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# Metabolism of Chloroform by Cytochrome P450 2E1 Is Required for Induction of Toxicity in the Liver, Kidney, and Nose of Male Mice

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**Chloroform is a nongenotoxic-cytotoxic liver and kidney carcinogen and nasal toxicant in some strains and sexes of rodents. Substantial evidence indicates that tumor induction is secondary to events associated with cytolethality and regenerative cell proliferation. Therefore, pathways leading to toxicity, such as metabolic activation, become critical information in mechanism-based risk assessments. The purpose of this study was to determine the degree to which chloroform-induced cytotoxicity is dependent on the cytochromes P450 in general and P450 2E1 in particular. Male B6C3F$_1$, Sv/129 wild-type (*Cyp2e1*+/+), and Sv/129 CYP2E1 knockout (*Cyp2e1*−/− or *Cyp2e1*-null) mice were exposed 6 h/day for 4 consecutive days to 90 ppm chloroform by inhalation. Parallel control and treated groups, excluding *Cyp2e1*-null mice, also received an i.p. injection (150 mg/kg) of the irreversible cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) twice on the day before exposures began and 1 h before every exposure. Cells in S-phase were labeled by infusion of BrdU via an implanted osmotic pump for 3.5 days prior to necropsy, and the labeling index was quantified immunohistochemically. B6C3F$_1$ and Sv/129 wild-type mice exposed to chloroform alone had extensive hepatic and renal necrosis with significant regenerative cell proliferation. These animals had minimal toxicity in the nasal turbinates with focal periosteal cell proliferation. Administration of ABT completely protected against the hepatic, renal, and nasal toxic effects of chloroform. Induced pathological changes and regenerative cell proliferation were absent in these target sites in *Cyp2e1*−/− mice exposed to 90 ppm chloroform. These findings indicate that metabolism is obligatory for the development of chloroform-induced hepatic, renal, and nasal toxicity and that cytochrome P450 2E1 appears to be the only enzyme responsible for this cytotoxic-related metabolic conversion under these exposure conditions.**



Water intended for human use and consumption is commonly disinfected with chlorine-based chemicals. Chloroform is usually the most prevalent by-product of these disinfection processes, and drinking water concentrations have been reported to range from 0.0001 to 0.3 ppm chloroform (Symons *et al.,* 1975; Uden and Miller, 1983; Craun, 1993). Chloroform can volatilize from water, and concentrations as high as 0.001 ppm have been measured in ambient air (Singh *et al.,* 1982; Jo *et al.,* 1990). Industrial processes such as bleaching of paper with chlorine can also yield chloroform that is released directly into the atmosphere or volatilized from waste waters (Butler and Dal Pont, 1992).

Chloroform induced liver cancer in male and female B6C3F$_1$ mice when given by gavage but not when given in the drinking water, induced kidney cancer in male but not female Osborne–Mendel rats when given by gavage or in the drinking water, induced kidney cancer in male but not female BDF$_1$ mice when administered by inhalation, and induced no cancer in F-344 rats when given by inhalation (National Cancer Institute, 1976; Jorgenson *et al.,* 1985; Yamamoto *et al.,* 1994). These studies have demonstrated that liver and kidney are target organs for chloroform-induced cancer and that tumor formation is highly dependent on genetic background, species, strain, gender, and rate of administration. Chloroform exposures have also produced hypertrophy and hyperplasia of the periosteum and irregular formation of new, immature bone in nasal passages of female and male B6C3F$_1$ mice (Larson *et al.,* 1994d, 1996).

Chloroform induces cancer by a nongenotoxic-cytotoxic mode of action (Butterworth *et al.,* 1995). Induced tumors arise secondary to events associated with cytolethality and regenerative cell proliferation (International Programme on Chemical Safety, 1994; Larson *et al.,* 1994a, b; International Life Sciences Institute, 1997). Therefore, pathways leading to toxicity,

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such as metabolic activation, become critical information in mechanistically based quantitative risk assessments.

