Chloroform Inhalation Exposure Conditions Necessary to Initiate Liver Toxicity in Female B6C3F1 Mice

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Chloroform is a nongenotoxic-cytotoxic carcinogen in rodent liver and kidney, including the female B6C3F1 mouse liver. Because tumors are secondary to events associated with cytolethality and regenerative cell proliferation, these end points are valid surrogates for tumor formation in cancer risk assessments. The purpose of the experiments presented here was to more clearly define the combinations of atmospheric concentration and duration of exposure necessary to induce cytolethality and regenerative cell proliferation in the sensitive female B6C3F1 mouse liver. Female B6C3F1 mice were exposed to chloroform by inhalation for 7 consecutive days using atmospheres of 10, 30, or 90 ppm and selected exposure times of 2, 6, 12, or 18 h/day. Bromodeoxyuridine (BrdU) was given over the last 3.5 days via an implanted osmotic pump to label cells in S-phase. Labeled hepatocytes were visualized immunohistochemically, and the labeling index (LI) was determined as the percentage of cells in S-phase. LI was a more sensitive indicator of cellular damage than histopathological examination and is the more conservative end point for use in risk assessments. Significant concentration and exposure time related increases in LI were observed at 30 and 90 ppm but not at any 10-ppm exposure. These data defined an empirical relationship for the combinations of airborne exposure concentration and duration needed to induce cytolethality. These results suggest that concentrations of about 10 ppm or below will not induce hepatotoxicity in these mice regardless of exposure duration. Thus, the rate of production of toxic metabolites and the subsequent rate of cellular damage produced by a continual exposure of approximately 10 ppm chloroform are less than the maximum rates at which hepatocytes can detoxify those metabolites and repair any induced cellular damage. A physiologically based pharmacokinetic (PBPK) dosimetry model was used to compare anticipated responses in mice and humans and predicted that chloroform concentrations of approximately an order of magnitude greater than 10 ppm would be required to induce human liver toxicity. Thus, no safety factor to account for species to species extrapolation should be required in formulating a chloroform inhalation cancer risk assessment based on the dose × time inhalation data presented here.

Key Words: chloroform; dose × time; inhaled chloroform toxicity; chloroform risk assessment.

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Disinfecting drinking water intended for human consumption with chlorine-based chemicals is common practice worldwide. Chloroform (CHCl₃) is usually the most prevalent by-product of those disinfection processes, and drinking water concentrations have been reported to range from 0.0001 to 0.3 ppm chloroform (Craun, 1993; Symons et al., 1975; Uden and Miller, 1983). Chloroform volatilizes from water, and concentrations as high as 0.01 ppm have been measured in ambient air. Air concentrations in shower stalls and above swimming pools have been measured as high as 0.06 and 0.1 ppm, respectively (Aggazzotti et al., 1990; Jo et al., 1990; Singh et al., 1982). Bleaching of paper by some processes can also yield chloroform that is released directly into the atmosphere or can be volatilized from wastewater (Butler and Dal Pont, 1992).

Exposure to chloroform is of concern because it has produced cancer in several long-term rodent bioassays when administered by gavage, in the drinking water, or by inhalation. These studies demonstrated that liver and kidney are the target organs for chloroform-induced cancer and that tumor formation is highly dependent on the route and rate of administration, genetic background, species, strain, and gender. To maintain perspective, the conditions that produced chloroform-induced toxicity and eventually cancer in the laboratory are orders of magnitude above the exposures that are normally found in the environment to which people are exposed (U.S. EPA, 1998).

A large body of evidence indicates that chloroform produces cancer via a nongenotoxic-cytotoxic mode of action and that tumors are produced secondary to events associated with cytotoxicity and regenerative cell proliferation (see the reviews: Butterworth and Bogdanffy, 1999; International Life Sciences Institute, 1997; International Programme on Chemical Safety, 1994; U.S. EPA 1998). The weight of evidence indicates that DNA reactivity or direct mutagenic activity is not a property of chloroform, including studies in the whole animal at doses and in target organs where cancer induction is observed (Larson et al., 1994a; Templin et al., 1997).

