Pulmonary Toxicity and Carcinogenicity of Trichloroethylene: Species Differences and Modes of Action

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Trichloroethylene (TCE) is both acutely toxic and carcinogenic to the mouse lung following exposure by inhalation. In contrast, it is not carcinogenic in the rat lung and is markedly less toxic following acute exposure. Toxicity to the mouse lung is confined almost exclusively to the nonciliated Clara cell and is characterized by vacuolation and increases in cell replication. Chloral, a metabolite of TCE that accumulates in Clara cells and has been shown to be the cause of the toxicity, also causes aneuploidy in some test systems. Cytotoxicity, increased cell division, and aneuploidy are known risk factors in the development of cancer and provide a plausible mode of action for TCE as a mouse lung carcinogen. All acute and chronic effects of TCE on the mouse lung are believed to be a direct consequence of high cytochrome P450 activity and impaired metabolism of chloral in Clara cells. Comparisons between species suggest that the ability of the human lung to metabolize TCE is approximately 600-fold less than that in the mouse. In addition, the human lung differs markedly from the mouse lung in the number and morphology of its Clara cells. Thus, the large quantitative differences between the metabolic capacity of the mouse lung and the human lung, together with the species differences in the number and morphology of lung Clara cells, suggest that the risks to humans are minimal and that other tumor sites should take precedent over the lung when assessing the potential risks to humans exposed to TCE. *Key words*: carcinogenicity, lung, mode of action, species comparisons, toxicity, TCE, trichloroethylene. — *Environ Health Perspect* 108(suppl 2):261–264 (2000).
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Exposure to trichloroethylene (TCE), a volatile liquid, occurs mainly by inhalation, a process in which absorption from the airways is both rapid and extensive. Following exposure, unmetabolized TCE is eliminated by exhalation. Furthermore, significant amounts of TCE are exhaled following dosing of laboratory animals via the gastrointestinal tract or by injection (*1*). Consequently, the pulmonary airways are exposed to this chemical irrespective of the route of administration, and at high dose levels both toxicity and cancer have been observed in laboratory animals. The effects are species specific; the mouse is far more sensitive to the acute toxicity of TCE than the rat and is also the only species in which an increased incidence of lung tumors has been seen. As a result of this difference in sensitivity between laboratory animals, a number of investigators have sought to explain the mechanistic basis of the both toxicity and carcinogenicity in mice and the relevance of these data to humans exposed to this chemical. In this article the effects of TCE on the lung are reviewed together with proposed modes of action for both acute toxicity and cancer.

Lung Cancer in Animals

Increases in pulmonary adenomas and adenocarcinomas have been observed in two studies in which mice were exposed to TCE by inhalation. Fukuda et al. (*2*) reported an increase in both pulmonary adenomas and adenocarcinomas following exposure of female CD-1 mice (males were not tested) to 150 and 450 ppm TCE (6 hr/day,

5 days/week for 104 weeks) and, in a shorter study, Maltoni et al. (*3,4*) reported an increase in pulmonary adenomas in male Swiss and female B6C3F1 mice exposed to 600 ppm, 7 hr/day, 7 days/week for 78 weeks. In contrast to the inhalation studies, lung tumor incidences were not increased in B6C3F1 or Ha:ICR mice given large doses (up to 2,339 mg/kg) of TCE by gavage in corn oil, 5 days/week for up to 104 weeks (*5–8*). Lung tumors were not increased in Sprague-Dawley rats of either sex exposed to 450 or 600 ppm by inhalation (*2–4*) nor were they increased in six different strains of rats administered TCE by gavage at dose levels up to 1,098 mg/kg (*5–7,9*). In summary, lung tumors have been seen in mice following inhalation exposure but not after gavage dosing. They have not been seen in rats exposed by either route.

Toxicity to the Lung

A number of studies (*10–20*) have shown that TCE is toxic to the bronchiolar epithelium of mice and rats following acute exposure either by inhalation or by intraperitoneal (i.p.) injection. As in the cancer bioassays, a marked difference in sensitivity between mice and rats was noted. In mice, pulmonary toxicity was seen following exposures as low as 20 ppm (*18*), whereas in rats, toxicity was not seen at dose levels below 1,000 ppm (*14,18*). The lack of toxicity or carcinogenicity in the lungs of mice after oral dosing is presumably due to extensive hepatic metabolism reducing the amount of TCE reaching the lungs.