Chloroform-induced hepatotoxicity is associated with metabolism by a cytochrome P450 oxygen-dependent pathway (Mansuy *et al.,* 1977; Smith *et al.,* 1983; Smith and Hook, 1984). In the liver and to a lesser degree in the kidney, biotransformation of chloroform produces the toxic metabolites phosgene and HCl (Mansuy *et al.,* 1977; Pohl *et al.,* 1977). Phosgene is then believed to attack nucleophilic sites on proteins, leading to cell death (Ilett *et al.,* 1973; Smith *et al.,* 1983; Cowlen *et al.,* 1984). Chloroform-induced cytotoxicity has been proposed to be dependent on the rate at which the target tissue dose is metabolized (Corley *et al.,* 1990; Reitz *et al.,* 1990). The degree of organ-specific damage is believed to be dependent on the rate of production of toxic metabolites and subsequent cellular damage versus the rates at which the cell can detoxify those metabolites or repair any induced cellular damage (Conolly and Andersen, 1991; Conolly and Butterworth, 1995).

The extent to which chloroform versus its metabolites are responsible for induced cytolethality has not been rigorously determined. This information is critical in the formulation of a comprehensive risk assessment model.

The objectives of this study were (1) to establish whether chloroform biotransformation is required to produce cytotoxicity and induce regenerative cell proliferation in the liver, kidney, and nasal passages of mice and (2) to determine the degree to which one specific cytochrome P450, namely Cyp2e1, is responsible for chloroform-induced cytotoxicity. These studies used an irreversible inhibitor of cytochromes P450 as well as genetically engineered mice deficient in active Cyp2e1 (*Cyp2e1*−/− or *Cyp2e1*-null mice). Male B6C3F$_1$ mice, which have been used extensively in previous chloroform studies, provided a comparison for results observed with the Sv/129 mouse strain.

## MATERIALS AND METHODS

***Chemicals.*** Chloroform, >99.5% purity and stabilized with 0.006% amylenes, was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI) and stored in stainless steel pressure vessels. 1-Aminobenzotriazole (ABT), >99% purity, was purchased from Sigma Chemical Company (St. Louis, MO) and prepared as a 12 mg/ml solution in saline and filter sterilized. ABT solutions were prepared fresh prior to administration. A 16 mg/ml solution of 5-bromo-2′-deoxyuridine (BrdU) (Sigma Chemical Co.) was prepared in phosphate-buffered saline (PBS) with stirring at 37°C and filter sterilized. BrdU solutions were prepared on the days they were dispensed into the osmotic pumps.

***Animals and husbandry.*** All animal experiments in these studies were conducted under federal guidelines for the humane use and care of laboratory animals (National Research Council, 1996) and were approved by the Chemical Industry Institute of Toxicology (CIIT) Institutional Animal Care and Use Committee. Three different types of mice were used in this study: male B6C3F$_1$ mice, male Sv/129 wild type mice, and male genetically engineered Sv/129 *Cyp2e1*-null mice. Male B6C3F$_1$ mice (B6C3F$_1$/CrlBR) mice were purchased from the Charles River Breeding Laboratories, Inc. (Raleigh, NC) at 11 weeks of age and allowed to acclimate for 1 week before the start of the study. The *Cyp2e1*-null (about 13 weeks old) and wild-type (about 11 weeks old) strains of mice were F$_4$ generation Sv/129 mice that were bred and reared at the National Cancer Institute and shipped to CIIT for the conduct of these studies. Animals were allowed to acclimate for 1 week. Previous studies have demonstrated a complete absence of Cyp2e1 protein expression and disruption of the CYP2E1 mRNA in the livers of these *Cyp2e1*−/− mice (Lee *et al.,* 1996; Valentine *et al.,* 1996). All mice were housed in humidity- and temperature-controlled facilities accredited by the American Association for Accreditation of Laboratory Animal Care. Mice of each strain were randomized by weight, assigned to various treatment 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, 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.

