wraps. Levels of monomeric 1,1-DCE have been detected in food wrapped with plastic wrapping such as Saran Wrap [2]. Moreover, workers in manufacturing facilities are also exposed to 1,1-DCE [3–6] and this compound has been identified as a contaminant in the atmospheres of submarines and spacecraft [7].

Animal studies with 1,1-DCE indicate that it may be a potential hazard to human health. Following its administration, liver [8–10] and kidney damage [11] ensue. It has been shown to be both a mutagen [12–13] and a potential carcinogen [14, 15]. Recently, it has been implicated as embryo- and fetotoxic [16] in studies with rats and rabbits.

Exposure to 1,1-DCE in humans is usually by inhalation or ingestion. In experimental animals, regardless of the method of administration (gastric intubation, intravenous, or intraperitoneal injection) or of the vehicle (mineral oil, corn oil, or aqueous Tween 80), the main route of elimination of unmetabolized monomer is through the lungs [17, 18]. In spite of the availability of this information, there are few reports on the pulmonary effects of 1,1-DCE. In one long-term inhalation study involving rats, guinea pigs, monkeys, rabbits, and dogs the results of the toxic effects on the lung were inconclusive [10]. In another long-term investigation, bronchoalveolar adenomas were observed in

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several mice but were considered to be of limited significance [15]. Despite the varied toxic effects of this compound in experimental animals, and the common exposure of humans to 1,1-DCE, there are no published reports on the acute toxic actions of this compound on the lung.

In this investigation, we evaluated the acute consequences of toxic doses of 1,1-DCE on the lungs of mice.

METHODS

Male C57B1/6 mice (Timco Breeding Labs, Houston, TX) weighing 20–25 g were maintained on a 12-hr light–dark cycle and allowed food (Wayne Lab-Blox, Allied Mills, Inc., Chicago, IL) and water ad libitum until death.

At 10:00 A.M. of the first day of the experiment, 1,1-DCE (99% purity, Aldrich Chemical Co., Milwaukee, WI) was administered by gastric intubation. Three to five animals in each group received either 100 mg/kg or 200 mg 1,1-DCE/kg in mineral oil. Control animals received only the vehicle. Mice were killed at 6, 12, 24, 48, 96, and 168 hr after administration of the chemical.

After cervical dislocation, a midline incision was made and a pneumothorax produced by cutting the diaphragm on both sides. The trachea was then exposed and cannulated with an intravenous cannula (Argyle Medicut Cannula, Sherwood Medical Industries, St. Louis, MO). The lungs were inflated via the airway with 1% glutaraldehyde in 0.1 M Pipes buffer (pH 7.35, 360 mOsm) at a hydrostatic pressure of 15 cm H_2O. After ligating the trachea, the lungs were removed en bloc together with the heart, and immersed in the fixative for 4 hr. The tissues were then stored in 0.1 M Pipes buffer overnight. Slices of lung (1-mm thick) were obtained from each lobe, post-fixed for 2–5 hr in 1% osmium tetroxide in 0.1 M Pipes buffer, stained en bloc with uranyl acetate for 2–5 hr and dehydrated in a graded series of ethanol and propylene oxide. Infiltration was achieved slowly, starting from a mixture of 75% propylene oxide and 25% Epon, continued through 50% propylene oxide and 50% Epon and terminated in 100% Epon. Each step was processed for 4 hr. Slices of lung were then embedded flat in the cap of a Beem capsule and the barrel filled with epoxy resin using a method similar to that described by Stephens and Evans [19].

The technique of embedding slices of lung tissue utilized in this study permitted specimens to be thick sectioned (0.5 μm) that were up to 5-mm wide. This facilitated the scrutiny of large pieces of lung tissue and therefore, also of a number of intrapulmonary airways within a section.

Polymerization was achieved at a temperature of 60°C for 24 hr for thick sections. Blocks that were selected for thin sectioning were cured at 60°C for an additional 48 hr.

For light microscopy 0.5-μm sections were cut with glass knives on a Sorvall MT-2 Ultramicrotome and stained with toluidine blue. For electron microscopy, thin sections were cut on an LKB Ultrotome III, with a Dupont diamond knife and stained with uranyl acetate and Reynolds' lead citrate [20]. Sections were examined with a Philips 400 or 201 Electron Microscope.

Blood Gases

Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (Nembutal) at a dose of 0.1 mg/g animal weight. The abdomen was exposed and arterial blood drawn from the abdominal aorta with a 26-gauge needle into a heparinized syringe. Blood gases were immediately determined in a blood gas analyzer (Instrumentation Laboratories, Model 813).

er Enzymes

Mice were lightly anesthetized with ether and blood obtained from the inferior vena cava. The serum was analyzed for glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT). Reactions were performed by standard techniques, utilizing Smith Kline Instruments, Inc. "Spin Chem" reagents.