There are no reports of adverse effects on the human lung.

The primary effects of TCE on the mouse lung in all studies have been morphological and biochemical changes in the nonciliated Clara cells. Milder responses, including reduced lamellar bodies and distortion of microvilli, have been reported in alveolar type II cells following administration of very high dose levels, 2,500 mg/kg by i.p. injection (*13*) or 9,000 ppm by inhalation (*10*). The morphological changes in the Clara cells were characterized by dilation of the endoplasmic reticulum cisternae, which ultimately resulted in large hydropic vacuoles formed by coalescence of smaller vacuoles. In some instances the hydropic vacuoles occupied large areas of the cell. A dose-dependent increase was seen both in the extent of the damage to individual cells and in the spread of damage throughout the airways. The toxicity, which was seen within 24 hr of a single 30-min exposure, had significantly recovered by 48 hr and was fully resolved within 5 days (*17*). Clara cell damage was also seen after the first exposure of each week, using a protocol in which CD-1 mice were exposed to 450 ppm 6 hr/day, 5 days/week for 2 weeks. The lungs were morphologically normal at the end of each week after five consecutive daily exposures (*18*).

The only other toxicological responses noted in the lung after exposure to TCE were fibrosis in the mouse (*21*) and a decrease in surfactant phospholipid following exposure of rats and mice to very high dose levels of TCE. In mice, the decreases were seen following i.p. injection of 3,000 mg/kg (*15*) and, in rats, following exposure to 9,000 ppm by inhalation (*10,11*). Similar responses were seen in the lungs of fetal and neonatal mice following an i.p. dose of 3,000 mg/kg on day 17 of pregnancy (*19*). In vitro, TCE has been reported to inhibit 5-hydroxytryptamine uptake in the isolated perfused rat lung (*22,23*).

TCE-induced damage to mouse lung Clara cells was accompanied by a reduction in

the metabolic capacity of the lung. Forkert et al. (13) found a reduction in both cytochromes P450 (to 10–20% of control) and aromatic hydrocarbon hydroxylase activities (to 40–50% of control) in the lungs of exposed mice. Similarly, Lewis et al. (12) found NADPH–cytochrome c reductase activity to be reduced to 67% of control following exposure of mice to 10,000 ppm for 4 hr and Odum et al. (18) also found a dose-dependent reduction in a number of cytochrome P450 activities in mice exposed to 450 ppm TCE daily for 5 days. Loss of cytochrome P450 activity and the morphological recovery of the Clara cells with repeated daily exposure to TCE suggest that loss of metabolic capacity in these cells is an adaptive response.

Mechanisms of Toxicity

The molecular basis of the Clara cell lesion seen in mice has been investigated by Odum et al. (18). Clara cells isolated from the mouse lung were shown to efficiently metabolize TCE to chloral and trichloroacetic acid, chloral being the major metabolite in these cells. Trichloroethanol glucuronide, which is the major metabolite of TCE in mice in vivo and in mouse hepatocytes in vitro, was not formed in Clara cells due to a lack of the glucuronosyltransferase enzyme catalyzing the reaction between trichloroethanol and glucuronic acid (Figure 1). Alcohol dehydrogenase, the enyme responsible for the metabolism of chloral to trichloroethanol, is also known to have low activity in the lung compared to the liver (24). When chloral, trichloroethanol, and trichloroacetic acid were administered separately to female CD-1 mice, chloral caused a lesion in the Clara cells that was identical to that seen with TCE. The other metabolites were inactive. The authors

proposed that the failure of Clara cells to conjugate trichloroethanol led to an accumulation of chloral and the observed toxicity.

The chloral-induced cytotoxicity in the Clara cell from mouse lung also results in an increase in cell division in the bronchiolar epithelium. Villaschi et al. (17) found a 13.5% increase 48 hr after exposures to 500–7,000 ppm TCE for 30 min, and Green et al. (20) found up to 10-fold increases after 5 and 10 daily exposures to 450 ppm TCE. Increased cell division was not seen in rats following Clara cell damage induced by exposure to 9,000 ppm TCE for 30 min (10).

The Clara Cell and the Development of Lung Tumors

A significant number of acute studies (10–20) have shown that TCE very selectively targets the mouse lung Clara cell. The question therefore arises as to the role of this cell in the subsequent development of the TCE-induced mouse lung tumors.