***Generation and characterization of atmospheres.*** Target exposure concentrations of chloroform were 0 and 90 ppm. The average chloroform concentration was 92 ± 4 ppm, as measured at the inlet to the exposure system. 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 in 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. These exposure systems were placed inside separate 1-m$^3$ Hinners-style inhalation chambers that served as a secondary containment hood to reduce the chance of any cross contamination. Approximately 15 min before the exposure, the mice were transferred from their home cages to the inhalation tubes. Relative humidity and temperature were monitored in a representative tube that contained a mouse, using an Omega RH 30 temperature–humidity indicator (Omega Engineering Inc., Stamford, CT). Following exposures, all the mice were exposed to control air for approximately 30 min before being returned to their home cages.

***Experimental design.*** Male mice were chosen to investigate the role of CYP2E1 since they are susceptible to both hepatic and renal toxicity following chloroform inhalation exposure. The chosen inhalation exposures in this study were based on previous experiments performed at CIIT. Exposures of 6 h/day for 4 consecutive days to 90 ppm chloroform were known to produce the desired cytotoxic effects in the known target organs (Larson *et al.,* 1994d, 1996). Previous studies conducted in our laboratory demonstrated that the production of frank necrosis and regenerative cell proliferation in target tissues after 4 days of exposure is representative of the sustained degree of response for exposure periods of over 90 days (Larson *et al.,* 1996).

Parallel control and treated groups (5 animals/group), excluding *Cyp2e1*-null mice, also received an i.p. injection of either saline or an irreversible inhibitor of cytochrome P450 activity, ABT (150 mg/kg), twice on the day before exposures began and once every following morning 1 h prior to chloroform exposure (days 1–4). ABT is known to act as a suicide inhibitor of cytochromes P450 (Mico *et al.,* 1988). ABT treatment does not result in overt clinical toxicity and produces only small increases in relative liver weight and mild hepatocytic hypertrophy (Meschter *et al.,* 1994). Hence, toxicity was evaluated based on the specific role of CYP2E1 in *Cyp2e1*-null mice versus the aggregate role of cytochromes P450 in ABT-treated wild-type mice.

***Labeling cells in S-phase.*** Chloroform-induced regenerative cell proliferation was evaluated by the immunohistochemical detection of the incorporation of the thymidine analogue BrdU into the nuclei of cells in S-phase. A 16 mg/ml BrdU solution was delivered at a flow rate of 1 μl/h for approximately 3.5 days prior to necropsy by an implanted osmotic pump (Alzet® Model 2001, Alza Corporation, Palo Alto, CA) to label the DNA of cells in the S-phase of the cell cycle. Osmotic pumps were implanted under isofluorane anesthesia and aseptic conditions as described in Eldridge *et al.* (1990).

***Necropsy.*** At necropsy, mice were weighed, anesthetized with sodium pentobarbital, and euthanized by exsanguination. Whole livers and both kidneys were immediately removed, weighed, and examined macroscopically.

Longitudinal sections of the left and right median lobes of the liver, transections of the left and right kidney, and a 2- to 3-mm section of the duodenum were dissected and fixed in 10% neutral buffered formalin (NBF) for 48 h and then stored in 70% ethanol until processing.

Immediately following removal of the liver and kidneys, the heads containing the nasal cavity were prepared by removing the lower jaw, removing excess soft tissue, and then flushing the nasal cavity with 10% NBF via the trachea. Following at least 1 week fixation in 10% NBF, the head was decalcified in 5% formic acid with ion exchange resin (Rexyn®101(H), Fisher Scientific) and 6 transverse blocks cut and embedded in paraffin according to Morgan (1991).