Statistical Analysis

Blood gases were analyzed by the unpaired *t* test; 95% confidence limits for geometric means of liver transaminases were compared by the Cochran approximation of *t* values for grouped data with unequal variances [21].

RESULTS

Administration of 100 mg 1,1-DCE/kg resulted in a decrease of 9% of body weight within 24 hr; 200 mg/kg caused a reduction of 11% in body weight over that of control animals by the same time period.

Histology

Examination of the lung by light microscopy revealed differences in morphology of the small airways between control and treated animals (Fig. 1). Bronchioles in control animals showed an epithelial lining composed of low cuboidal ciliated cells and columnar nonciliated (Clara) cells with prominent apices which projected into the lumen [Fig. 1(a)]. With a dose of 100 mg/kg 1,1-DCE, the bronchiolar epithelium showed the presence of a few vacuolated cells at 12 hr after gavage. By 24 hr it was apparent that the primary target of 1,1-DCE on the bronchiolar epithelium was the Clara cells for they prominently displayed cytoplasmic vacuoles [Fig. 1(b)], while ciliated cells did not appear to be affected. By 48 hr, recovery had occurred and the bronchioles displayed a normal and intact epithelial lining.

The main bronchi and trachea did not display any prominent changes in their epithelial lining upon administration of 1,1-DCE.

The 200 mg 1,1-DCE/kg dosage induced necrosis of both ciliated and Clara cells of the bronchiolar epithelium. Some epithelial cells in the small airways sloughed off as early as 6 hr. By 24 hr, the lesion had increased in severity and areas of bronchioles were denuded of epithelium. Residual epithelium appeared flattened and attenuated and cellular debris was present in the airway lumen [Fig. 1 (c)]. Peribronchial and perivascular edema were also observed and the latter appeared to affect the larger peribronchial vessels. In spite of the apparent severe injury, recovery was accomplished by 7 days, as the epithelium was similar to that of control animals at that time.

The main bronchi and trachea exhibited changes similar to those observed in the bronchioles, with epithelial lining cells undergoing degenerative changes and exfoliation.

Ultrastructure

Electron microscopic examination of the lung confirmed that the vacuolated cells present in the bronchiolar epithelium following 100 mg 1,1-DCE/kg, were nonciliated Clara cells and that the ciliated cells appeared unaffected. These cells in the control animals possessed the characteristic morphology attributed