Chloroform-induced tumor formation is driven by initiation and promotional events associated with induced cytolethality and regenerative cell proliferation. Without exception, chloroform-induced cytolethality and regenerative cell proliferation have been demonstrated with the dosing regimen that eventu-
ally produced tumors in every instance of chloroform-induced cancer, including in B6C3F1 mice (Jorgenson et al., 1985; Larson et al., 1994b,c; National Cancer Institute, 1976), Osborne-Mendel rats (Hard et al., 2000; National Cancer Institute, 1976; Templin et al., 1996a); and BDF1 mice (Templin et al., 1998; Yamamoto et al., 1994).

Thus, because of the obligatory role they play, induced cytolethality and regenerative cell proliferation become valuable surrogates for induced cancer in risk assessments. Protecting against chloroform toxicity will also protect against chloroform-induced cancer. Quantitative cell proliferation data may be used to interpolate or extrapolate a predicted cancer response in cases where no cancer data are available, such as the response in the B6C3F1 mouse liver to inhaled chloroform or dose × time relationships.

In obtaining data as a basis for risk assessments, cytolethality and regenerative cell proliferation can be more precisely and more easily measured than induced cancer. Thus, these endpoints become valuable in assessing the many factors that influence whether chloroform exposure will lead to tumor formation. The following examples show the direct correlation between induced cell proliferation and the tumor response. Chloroform given to B6C3F1 mice by gavage induces hepatic regenerative cell proliferation, with continued lifetime administration, resulting in liver tumor formation (National Cancer Institute, 1976; Larson et al., 1994c). When similar daily doses were given in the drinking water, neither liver cell proliferation nor cancer was observed (Jorgenson et al., 1985; Larson et al., 1994c). Chloroform given by inhalation induces necrosis, regenerative cell proliferation, and eventually cancer in the kidneys of male BDF1 mice, but neither toxicity nor cancer in the kidneys of female BDF1 mice (Templin et al., 1998; Yamamoto et al., 1994).

Reitz et al. (1990) suggested that the mechanism of chloroform-induced cytotoxicity is dependent upon the rate at which the target tissue dose is metabolized. Studies with CYP 2E1-null mice indicate that metabolism is obligatory for the development of chloroform-induced hepatic and renal toxicity and that cytochrome P450 2E1 appeared to be the only enzyme responsible for this cytotoxic-related metabolic conversion under the inhalation exposure conditions used in those studies (Conolly and Butterworth, 1995). Thus, establishing those conditions of chloroform exposure that begin to induce cytolethality provides critical data in formulating a PBPK-PD model as the basis for more realistic cancer risk assessments. The purpose of the experiments presented here was to more clearly define the combination of dose and duration of inhalation exposure necessary to trigger target organ cytolethality and regenerative cell proliferation. Because of the high degree of variability in response between different routes of administration, the chloroform inhalation risk assessment should be based on inhalation studies. The Corley et al. (1990) chloroform PBPK model was used to help design the current dose × time studies to obtain several exposure conditions that resulted in approximately the same total amount of chloroform being metabolized (Fig. 1). The female B6C3F1 mouse liver was chosen for this work because it is highly susceptible to chloroform-induced

![FIG. 1. PBPK model simulations showing the metabolism rate for a 25-g female B6C3F1 mouse exposed to chloroform via 3 different inhalation-exposure regimens used in the study. The 3 exposure conditions resulted in similar amounts of chloroform being metabolized (or area under the curve, approximately 9.4 μmol per exposure), while the greatest rate of metabolism reached by each exposure differed.](image-url)
toxicity and eventually cancer (Larson et al., 1994c; National Cancer Institute, 1976). With daily dosing, chloroform-induced regenerative cell proliferation increases rapidly and remains at about the same level for durations of exposure of at least 90 days (Larson et al., 1996; Templin et al., 1996b, 1998). Thus, the one-week exposures conducted here are presumed to be indicative of the degree of hepatotoxicity expected with longer exposures. The data generated in these studies help further refine rodent and human PBPK-PD models for inhaled chloroform. Another purpose of these studies was to use the rodent and human PBPK models to assess how large a safety factor might be appropriate in extrapolating from the cytotoxic exposure conditions measured in the mouse liver to those conditions that might be expected to induce cytotoxicity in the human liver.