***Histopathology.*** Histological examinations were performed by light microscopy on hematoxylin and eosin (H&E) stained sections of liver, kidney, and nasal tract to document the relationships between strain, dose, toxic response, and proliferative response in mice exposed to chloroform vapors for 4 days. H&E-stained sections were read by a pathologist, first with knowledge of treatment group followed by blinded reading of slides.

***Evaluation of regenerative 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 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 anti-mouse IgG (Vector Labs, Burlingame, CA). Slides were then incubated for 30 min at room temperature with a strepavidin–alkaline phosphatase complex (Zymed Laboratories, South San Francisco, CA), and BrdU incorporation was visualized by a final incubation with the chromagen Stable Fast Red (Research Genetics, Huntsville, AL), with surrounding tissue counterstained with hematoxylin.

The labeling index was determined microscopically by capturing images from histological sections stained for BrdU incorporation using the Imascan software (Imagraph, Chelmsford, MA). The Cytology/Histology Recognition Information System (CHRIS) software package (Sverdrup Medical/Life Sciences Imaging Systems, Fort Walton Beach, FL) was used to assess regenerative cell proliferation in the captured images. A minimum of 10 fields were captured from the liver, which provided at least 1000 hepatocyte nuclei for analysis. In the kidney, a minimum of 15 fields were captured from the cortex and outer stripe of the outer medulla (OSOM), which provided a minimum of 2000 cell nuclei for analysis. These images were calibrated for labeled cells, unlabeled cells, and cytoplasm and processed according to the *CHRIS User's Guide.*

For the liver and kidneys, 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 was expressed as a percentage. In the liver, a section of the left hepatic lobe was used to determine the hepatocyte LI as described in Larson *et al.* (1994b). In the kidney, the LI was evaluated in the cortex and OSOM, the two zones where chloroform-induced cytotoxicity occurs because of the presence of the proximal tubules. These two zones were counted as a single region with field areas counted diagonally across the cortex and outer stripe according to Larson *et al.* (1994d). A section of duodenum was included with each tissue to confirm systemic delivery of BrdU to the tissues.

The region of interest to evaluate nasal periosteal cell proliferation was the proximal portion of the dorsal scroll and base of the first endoturbinate, adjacent to the lateral wall. This region was selected on the basis of histopathology and qualitative assessment of cell replication sites selected in Méry *et al.* (1994). Regenerative cell proliferation was quantified as a unit length labeling index as follows. Proliferation in a 1.5- to 4-mm section of a region bordered by the periosteum and the base of the epithelium was assessed starting at the margin of the growth plate and progressing toward the diaphysis. Cell types counted included primarily periosteal and Bowman's gland cells. The number of labeled cells, from growth plate to growth plate, was assessed visually, and the distance in millimeters was determined. Estimates of cell regeneration were expressed as the number of labeled cells per millimeter of turbinate, according to Larson *et al.* (1996).

***Statistics.*** For liver and kidney tissues, an arcsine transformation was applied to the LI data prior to statistical analysis to meet criteria for normality and homogeneity. A multiple comparisons ($p \leq 0.01$) test on organ weights and LI was used to compare the control group, which received no ABT pretreatment and 0 ppm chloroform, with the other 3 treatment groups for each mouse strain. To determine the effect of inhibitor pretreatment, analyses were performed using Student's $t$ test ($p \leq 0.01$) between the two chloroform exposure groups that received the same pretreatment. Following statistical analysis, the data were back-transformed and are reported on a percentage scale.