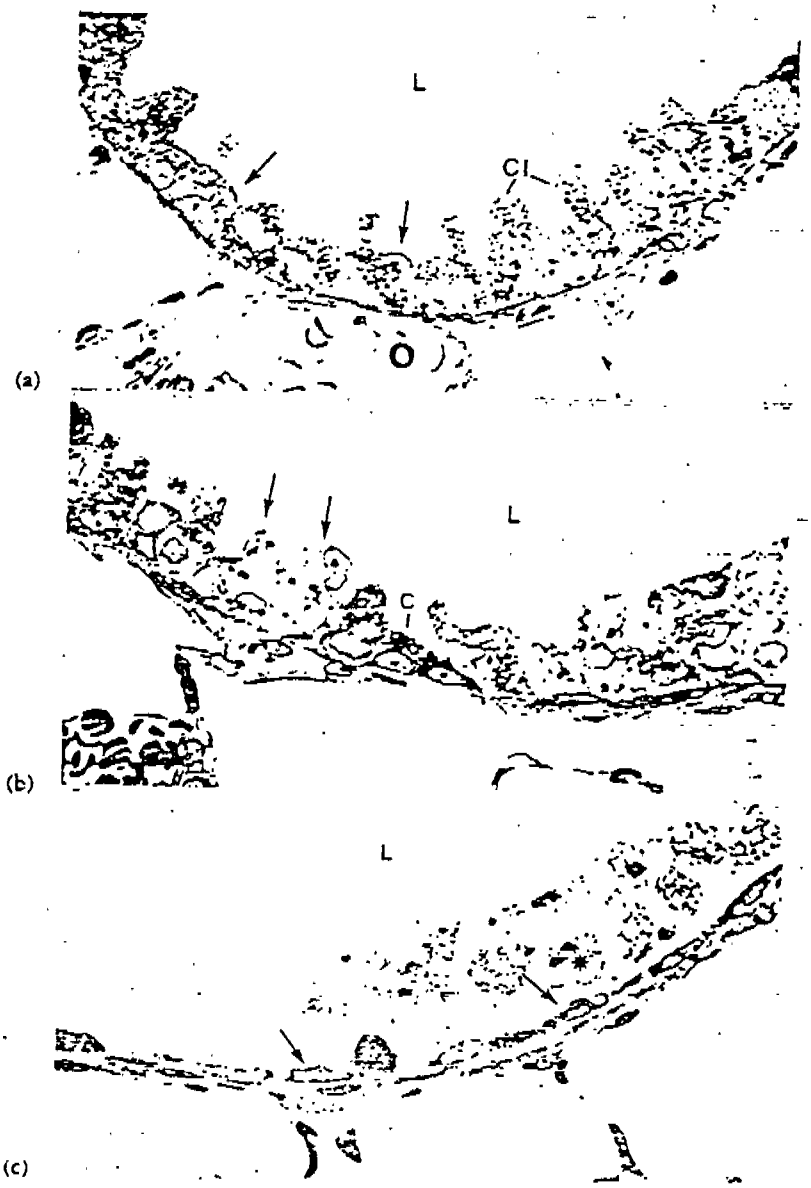
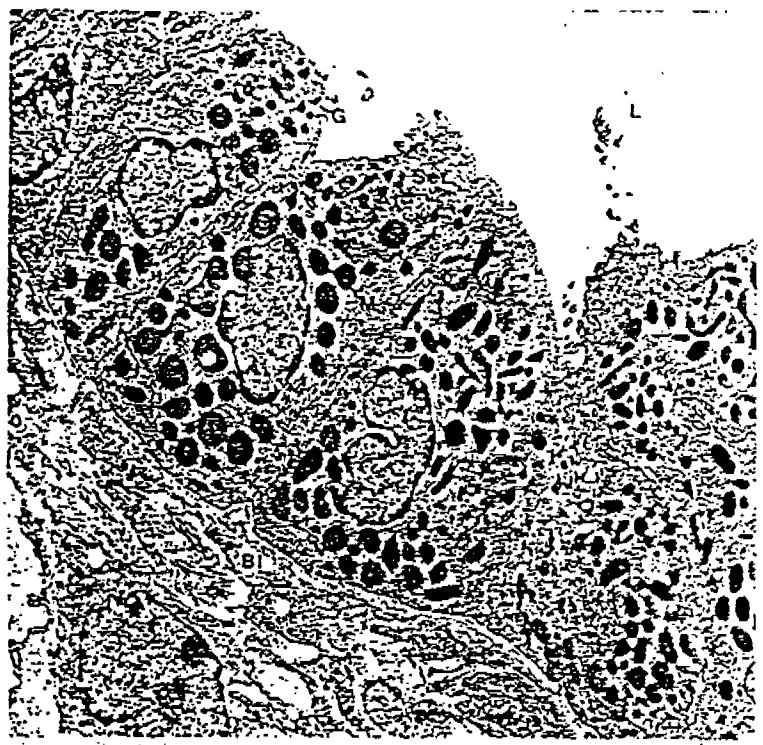


Figure 1 Bronchiole in the lung of control mouse (a), 100 mg/kg (b) and 200 mg/kg (c) 1,1-DCE. Toluidine blue stain (1000×). (a). In controls, Clara cells (Cl) have prominent apices which project into the lumen (L) of the airway. A small number of ciliated cells are present (arrows). (b). At 24 hr after exposure to 100 mg 1,1-DCE/kg, numerous Clara cells are vacuolated (arrows). The ciliated cells (C) appear normal. (L) lumen. (c). At 24 hr after 200 mg 1,1-DCE/kg numerous epithelial cells have sloughed off. The ciliated cells have flattened (arrows) and cellular debris (asterisk) is present in the lumen (L).

to Clara cells (Fig. 2). The nucleus is indented and basally located. The mitochondria are pleomorphic, varying in electron density and shape. The endoplasmic reticulum tends to form arrays encircling the mitochondria. While the smooth endoplasmic reticulum forms extensive networks in the apical portion of the cell, the rough variety tends to be concentrated in the lower half of the cell. Dense secretory granules were found in the apex of the cell close to the plasma membrane. Following 100 mg 1,1-DCE/kg, the extensive vacuolization in Clara cells consisted of dilated cisternae of both the smooth and rough endoplasmic reticulum (Fig. 3). The rough endoplasmic reticulum also appeared degranulated and polyribosomes disaggregated. Perinuclear cisternae were also distended and the nuclei assumed a more rounded profile. Mitochondria of affected cells were swollen and showed a reduction in numbers of cristae, and the distribution of organelles in the cytoplasm appeared disorganized. Electron-dense aggregates, approximately 10–15 nm in cross-section, appeared in the cytoplasm adjacent to tubules of smooth endoplasmic reticulum. These aggregates formed a fuzzy coat on their cytoplasmic surface. Transitional forms between "coated" and normal smooth endoplasmic reticulum were seen in some tubular profiles (Fig. 4). A web of filaments was often present in the cytoplasmic matrix