MATERIAL AND METHODS

Animals and husbandry. This study was conducted under the guidelines for the humane use and care of laboratory animals (National Research Council, 1996) and was approved by the CIIT Institutional Animal Care and Use Committee. Mice were housed in humidity- and temperature-controlled facilities accredited by the American Association for Accreditation of Laboratory Animal Care. Ten-week-old female B6C3F1 mice were obtained from the Charles River Breeding Laboratories, Inc. (Raleigh, NC) and allowed to acclimate for 2 weeks. The mice were randomized by weight, assigned to control or chloroform exposure groups, and housed individually in stainless steel, hanging wire cages contained within H-1000 stainless steel chambers. Control and chloroform-exposed animals were housed in separate chambers. The room in which the chambers were maintained was on a 12-h light-dark cycle at 22.2 ± 4°C and 60 ± 15% relative humidity. NIH-07 rodent chow (Ziegler Bros., Gardener, PA) and deionized, filtered tap water were available ad libitum except during exposure periods, as described below.

Physiologically based pharmacokinetic (PBPK) modeling. A flow-limited PBPK model (Corley et al., 1990) was used to determine appropriate exposure conditions that would enable a comparison between exposures that produce equal amounts of chloroform metabolism but via different rates of metabolism (Fig. 1). The Corley et al. (1990) model was slightly modified to incorporate the female B6C3F1 mouse metabolism rate and tissue to blood partition coefficients obtained from experiments performed at CIIT as follows: Vmax = 13.5 mg/h/kg; blood/air = 24.3; liver/air = 15.2; kidney/air = 12.1. The model code was written in advanced continuous simulation language (ACSL), and computer simulations were performed with the optimization software package SimuSolv (The Dow Chemical Company, Midland, MI) using a VAX 4000/300 computer.

Generation and characterization of atmospheres. Target exposure concentrations of chloroform were 0, 10, 30, and 90 ppm. The overall average concentration did not deviate from the target concentrations by more than 2% with a coefficient of variation of less than 7%. Chloroform, > 99.5% purity and stabilized with 0.006% amylenes (Aldrich Chemical Company, Inc., Milwaukee, WI) was stored in stainless steel pressure vessels. The exposure atmospheres were generated by a dilution technique using HEPA-filtered air.

Exposures were conducted in glass, single-animal, whole-body exposure tubes similar to those described by Dorman et al. (1996). The total airflow through each exposure system was maintained at approximately 32 l/min, providing a flow of approximately 2.0 l/min through each of the 16 exposure tubes per system. A separate exposure system was used for each exposure concentration. These exposure systems were placed inside separate 1-m³, Hinners-style inhalation chambers, which served as secondary containment hoods to reduce the chance of any cross contamination. Approximately 15 min before the exposure, the mice were transferred from their home cage to the inhalation tubes. Relative humidity and temperature were monitored using an Omega RH 30 temperature and humidity indicator (Omega Engineering, Inc., Stamford, CT) in a representative tube that contained a mouse. Following exposure, all the mice were then exposed to control air for approximately 30 min before being returned to their home cages.

Experimental design. Several combinations of concentration and exposure duration were used to evaluate a range of exposure conditions (5 animals/group) (Table 1). Selected groups of mice were exposed to chloroform vapors of 0, 10, 30, and 90 ppm for 2, 6, 12, or 18 h/day for 7 consecutive days. Feed (1–3 pellets) and gelatin-based water were available during the inhalation exposures in each single-animal tube. The 12-h light period began 1 h prior to exposure start, except for the 12- and 18-h exposures, which began during the night and encountered the change in the light-dark cycle at 7:00 a.m. For these exposures, animals were loaded into the single-animal tubes at approximately 8:45 P.M. of the evening that exposures were scheduled. Two groups of 5 mice each were used as controls and exposed to 0 ppm chloroform for either 6 or 18 h. The 18-h control group served as a control for the 12 and 18 h exposures, which encountered a change in the light-dark cycle and extended confinement in the single-animal tubes. The 6-h control group served as a control for the 2- and 6-h exposure groups. Approximately 18 h after the last exposure, the mice were euthanized and necropsied for tissue collection.