## RESULTS

*Clinical observations and histopathology.* Clinical signs of toxicity, such as lethargy and a roughened coat, were observed in $B6C3F_1$ and Sv/129 wild-type (*Cyp2e1*+/+) mice exposed to 90 ppm chloroform without ABT pretreatment. One *Cyp2e1*+/+ mouse that received 90 ppm chloroform and no ABT was found moribund following the third day of exposure and was euthanized. Upon necropsy and visual examination, this mouse had an accentuated hepatic lobular pattern, and the kidneys had moderate diffuse pallor, both attributed to chloroform exposure. No clinical signs of adverse health effects associated with treatment were evident in mice from other exposure groups, including the Sv/129 *Cyp2e1*-null mice that were exposed to 90 ppm chloroform. No significant change in body weight was observed in any exposure group. Chloroform induced small treatment-related increases in relative liver and kidney weights (Table 1). ABT also induced small treatment-related increases in relative liver weights (Table 1).

Sections of liver, kidney, and nasal tissues revealed homogeneity between animals within groups. Liver sections from $B6C3F_1$ mice pretreated with saline and exposed to 90 ppm chloroform had moderate centrilobular to midzonal hepatocytic vacuolar degeneration similar to chloroform induced lesions reported earlier in this strain (Larson *et al.,* 1994b,c, 1996). This degenerative lesion was absent in air-exposed control animals and in all animals pretreated with ABT. ABT treatment alone was associated with minimal centrilobular hepatocytic hypertrophy and intracytoplasmic vacuolization.

Sv/129 *Cyp2e1* wild-type mice exposed to 90 ppm chloroform had marked centrilobular hepatocytic hydropic degeneration with necrosis. The lesions in this strain were more severe than the hepatocytic changes found in the $B6C3F_1$ animals. This lesion was not present in air-exposed control animals or in animals pretreated with ABT and exposed to either air or chloroform. The ABT treatment was associated with minimal centrilobular hepatocytic hypertrophy and intracytoplasmic vacuolization. Hepatic tissues from Sv/129 *Cyp2e1*-null mice exposed to 90 ppm chloroform had an identical histological appearance to those from air-exposed controls.

**TABLE 1**
**Relative Liver and Kidney Weights for Chloroform Exposed Mice**

| Strain | Treatment group | | Relative liver weight (% of body wt) | Relative kidney weight (% of body wt) |
|---|---|---|---|---|
| | Pretreatment | Chloroform (ppm) | | |
| $B6C3F_1$ | ABT[a] | 0[b] | 6.4 ± 0.3[c] | 1.5 ± 0.0 |
| $B6C3F_1$ | ABT | 90 | 6.6 ± 0.5 | 1.5 ± 0.1 |
| $B6C3F_1$ | Saline | 0 | 5.4 ± 0.1[d] | 1.6 ± 0.1 |
| $B6C3F_1$ | Saline | 90 | 6.9 ± 0.6[e] | 1.8 ± 0.1[e] |
| Sv/129 wild type | ABT | 0 | 6.2 ± 0.2 | 1.6 ± 0.1 |
| Sv/129 wild type | ABT | 90 | 6.3 ± 0.3 | 1.6 ± 0.1 |
| Sv/129 wild type | Saline | 0 | 5.1 ± 0.4[d] | 1.7 ± 0.1 |
| Sv/129 wild type | Saline | 90 | 6.6 ± 0.4[e] | 1.8 ± 0.1 |
| Sv/129 *Cyp2e1*−/− | — | 0 | 4.9 ± 0.1 | 1.6 ± 0.1 |
| Sv/129 *Cyp2e1*−/− | — | 90 | 4.8 ± 0.7 | 1.7 ± 0.2 |

[a] ABT, 1-aminobenzotriazole, an irreversible cytochrome P450 inhibitor.
[b] Chloroform exposures were 6 h/day for 4 consecutive days.
[c] Values are means ± SD ($n = 5$ mice with the exception of the Sv129 wild type ABT 0 ppm and saline 90 ppm, which were $n = 4$).
[d] Significantly lower than other respective groups using Dunnett's multiple comparisons test ($p \leq 0.01$).
[e] Significantly greater than respective control group, Student's $t$ test ($p \leq 0.01$).