Figure 2 Electron micrograph of bronchiolar epithelium of lung in control mouse. The ciliated cells are low and cuboidal; the Clara cell is columnar, has numerous mitochondria (M) and dense secretory granules (G) and abundant rough (ER) and smooth endoplasmic reticulum (Ser). (Bl) basal lamina. Uranyl acetate and lead citrate stains (6,200×).



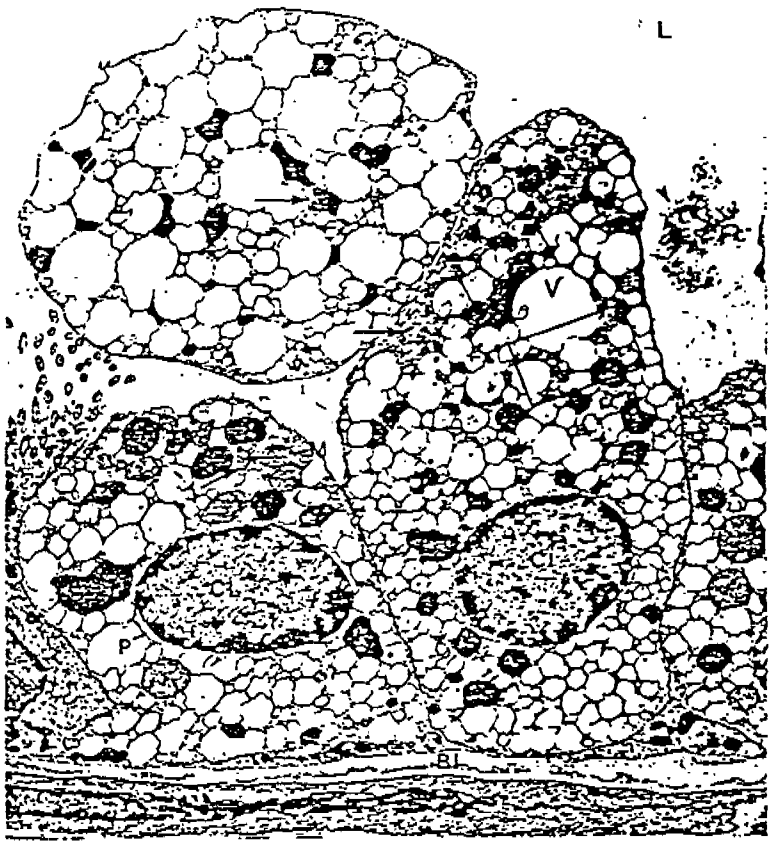


Figure 3 Electron micrograph of bronchiolar epithelium of lung in mouse administered 100 mg 1,1-DCE/kg. At 24 hr after gavage, the Clara cell shows degenerative changes. The endoplasmic reticulum shows extensive dilatation with the formation of numerous vacuoles (V). The mitochondria are swollen with rarified matricies. Perinuclear cisternae (P) have formed around the rounded nucleus. Coated tubular aggregates are present in the cytoplasm (arrows). Debris (arrowheads) of degenerated cells are seen in the lumen of the airway. Uranyl acetate and lead citrate stains (6,200×).

adjacent to these aggregated tubular profiles. The aggregates were rather scattered randomly throughout affected cells and were also present in autophagic vacuoles.

Pulmonary edema was also observed in the peribronchial and perivascular areas. The perivascular edema appeared to be most severe in larger vessels in the peribronchial interstitium. Although interstitial edema was found surrounding small vessels, this was focal and rather minimal.