Regenerative cell proliferation was evaluated by administering the thymidine analog bromodeoxyuridine (BrdU) via subcutaneously implanted osmotic pumps approximately 3.5 days prior to necropsy (Alzet Model 2001, 1 μl/h delivery rate, Alza Corporation, Palo Alto, CA). The osmotic pumps contained a solution of 16 mg/ml, filter-sterilized BrdU (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline. Osmotic pumps were implanted under aseptic conditions, as described in Eldridge et al. (1990).

At necropsy, mice were weighed and anesthetized with sodium pentobarbital and euthanized by exsanguination. Whole liver was immediately removed, weighed, and examined macroscopically. Longitudinal sections of the left and right median lobes and a 2- to 3-mm section of the duodenum were dissected and fixed in 10% neutral buffered formalin for 48 h and then stored in 70% ethanol until processing.

Histopathology. Hematoxylin and eosin (H&E)-stained sections of liver from mice were evaluated, first with knowledge of treatment group followed by blinded reading of slides to confirm presence and absence of lesions.

Cell proliferation. Immunohistochemical staining for BrdU incorporation was performed on liver and duodenum, the duodenum being used to confirm systemic delivery and staining of BrdU. These tissues were mounted on ProbeOn Plus® Slides (Fisher Scientific, Pittsburgh, PA) to ensure adhesion

<table>
<thead>
<tr>
<th>Exposure (h)</th>
<th>Concentration (ppm)</th>
<th>ppm-h</th>
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</thead>
<tbody>
<tr>
<td>2.0</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>2.0</td>
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<td>180</td>
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<td>30</td>
<td>360</td>
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<tr>
<td>18.0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>18.0</td>
<td>10</td>
<td>180</td>
</tr>
</tbody>
</table>

*Product of exposure duration and chloroform concentration expressed as ppm-h. These values were used to compare equal amounts of chloroform exposure.
during processing. The immunohistochemical detection of BrdU-labeled cells has been previously described (Eldridge et al., 1990). Briefly, sections were incubated for 1 h at room temperature with a 1:1500 dilution of an anti-BrdU monoclonal antibody (Caltag Laboratory, South San Francisco, CA). After incubation with primary antibody, the slides were incubated for 30 min at room temperature with a 1:200 dilution of biotinylated horse antimouse IgG (Vector Laboratories, Burlingame, CA). Slides were then incubated for 30 min at room temperature with a strepavidin-alkaline phosphatase complex (Zymed Laboratories, South San Francisco, CA). BrdU incorporation was visualized by a final incubation with the chromagen Stable Fast Red (Research Genetics, Huntsville, AL), and the nuclei were counterstained with hematoxylin.

The labeling index (LI, percentage of cells in S-phase) was calculated by dividing the number of hepatocyte nuclei that stained positive for BrdU incorporation by the total number of hepatocyte nuclei counted, and the result were expressed as a percentage. A section of the left hepatic lobe was used to determine the hepatocyte LI, as described in Larson et al. (1994c). The LI was determined by microscopically scoring 10 computer-generated, random fields from each slide, which provided approximately 1000 hepatocyte nuclei for analysis for each animal. Statistical significance of the LI data were evaluated using the Mann-Whitney U test, with a significance level of $p < 0.05$.

### RESULTS

#### Clinical Observation and Histopathology

Lethargy and a roughened coat were the only clinical signs of toxicity encountered in this study, and only observed in mice exposed to 30 ppm chloroform for 12 h and to 90 ppm chloroform for 6 h. These were noted following the second day of exposure and continued with approximately equal severity through the remaining 5 days of the study. No clinical signs of adverse health effects were evident in mice from other exposure groups, and no significant difference in body weight gain was observed among any exposure groups (Table 2). A significant increase in relative liver weight was found in mice exposed to chloroform at 10 ppm for 6 and 18 h, 30 ppm for 12 h, and 90 ppm for 2 and 6 h (Table 2).