Severe acute tubular coagulative necrosis, especially in the epithelium of proximal convoluted tubules, was noted in the kidneys of both $B6C3F_1$ and Sv/129 *Cyp2e1* wild-type mice exposed to 90 ppm chloroform. Affected tubules were lined by hyaline eosinophilic cells with pyknotic and missing nuclei. Occasional flattened basophilic cells indicative of regenerating epithelium were found. No significant renal pathology was noted in Sv/129 *Cyp2e1*-null mice or mice pretreated with ABT in chloroform or air exposed groups.

Nasal histopathologic changes were limited to chloroform-exposed $B6C3F_1$ and Sv/129 *Cyp2e1* wild-type mice. These lesions were minimal in severity and consisted of submucosal edema and focal periosteal cell proliferation in the ventral and lateral regions of the ethmoid turbinates and adjacent nasal wall as described in earlier studies (Larson *et al.*, 1994d, 1996; Méry *et al.*, 1994). No histopathologic lesions were present in Sv/129 *Cyp2e1*-null mice or mice pretreated with ABT in chloroform or air-exposed groups.

*Induced cell proliferation.* $B6C3F_1$ and Sv/129 *Cyp2e1* wild-type mice exposed to 90 ppm chloroform alone exhibited significant hepatic and renal regenerative cell proliferation (Figs. 1 and 2). No such regenerative cell proliferation was observed in the livers or kidneys of those mice that were first administered ABT. Hepatic and renal cytotoxicity and induced regenerative cell proliferation were also absent in *Cyp2e1*-null mice exposed to 90 ppm chloroform (Figs. 1 and 2).

Periosteal cell proliferation was observed in the nasal passages of $B6C3F_1$ and Sv/129 *Cyp2e1* wild-type mice exposed to 90 ppm chloroform but was not observed in animals given ABT or in *Cyp2e1*-null mice (Fig. 3).

## DISCUSSION

Exposure of male $B6C3F_1$ mice and male Sv/129 *Cyp2e1* wild-type mice to 90 ppm chloroform resulted in severe toxicity in the liver and kidney and minimal toxicity in the nasal passages. No histologic changes or increases in cell proliferation were observed in target tissues of the same strains of similarly exposed mice given the general P450 inhibitor ABT, confirming that metabolites of chloroform, and not the parent compound, are responsible for its toxic effects. Further, no histologic changes or increases in cell proliferation were seen in target tissues of the *Cyp2e1*-null mice following chloroform inhalation exposures identical to those that produced severe pathological lesions and significant regenerative cell proliferation in *Cyp2e1* wild-type mice. Lee *et al.* (1996) previously confirmed the absence of hepatic Cyp2e1 protein and disruption of CYP2E1 mRNA in *Cyp2e1*−/− mice. Those authors also demonstrated that P450s in the CYP1A, CYP2A, CYP2B, CYP2C, and CYP3A subfamilies were expressed in the *Cyp2e1*-null mice at similar levels to those found in *Cyp2e1* wild-type mice (Lee *et al.*, 1996). Studies performed by Valentine *et al.* (1996) also demonstrated that Cyp2e1 protein was absent in the *Cyp2e1*-null mice and that no induction of Cyp2e1 occurred in these mice following benzene exposure. Further, exposures in the current study were for 4 consecutive days, and no induction of other P450 enzymes capable of metabolizing chloroform were evident. Thus, the findings presented here indicate that metabolism is obligatory in chloroform-induced hepatic, renal, and nasal toxicity and that Cyp2e1 is the only enzyme responsible for this target tissue-related metabolic conversion under these conditions of exposure.