Ultrastructural examination of the lungs of animals administered 200 mg 1,1-

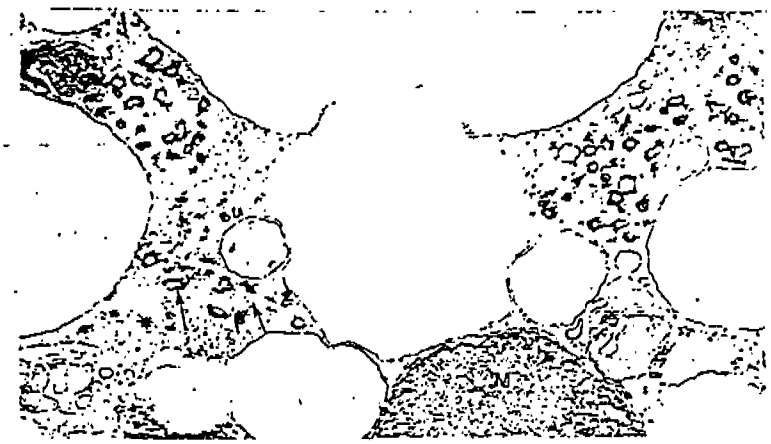


Figure 4 - Electron micrograph of the area boxed in Fig. 3. The coated tubular aggregates appear similar in cross-section to coated vesicles (arrowhead) but in longitudinal section, represent tubules of endoplasmic reticulum (arrows). A filamentous web (asterisk) is frequently seen in the vicinity of these aggregates. Uranyl acetate and lead citrate stains (49,300×).

DCE/kg revealed more severe degenerative and inflammatory responses than those exhibited by animals gavaged with 100 mg 1,1-DCE/kg. Ciliated and nonciliated cells displayed extensive vacuolization by 6 hr and some areas of the epithelium were denuded by this time. By 24 hr, numerous Clara cells had sloughed off exposing patches of bare interstitium and basal lamina. Ciliated cells were also similarly affected, albeit not to the degree demonstrated by Clara cells, since the residual epithelial lining was formed by flattened ciliated cells, which had stretched out over the interstitium. These ciliated cells showed a reduction in ciliary profiles and the mitochondria were swollen and decreased in number. Peribronchial and perivascular edema was also observed (Fig. 5). A dosage of 200 mg 1,1-DCE/kg did not consistently produce pulmonary injury to the same extent. The lesions ranged in severity from bronchioles devoid of epithelium to extensive damage involving the whole lung. The latter included severe alveolar edema in conjunction with frank necrosis of bronchiolar epithelium. The endothelial lining of blood vessels was disrupted and exhibited gaps. Subendothelial blebs were also observed together with large cytoplasmic vacuoles. Areas of atelectasis and hemorrhage were seen and gaps were present in the alveolar lining formed by Type I alveolar lining cells. Infiltration of leukocytes and monocytes into the lung was extensive in the latter instance. Mortality among animals given 200 mg 1,1-DCE/kg was approximately 25%.

Functional Correlation

Arterial blood gases determined at 24 hr, a time coincident with peak morphologic injury, revealed a mean decrease of 13% in the arterial pO_2 level with 100 mg 1,1-DCE/kg and a 27% decrease with 200 mg 1,1-DCE/kg (Table 1).