Three of the 9 treatment groups had treatment-related histopathological alterations. The most severe changes were noted in the 6-h, 90-ppm group. All (5/5) animals had a similar mild hepatopathy consisting of centrilobular and midzonal hepatocytic vacuolar degeneration. Individual cell necrosis was evident in swollen, vacuolated hepatocytes, and frequent mitotic figures were noted. Minimal hepatocytic degenerative changes were found in livers from 5/5 animals exposed for 12 h to 30 ppm chloroform. These changes consisted of centrilobular tinc torial alterations (loss of eosinophilia) with midzonal vacuolar change. Occasional mitotic figures were evident scattered throughout the hepatic parenchyma. Most (4/5) animals exposed for 6 h to 30 ppm had very subtle hepatic parenchymal change consisting of a centrilobular loss of eosinophilia, which appeared as an accentuated lobular pattern.

#### Regenerative Hepatocyte Proliferation

Chloroform-induced regenerative cell proliferation was observed in the livers of female mice in a manner clearly dependent on both exposure concentration and duration of exposure (Table 3). Statistically significant increases in the hepatocyte LI were observed in mice exposed to 30 ppm chloroform for 6 and 12 h and to 90 ppm chloroform for 2 and 6 h. The LI was elevated approximately 30-fold over control in mice exposed to 90 ppm for 6 h, 15-fold over control in mice exposed to 30 ppm for 12 h, and 5-fold over control in mice exposed to 90 ppm chloroform for 2 h and 30 ppm for 6 h. No significant increase in the LI was found in mice exposed to any of the other exposure groups in the study.

### DISCUSSION

For carcinogens such as chloroform that act via a nongenotoxic-cytotoxic mode of action, no increased cancer risk would

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Exposure time (h)</th>
<th>LI</th>
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<tbody>
<tr>
<td>0</td>
<td>6*</td>
<td>0.55 ± 0.27</td>
</tr>
<tr>
<td>0</td>
<td>18*</td>
<td>0.70 ± 0.22</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.93 ± 0.32</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>1.83 ± 1.05</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>1.50 ± 1.23</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>2.94 ± 1.95*</td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>10.04 ± 3.96*</td>
</tr>
<tr>
<td>90</td>
<td>2</td>
<td>2.82 ± 1.10*</td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>19.92 ± 4.00*</td>
</tr>
</tbody>
</table>

* Statistically significant increase from the respective control group (Mann-Whitney U test, $p < 0.05$).
be anticipated at nontoxic doses (Butterworth et al., 1995). Induced regenerative cell proliferation is an indicator of the many initiation and promotion activities associated with sustained cytotoxicity, and as such it becomes a valid surrogate for the tumor response in risk assessments. This allows precise measurements that would be time- and cost-prohibitive if one were to try to obtain the data using the traditional 2-year cancer bioassay. For example, a chloroform inhalation risk assessment should be based on inhalation data. Yet, there are no chloroform inhalation cancer data in B6C3F1 mice. Further, no dose × time cancer bioassays have been conducted with chloroform. Thus, the inhalation data presented here facilitate and serve as a relevant basis for formulating a chloroform inhalation risk assessment.

Comparison of induced cell proliferation as LI and histopathological changes in mice from this study indicate that measurement of regenerative cell proliferation is a more sensitive indicator of chloroform-induced cellular damage than histopathological examination. Thus, at least for chloroform, the LI is the more conservative end point as the basis for risk assessments. As the most sensitive cells in a target organ begin to die and be replaced, the increase in the labeling index can be measured before overt histological changes can be observed. In our experience, including the current studies, increased cell proliferation can usually be seen about one dose lower than observed histological changes such as necrosis. Previous studies have shown that with daily dosing, chloroform-induced regenerative cell proliferation at any given dose increased rapidly and remained at about the same level for exposure periods of at least 90 days (Larson et al., 1996). Thus the one-week LI values noted here can be extrapolated to sustained rates over longer periods of time.