At the present time there is no direct evidence that the mouse lung tumors are derived from Clara cells. However, the complete lack of acute morphological response or changes in cell division rates in other cell types such as alveolar type II cells suggests that the Clara cell plays a key role in the development of the lung tumors in mice exposed to TCE. Similarly, the differences in metabolic capacity of Clara cells in mice and rats are consistent with the species differences in toxicity and carcinogenicity. No such argument can be made for other cell types.

Clara cells have been identified as the cell of origin of some chemically induced mouse lung tumors (24–31); equally a large number of mouse lung tumors express alveolar type II surfactant apoprotein, suggesting that type II cells are the origin of many chemically

induced mouse lung tumors (32). Whether surface antigens are a reliable marker for the identification of the cell of origin of tumors that consist of dedifferentiated cells remains uncertain. Furthermore, Clara cells exposed to nitrosamines have been reported to develop the same surfactant-secreting organelles found in untreated alveolar type II cells (33). In the case of TCE, evidence from antigenic staining or more detailed morphological characterization of the tumors is lacking. A third option is also possible or even likely: mouse lung tumors develop from pluripotent stem cells that are influenced by the changes in Clara cells. In the lung, basal cells are generally considered to be the primary stem cell for the repair and regeneration of new airway epithelium, although there is now evidence that the Clara cells also serve as progenitor cells for the lower airway epithelium (34).

Possible Modes of Action of TCE as a Mouse Lung Carcinogen

Cytotoxicity and increased cell division form the basis of a plausible mode of action for TCE-induced mouse lung tumors. Both are known risk factors for carcinogenesis, particularly in organs such as the mouse lung that have significant background tumor incidences. In addition, chloral also appears to have some genotoxic potential. Tests for mutagenicity have given conflicting results (35–37), but those for aneuploidy are more consistently positive (36). However, at the present time it is not known whether aneuploidy occurs in the mouse lung. Studies in specific cell populations such as Clara cells, which account for only a small percentage of total cell types in the lung, are technically difficult and have not been conducted for either TCE or chloral. In the whole lung, two studies failed to find evidence of DNA binding in mice exposed to TCE (16,38).

The observation of Clara cell toxicity, increased cell replication, and the development of lung tumors in the mouse are by no means unique to TCE. Acrylonitrile, bromobenzene, carbon tetrachloride, 1,1-dichloroethylene, dichloromethane, 4-ipomeanol, naphthalene, 1-nitronaphthalene, O,O,S-trimethylphosphorothioate, styrene, and a number of other chemicals selectively target the Clara cells of the pulmonary airways; a number of these chemicals cause an increase in pulmonary tumors (39–48). It seems improbable therefore that the observed acute effects in Clara cells are unrelated to the subsequent development of these tumors.

In conclusion, a number of known risk factors for the development of tumors—cytotoxicity, increased cell replication, and possibly aneuploidy—correlate well with

Mouse Hepatocytes

Mouse Lung Clara Cells
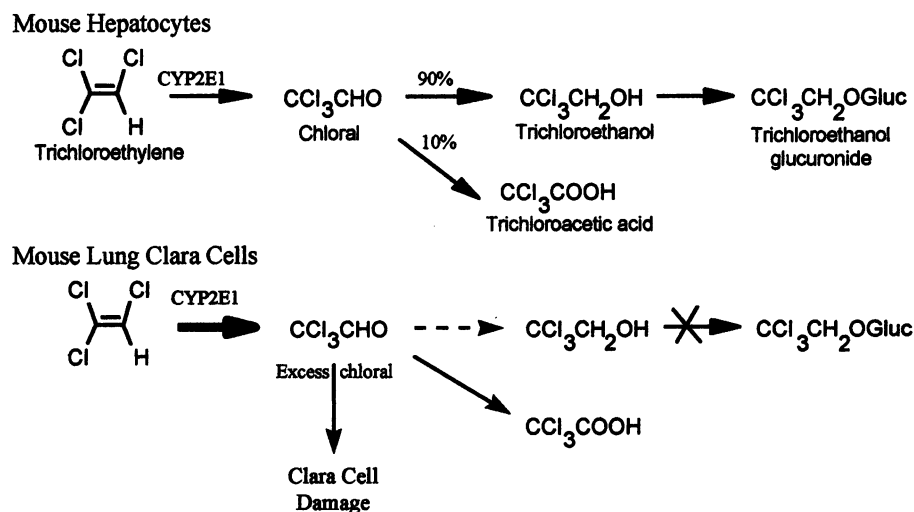
Figure 1. The comparative metabolism of TCE in mouse hepatocytes and lung Clara cells [based on the work of Odum et al. (18)]. % refers to metabolism of TCE by each pathway in control mouse hepatocytes.