Both doses of 1,1-DCE caused concurrent hepatic necrosis as evidenced by

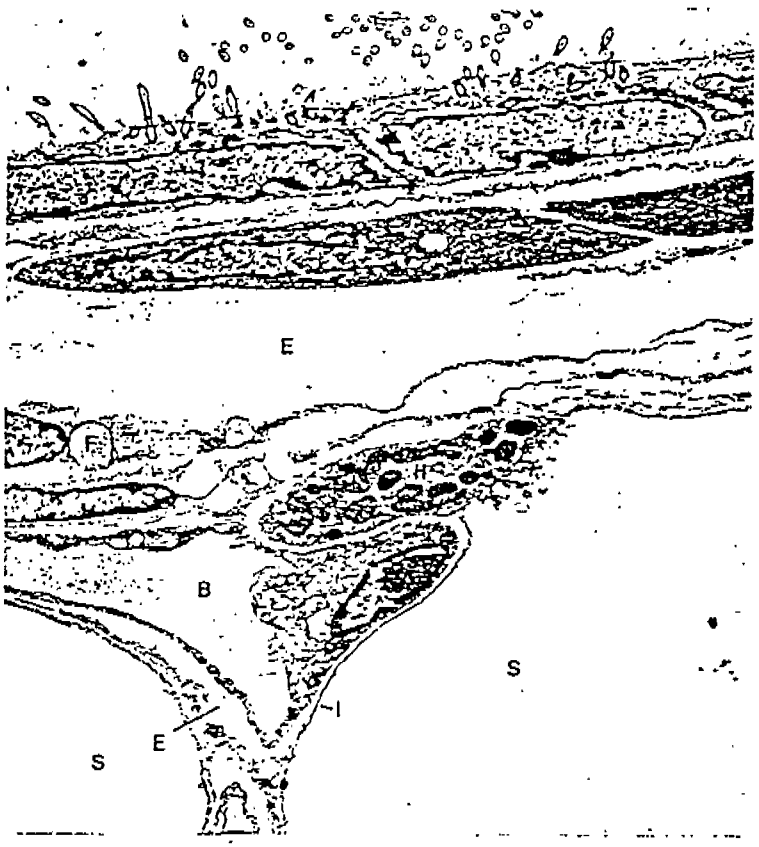


Figure 5 Electron micrograph of bronchiole in lung of mouse administered 200 mg 1,1-DCE/kg. Epithelial cells are predominantly ciliated and they have attained an elongated and flattened appearance. Interstitial edema (E) is present in areas surrounding the bronchiole and blood vessels (B). The interstitial cells (F) contain large homogeneous inclusions. Type I (I) and Type II (II) alveolar cells line the alveolar spaces (S). Uranyl acetate and lead citrate stains (6,200×).

increased liver enzyme activity in the serum. Following 100 mg 1,1-DCE/kg, SGOT and SGPT increased five- and nineteen-fold, respectively. The 200 mg 1,1-DCE/kg dosage caused 16-fold increases in SGOT and 31-fold elevations in SGPT (Table 2).

DISCUSSION

Preparation of lung tissue for ultrastructural examination has utilized fixation either by vascular perfusion or tracheal instillation [22–24]. As our preliminary studies were focused on Clara cells in the bronchioles, the procedure of inflation fixation through the trachea was preferred, since this method facilitated preser-

Table 1: Blood gases 24 hr after 1,1-DCE

Dosage	Arterial pO_2 (mmHg)[a]
Control	100 ± 11
100 mg/kg	88 ± 24
200 mg/kg	73 ± 8[b]

[a]Values are arithmetic means ± standard deviation of fed animals (5 in each group).

[b]Significantly different from control values, $P < 0.05$.

Table 2 Glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) in serum measured in international units per liter

Dosage	SGOT[a]	SGPT[a]
Control (7)[b]	68 ± 6	47 ± 6
100 mg 1,1-DCE/kg (5)	314 ± 68[c]	875 ± 143[c]
200 mg 1,1-DCE/kg (5)	1063 ± 491[c]	1465 ± 1245[c]

[a]Values are geometric means ± standard error of fed animals measured at 24 hr after gavage with 1,1-DCE.

[b]The number of animals in each group is given in parentheses.

[c]Significantly different from control values, $P < 0.05$.

vation of Clara cell morphology, especially of the endoplasmic reticulum [23, 24]. Instillation fixation has been reported to cause perivascular and interstitial edema [25]. The 100 mg 1,1-DCE/kg dosage caused more prominent peribronchial and perivascular edema than that seen in the lungs of control animals, and as dose increased, injury increased. Following the 200 mg 1,1-DCE/kg dose necrosis was more severe in some animals as evidenced by flooding of alveoli by edema fluid. Thus, in the case of 1,1-DCE, edema appears to be a consequence of chemical administration rather than a fixation artifact.

Since pulmonary edema was present in varying degrees, blood gases were analyzed to assess the functional integrity of the lungs in response to 1,1-DCE. Furthermore, Clara cells in lungs of llamas living at 4720 m above sea level, where the partial pressure of oxygen in ambient air is 82 mmHg [26–27], exhibited morphology similar to 1,1-DCE-exposed mice. Since the Clara cells in the lungs of llamas living at sea level did not display such morphology, the differences in appearance were attributed to the environment [27]. Moreover, rats that have been subjected to acute hypoxia show hyperactivity in the Clara cell population [28]. This suggests the possibility that the changes observed following exposure to 1,1-DCE are in response to hypoxia.

A number of chemical agents have been reported to cause airway injury. Necrosis of bronchiolar epithelium is a consequence of administration of bromobenzene [29], although it is unclear from the report which cell type was involved. 4-ipomeanol [30], 3-methylfuran [31] and carbon tetrachloride [32] all cause selective damage to the Clara cells of the bronchiolar epithelium. In addition to bronchiolar injury, parenchymal changes were also observed. In the

mouse, 4-ipomeanol [33] causes severe pleural effusion and alveolar edema. However, Clara cell damage is the primary event and the edematous changes were secondary effects [30]. Damage following carbon tetrachloride also involves endothelial and Type II alveolar cells, with resulting pulmonary edema and hemorrhage [34, 35]. It is not clear in the dose-response relationships with carbon tetrachloride-induced necrosis, whether Clara cell injury occurs at lower doses, with the exclusion of parenchymal changes. In mice, administration of 100 mg 1,1-DCE/kg primarily affected the Clara cells with no obvious changes elsewhere in the parenchyma. This parallels the lesion present in lungs of animals dosed with 4-ipomeanol, where necrosis of Clara cells precedes the onset of edema and congestion, as dose increases. In contrast to carbon tetrachloride where alveolar Type II cells were damaged [34], 1,1-DCE caused gaps to occur in the attenuated epithelial lining of Type I cells. However, the initial sites of reaction, at least to 4-ipomeanol and 1,1-DCE, appear to be within Clara cells. Therefore, pulmonary reactivity in response to 1,1-DCE is qualitatively similar to that produced by other chemical toxins.