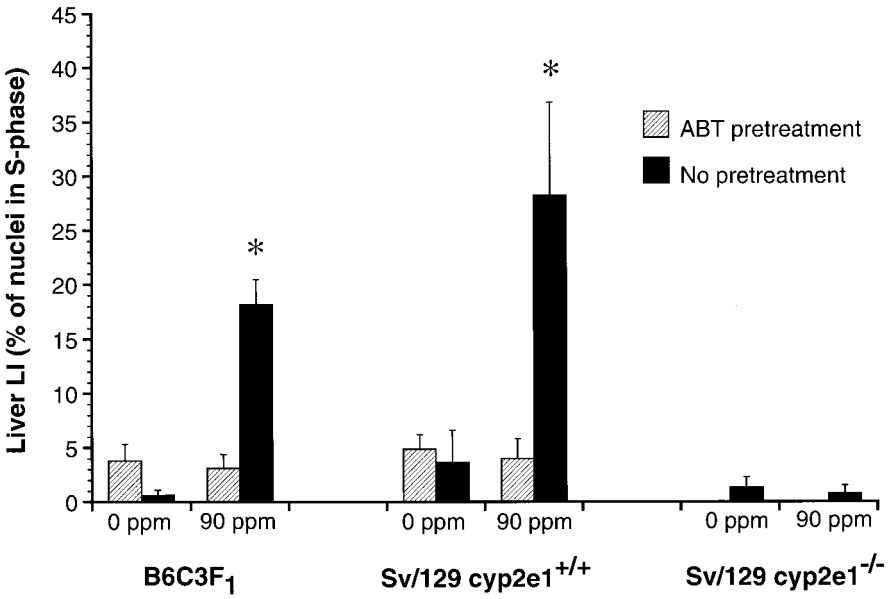


**FIG. 1.** Labeling indices in the livers of control and chloroform-exposed wild-type and Sv/129 *Cyp2e1*-null mice. Treated mice were exposed to 90 ppm chloroform for 6 h/day for 4 consecutive days. ABT mice (▨) were pretreated with the P450 inhibitor ABT. No regenerative cell proliferation was seen in mice pretreated with ABT or mice that lacked the enzyme Cyp2e1.

The weight of evidence indicates that chloroform induces cancer by a nongenotoxic-cytotoxic mode of action (Butterworth *et al.*, 1995). Tumor formation is driven secondary to events associated with cytolethality and regenerative cell proliferation (International Programme on Chemical Safety, 1994; International Life Sciences Institute, 1997; U.S. EPA, 1998). Therefore, pathways leading to toxicity, such as metabolic activation, are rate-limiting steps and become critical information in mechanistically based quantitative risk assessments. Simply put, an individual would suffer symptoms of chloroform toxicity long before such toxicity would lead to cancer. Similarly, target tissue rates of metabolism must be considered in extrapolating from responses in rodents to predicted responses in humans.