the observed species-specific pulmonary carcinogenicity of both TCE and a number of other chemicals. Furthermore, the tumors are not seen in those species or by those routes of administration where toxicity does not occur. Since all of these responses are a consequence of high localized (Clara cell) concentrations of chloral, the species-dependent pulmonary metabolism of TCE to chloral would appear to the most appropriate dosimeter for assessing human risk.

Species Differences in the Pulmonary Metabolism of TCE

The acute responses believed to be causally related to the development of the lung tumors in mice exposed to TCE have been attributed to the high metabolic capacity of the mouse lung Clara cells. Comparisons of the metabolic capacity of mouse, rat, and human lung tissue found that mouse lung microsomes metabolized TCE to chloral at a rate 23-fold higher than that in rat lung microsomes. A metabolic rate could not be detected in human lung (20). Using an antibody to cytochrome P450IIE1 (CYP2E1), the enzyme responsible for the metabolism of TCE to chloral (20,49), the highest concentrations of enzyme were found in Clara cells of the mouse lung. Significantly lower amounts were found in the Clara cells of rat lung. This enzyme could not be detected in human lung in any cell type, either in human lung tissue sections or by Western blotting (20). Other studies have reported the presence of trace amounts of CYP2E1 in human lung, usually only detectable by reverse transcriptase–polymerase chain reaction (50–52). In total, the cytochrome P450 content of human lung is reported to be only 3.7% (27-fold lower) that of rat lung (53), which is consistent with the lack of a measurable metabolic rate for TCE in the studies of Green et al. (20). Overall, the data available suggest a 600-fold difference in the capacity of mouse and human lung to metabolize TCE. The marked difference in metabolic capacity between mouse lung Clara cells and those of rats or humans is not unique to CYP2E1-catalyzed reactions. Most Clara cell toxicants are activated by cytochromes P450 and, for example, CYP2F2, the isoform primarily responsible for the metabolism of naphthalene, also shows a quantitative distribution pattern between species which is very similar to that of CYP2E1 (52,54). Inhibition of cytochromes P450 has also been shown to prevent the development of this type of lesion (48). Ultimately, a gene knockout experiment could potentially prove the role of CYP2E1 in the development of pulmonary toxicity and cancer in mice exposed to TCE.

Another factor should also be considered when comparing species and evaluating human risks in this area. Clara cells differ significantly between rodents and between rodents and humans both in number and structure. In mice they are numerous and are spread throughout the airways, whereas in rats they are significantly fewer in number, particularly in the terminal bronchiolar region. In human lung, Clara cells are rare, as they are found in small numbers in the distal bronchioles. They also differ morphologically: the mouse lung Clara cell is packed with endoplasmic reticulum, and the human Clara cell apparently is largely devoid of these membranes (34,55–58). This difference in morphology is consistent with the observed differences in cytochrome P450 activity—the endoplasmic reticulum is the membrane in which the cytochromes P450 enzymes are heavily localized. This membrane is also the origin of the lesion in the mouse.

Relevance of Mouse Lung Tumors for Assessing Human Risks

Trichloroethylene induces a range of responses in the mouse lung that are known risk factors for the development of cancer. The effects are not seen in mice following oral dosing nor are they seen in rats by any route of administration. Chloral, a metabolite of TCE that is produced in large quantities in the mouse lung Clara cell has been shown to be responsible for the observed effects (18).

The unique sensitivity of the mouse lung to both the acute and chronic effects of TCE is a direct consequence of high cytochrome P450 activity and impaired metabolism of chloral. The ability of the human lung to metabolize TCE is considerably less than that of the mouse lung, as much as 600-fold, based on the available data. In addition, the human lung differs markedly from the mouse lung in the number and morphology of its Clara cells. Thus, the large quantitative differences between the metabolic capacity of the mouse lung and the human lung, together with the species differences in the number and morphology of lung Clara cells, suggest that the risks to humans are minimal and that other tumor sites should take precedent over the lung when assessing the potential risks to humans exposed to TCE.

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