The presence of "coated" tubules in affected Clara cells was an obvious feature of the lesion induced by 1,1-DCE. These tubular aggregates were also found in liver parenchymal cells injured by the administration of carbon tetrachloride [36], vinyl chloride [37], and trichloroethylene [38]. It would, therefore, appear that these profiles were not specific to the Clara cell and probably represented denatured membranes [36]. However, the chemical agents administered were all chloroethylenes.

Previous investigation indicated that the Clara cell is a cellular site of cytochrome P-450 dependent mixed function oxidase activity in the lung. Indeed, P-450 has recently been localized in the apices of Clara cells [39]. The presence of lung metabolizing enzyme systems in the Clara cells may account for susceptibility to chemical insult and indicates that injury to this cellular population occurs because they can metabolize 1,1-DCE to a cytotoxic reactive electrophile.

1,1-DCE is also an acute hepatotoxin in male fasted rats. Liver necrosis induced by 1,1-DCE is characterized by swelling of mitochondria, disintegration of nuclear chromatin, and contraction of plasma membranes [40]. In contrast to the striking involvement of the endoplasmic reticulum of the Clara cells encountered in this study, the endoplasmic reticulum in injured hepatocytes remains unaffected. Indeed, among the chlorocarbon hepatotoxins, the morphologic pattern of injury following 1,1-DCE is unique, for the others, including carbon tetrachloride, vinyl chloride, trichloroethylene, and halothane, all appear to preferentially injure endoplasmic reticulum within liver cells [40, 41]. This apparent paradox in the case of 1,1-DCE may reflect different pathways of metabolism in the two organs. Different pathways and/or rates of detoxification of active metabolites formed or both may account for the difference. Further investigation will hopefully resolve this paradox.

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REFERENCES

1. IARC: IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. 19:439–459, 1979.
2. Gilbert J, Shepherd MJ, Startin JR et al.: Gas chromatographic determination of vinyldene chloride monomer in packaging films and in foods. J Chromat 197:71–78, 1980.

3. Jaeger RJ: Vinyl chloride monomer: Comments on its hepatotoxicity and interaction with 1,1-dichloroethylene. Amer NY Acad Sci 246:150–151, 1975.
4. Ott MG, Langner MS, Holder BD: Vinyl chloride exposure in a controlled industrial environment. A long term mortality experience in 594 employees. Arch Environ Health 30:333–339, 1975.
5. Kramer CG, Mutchler JE: The correlation of clinical and environmental measurements for workers exposed to vinyl chloride. Amer Ind Hyg Assoc J 33:19–30, 1972.
6. Ott MG, Fishbeck WA, Townsend JC, et al.: A health study of employees exposed to vinylidene chloride. J Occup Med 18:735–738, 1976.
7. Altman PL, Dittmer DS: Environmental Biology. Federation of American Societies for Experimental Biology, Bethesda, MD, pp. 326–328, 1966.
8. Jenkins LJ Jr., Trabulus MJ, Murphy SD: Biochemical effects of 1,1-dichloroethylene in rats: Comparison with carbon tetrachloride and 1,2-dichloroethylene. Toxicol Appl Pharmacol 23:501–510, 1972.
9. Jaeger RJ, Trabulus MJ, Murphy SD: Biochemical effects of 1,1-dichloroethylene in rats: Dissociation of its hepatotoxicity from a lipoperoxidative mechanism. Toxicol Appl Pharmacol 24:457–467, 1973.
10. Prendergast JA, Jones RA, Jenkins LJ Jr., et al.: Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol Appl Pharmacol 10:270–289, 1967.
11. Jenkins LJ Jr., Andersen ME: 1,1-dichloroethylene nephrotoxicity in the rat. Toxicol Appl Pharmacol 46:131–141, 1978.
12. Jones BK, Hathway DE: Tissue-mediated mutagenicity of vinylidene chloride in Salmonella typhimurium TA1535. Cancer Lett 5:1–6, 1978.
13. Bartsch H, Malaveille C, Montesano R, et al.: Tissue mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salamonella typhimurium*. Nature (Lond.) 255:641–643, 1976.
14. Maltoni C, Cotti G, Morisi L, et al.: Carcinogenicity bioassays of vinylidene chloride. Research plan and early results. Med Lavaro 68:241–262, 1977.
15. Lee CC Bhandari JC, Winston JM, et al.: Carcinogenicity of vinyl chloride and vinylidene chloride. J Toxicol Environ Health 4:15–30, 1978.
16. Murray FJ, Nitschke KD, Rampy LW, et al.: Embryotoxicity and fetotoxicity of inhaled or ingested vinylidene chloride in rats and rabbits. Toxicol Appl Pharmacol 49:189–202, 1979.
17. Jones BK, Hathway DE: The biological fate of vinylidene chloride in rats. Chem-Biol Interactions 20:187–198, 1978.
18. Chieco P, Moslen MT, Reynolds ES: Effect of administrative vehicle on oral 1,1-dichloroethylene toxicity. Toxicol Appl Pharmacol 57:146–155, 1981.
19. Stephens RJ, Evans MJ: Selection and orientation of lung tissue for scanning and transmission electron microscopy. Environ Res 6:52–59, 1973.
20. Reynolds ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 16:208–212, 1963.
21. Snedecor GW, Cochran WG: Statistical Methods, 6th Ed. Iowa State University Press, Ames, IA, 1967.
22. Gil J, Weibel ER: Extracellular lining of bronchioles after perfusion-fixation of rat lungs for electron microscopy. Anat Rec 169:185–200, 1971.