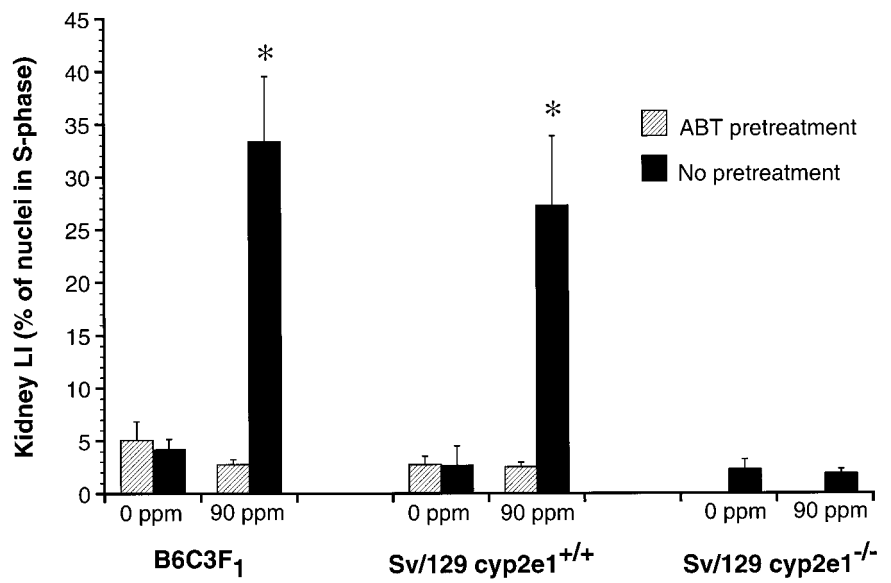


**FIG. 2.** Labeling indices in the kidneys of control and chloroform-exposed wild-type and Sv/129 *Cyp2e1*-null mice. Treated mice were exposed to 90 ppm chloroform for 6 h/day for 4 consecutive days. ABT mice (▨) were pretreated with the P450 inhibitor ABT. No regenerative cell proliferation was seen in mice pretreated with ABT or mice that lacked the enzyme Cyp2e1.

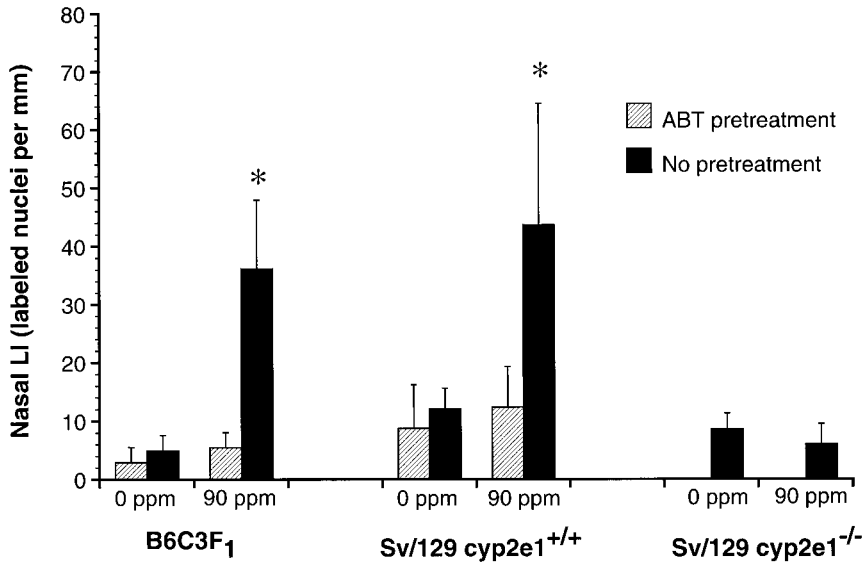


**FIG. 3.** Labeling indices in the nasal turbinates of control and chloroform-exposed wild-type and Sv/129 *Cyp2e1*-null mice. Treated mice were exposed to 90 ppm chloroform for 6 h/day for 4 consecutive days. ABT mice (▨) were pretreated with the P450 inhibitor ABT. No periosteal cell proliferation was seen in mice pretreated with ABT or mice that lacked the enzyme Cyp2e1.

Chloroform has a long and widespread history of use as an anesthetic in humans at doses that are orders of magnitude above those that might be found in the environment, such as a by-product resulting from drinking water disinfection. Chloroform anesthesia was associated with modest side effects in some individuals (Whitaker and Jones, 1965). Those studies show no increased sensitivity to chloroform intoxication among children compared with adults (Whitaker and Jones, 1965). Side effects from chloroform anesthesia include changes in respiratory and pulse rate and changes in cardiac rhythm and hypotension. The target tissue for chloroform-induced toxicity in people most consistently described is the liver (Davison, 1965; International Programme on Chemical Safety, 1994). Protecting against the toxic effects of chloroform would also protect against any carcinogenic effects, so that the toxic and cancer risk assessment considerations become the same (Butterworth and Bogdanffy, 1999). Knowledge of enzyme levels, metabolic activation and detoxification pathways, and patterns of toxicity in human beings thus becomes critical information for risk assessments. The central role of Cyp2e1 in producing chloroform toxicity is a key observation upon which to build further work.

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