It should be noted that cell proliferation measurements are quantitative and reproducible over time. Measurements of the patterns of chloroform-induced regenerative cell proliferation in the livers of B6C3F1 mice have been remarkably constant in various experiments over a several-year period (Larson et al., 1994c,d, 1996; Templin et al., 1997). In a 90-day B6C3F1 mouse chloroform inhalation study with daily 6 h/day exposures, the NOAEL for induced liver cell proliferation was 10 ppm (Larson et al., 1996), consistent with the NOAEL of 10 ppm observed in the current study (Table 3).

For DNA-reactive mutagens, there is the theoretical possibility that very low doses, including one molecule, may mutate a key gene related to cancer induction. For nongenotoxic-cytotoxic carcinogens, any genotoxic activity is secondary to induced toxicity. It is highly improbable that one molecule, acting at a non-DNA target, might kill a cell. Even a few additional cell divisions that do not raise the overall background of cell turnover are unlikely to have any significant effects on the tissue. Increased cancer associated with cytotoxicity appears to require a substantial increase in regenerative cell proliferation to drive tumor formation (Larson et al., 1994c; National Cancer Institute, 1976; Templin et al., 1997b; Yamamoto et al., 1994). In contrast to an end point such as DNA adducts, cell proliferation measurements do not require precision beyond measuring to the point of lack of statistical significance over background. In practice, toxicity and cancer risk assessments will use safety factors to account for species and interindividual variations that are on the order of 100- to 1000-fold below this NOAEL. While there may be some uncertainty as to the exact value of the NOAEL, there will clearly be no biologically significant increase in regenerative cell proliferation at the lower estimated safe doses. Thus, the profile of induced cell proliferation provides a sound scientific basis for a risk assessment.

The degree to which a given chloroform dosing regimen results in cell death within a target organ is dependent on a complex series of rates that define chloroform dosimetry. These include the rates of uptake and distribution to the target organ, of generation of the toxic metabolites phosgene and HCl, of detoxification of those products, of cellular damage by metabolites that are not detoxified, and of repair of that cellular damage. At sufficiently low doses and dose rates, detoxification and repair processes are protective, and no cytotoxicity will occur. As doses and dose rates are increased, a pointed will be reached where the protective capabilities of the cell are exceeded, and the more sensitive cells in the target organ will begin to die.

The experiments presented here were based on estimation of chloroform dosimetry described from the chloroform PBPK model (Corley et al., 1990). Figure 1 and Table 1 illustrate that the same total amount of chloroform would be expected to be metabolized by a 90-ppm, 2 h/day exposure; a 30-ppm, 6 h/day exposure; and a 10-ppm, 18 h/day exposure. However, there is an increasing rate of metabolism in moving from 10 to 30 to 90 ppm due to the increased tissue dose (Fig. 1). An objective of this study was to determine whether maintaining a minimum metabolic rate for a specific duration or a cumulative amount metabolized would be the critical factor needed to trigger regenerative cell proliferation. Of these 3 exposure conditions, only the 6-h, 30-ppm and 2-h, 90-ppm exposures significantly increased the hepatocyte LI. This observation supports the conclusions that a defined minimal rate of metabolism must be exceeded to produce cell death and that chloroform-induced toxicity is not cumulative. Inhalation exposures to 10 ppm or less of chloroform did not significantly induce cytotoxicity and regenerative cell proliferation in the female B6C3F1 mouse liver, even when exposure periods were up to 18 h per day (Table 3). At approximately this concentration and below, the rate of production of toxic metabolites appears to be low enough so that the rates of detoxification of metabolites and the repair of any cellular damage are within the capacity of the cell.

Similarly, the 30-ppm, 6 h/day exposure resulted in a LI of about 5-fold over background. Increasing the exposure concentration 3-fold to 90 ppm, 6 h per day resulted in a LI of about 30-fold over background. Clearly, the rate of metabolism...
associated with the higher dose, rather than the cumulative amount of chloroform exposure, appears to be the predominant factor in driving hepatocyte lethality.