23. Kuhn C III, Callaway LA, Askin FB: The formation of granules in the bronchiolar Clara cells of the rat 1. Electron Microsc Ultrastruct Res 49:387–400, 1974.
24. Etherton JE, Conning DM, Corrin B. Autoradiographical and morphological evidence for apocrine secretion of dipalmitoyl lecithin in the terminal bronchiole of mouse lung. Amer J Anat 138:11–35, 1973.
25. Staub NC: Pulmonary edema. Physiol Rev 54:678–811, 1974.
26. Heath D, Smith P, Harris P: Clara cells in the llama. Exp Cell Biol. 44:73–82, 1976.
27. Heath D, Smith P, Biggar R: Clara cells in llamas born and living at high and low altitudes. Br J Dis Chest 74:75–80, 1980.
28. Smith P, Heath D, Moosavi H: The Clara cell. Thorax 29:147–163, 1974.
29. Reid WD, Hett KF, Glick JM, et al.: Metabolism and binding of aromatic hydrocarbons in the lung. Relationship to experimental bronchiolar necrosis. Am Rev Resp Dis 107:539–551, 1973.
30. Boyd MR: Evidence for the Clara cell as a site of cytochrome P-450 dependent mixed-function oxidase activity in the lung. Nature (Lond.) 269:713–715, 1977.
31. Boyd MR, Statham CN, Franklin RB, et al.: Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant. Nature (Lond.) 272:279–271, 1978.
32. Boyd MR, Statham CN, Logo NS: The pulmonary Clara cell as a target for toxic chemicals requiring metabolic activation, studies with carbon tetrachloride. J Pharmacol Exp Ther 212:109–114, 1980.
33. Boyd MR: Role of metabolic activation in the pathogenesis of chemically induced pulmonary disease: mechanism of action of the lung-toxic furan, 4-ipomeanol. Environ Health Persp 16:127–138, 1976.
34. Gould VE, Smuckler EA: Alveolar injury in acute carbon tetrachloride intoxication. Arch Intern Med 128:109–117, 1971.
35. Chen WJ, Chi EY, Smuckler EA: Carbon tetrachloride-induced changes in mixed function oxidases and microsomal cytochromes in the rat lung. Lab Invest 36:388–394, 1977.
36. Reynolds ES, Ree HJ: Liver parenchymal cell injury. VII. Membrane denaturation following carbon tetrachloride. Lab Invest 25:269–278, 1971.
37. Reynolds ES, Moslen MT: Damage to hepatic cellular membranes by chlorinated olefins with emphasis on synergism and antagonism. Environ Health Persp 21:137–147, 1977.
38. Jaeger RJ, Reynolds ES, Conolly RB, et al.: Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital. Nature (Lond.) 252:724–726, 1974.
39. Serabjit-Singh CJ, Wolf CR, Philpot RM: Cytochrome P-450: Localization in rabbit lung. Science. 207:1469–1475, 1980.
40. Reynolds ES, Moslen MT, Szabo S, et al.. Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene. Role of mixed function oxidase system. Amer J Path 81:219–236, 1975.
41. Reynolds ES: Cellular injury: One-dimensional approaches to polydimensional problems. Texas Rep Biol Med 38:55–77, 1979.