The data in Table 3 approximate an empirical relationship that characterizes the exposure conditions necessary to initiate cytolethality and regenerative cell proliferation in the female B6C3F1 mouse liver (Fig. 2). The solid line in Figure 2 is drawn through those exposure combinations that just produce a borderline response and represent the minimum exposure conditions needed to increase cell proliferation. The shaded area above the line represents combinations of chloroform concentration × exposure duration that would be expected to induce cell death, regenerative cell proliferation, and, presumably, increased tumor incidence. These data suggest that a concentration of about 10 ppm is a NOAEL below which there will be no hepatotoxicity regardless of the duration exposure. The rate of chloroform metabolism at the point that separates the NOAEL from induced cytolethality is a critical parameter needed to link the PBPK chloroform model to the PD model describing tissue response and tumor formation in the mouse and serve as the basis for a biologically based cancer risk assessment.

To extrapolate the effects of chloroform exposure in humans, we used the chloroform mouse and the human PBPK models (Corley et al., 1990) to estimate hepatotoxic exposures in humans based on the results of the animal experiments presented here. The strategy was to identify human chloroform exposure conditions that result in similar rates of hepatic metabolism to those found to produce increased cytolethality in mice. For PBPK model simulations, human physiological parameters (i.e., organ weights, blood flows) and biochemical parameters specific for chloroform (i.e., \( V_{\text{max}} \), \( K_m \)) and partitioning coefficients were obtained from Corley et al. (1990). Hepatic metabolism was normalized by liver weight and measured on a per gram basis (\( \mu \text{mol/h/g} \)) to account for the difference in liver mass between humans and mice.

In estimating cytolethal chloroform exposures in humans, the following assumptions were made:

- The rate of formation of toxic chloroform metabolites is the driving force for chloroform-induced hepatotoxicity.
- Mouse and human hepatocytes are equally sensitive to the toxic effects of chloroform metabolites.
- The capability and rates of detoxification of metabolites and repair of cellular damage are equal for mouse and human hepatocytes.
- Defined conditions of metabolism rate on a per gram of liver basis, and an associated time of exposure will result in the same degree of liver cytolethality for the mouse and human.

With these assumptions, the metabolism rate associated with the 10-ppm NOAEL value on the mouse curve in Figure 2 was used to estimate the exposure concentration for humans that would result in the same hepatotoxic response.

The estimated NOAEL for chloroform-induced hepatotoxicity in humans is approximately 110 ppm. The human NOAEL is higher than the mouse value of 10 ppm for the following reasons. The PBPK model parameter for the amount of adipose tissue in the human (23.1% of body weight) is greater than the mouse (10.0% of body weight) and results in a greater distribution into the fat rather than the liver for the human. The blood/air partition coefficient, or relative solubility of chloroform in the blood, is approximately 3 times greater in the mouse than human, 24.3 and 7.43, respectively. The rate of metabolism or \( V_{\text{max}} \) is scaled to the \( \frac{1}{3} \) power, in extrapolating from mouse to human, to account for body size in cross-species comparisons.

The predicted lower susceptibility to chloroform toxicity of people compared to mice is supported somewhat by experience with chloroform as an anesthetic. Chloroform began to be used as an anesthetic gas in 1847 and was commonly used for the next 110 years (Davison, 1965; Whitaker and Jones, 1965). While the toxic effects of chloroform included liver failure, those cases occurred primarily during the early years and were the result of overdoses associated with inexperience and poor techniques of administration such as dropping the liquid on to a piece of gauze over the patients face. Davison (1965, p. 656) states “It is quite wrong to compare, as is so frequently done, the fatalities with chloroform one hundred years ago, with the success of halothane in the present era. It is the author’s belief that chloroform, administered by means of modern apparatus and techniques, is no more dangerous than halothane . . .” Under more modern administration techniques, the concentration most commonly administered to patients was approximately 2.25% (22,500 ppm). Under these conditions, Davison (1965) observed no cases of liver toxicity and Whitaker and
Jones (1965) reported one case in 1502 patients. A further analysis of that cohort of patients did not show any increased chloroform toxicity in children compared to adults. No kidney toxic effects were noted in the above reports. The limitation of these data are that exposures were generally for a period of only 30 min, with some extending to 2 h.

The PBPK comparison modeling suggests that mice will be more susceptible to the toxic effects of chloroform than humans. Therefore, no safety factor to account for species to species extrapolation should be required in formulating a chloroform inhalation cancer risk assessment based on the dose × time inhalation data presented here.

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