California Air Resources Board

Technical Support Document

Proposed Identification of

TRICHLOROETHYLENE

as a Toxic Air Contaminant

Part B
Health Assessment

State of California
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HEALTH EFFECTS OF TRICHLOROETHYLENE (TCE)

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Prepared By:

Environmental Sciences
Lawrence Livermore National Laboratory
University of California
Livermore, California

Reviewed and Edited by:

James F. Collins, Ph.D.

George V. Alexeeff, Ph.D.

Hanna H. Schunk, M.S.

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1. SUMMARY

The health effects of trichloroethylene have been reviewed and evaluated to determine if trichloroethylene (TCE) may be a toxic air contaminant as defined by California Health and Safety Code Section 39655. Trichloroethylene is a colorless, volatile organic hydrocarbon that is not flammable. It is widely used as an industrial solvent in the vapor degreasing and cold cleaning of fabricated metal parts, in textile cleaning, in solvent extraction processes, and as a chemical intermediate.

Trichloroethylene is lipophilic and readily diffuses across the surface of the lungs into the blood. Once in the blood, it is distributed to all tissues and is metabolized. The main metabolites found in humans are trichloroethanol (TCEL), trichloroethanol glucuronide, and trichloroacetic acid (TCA). With continuous exposure, blood and tissue levels of TCE, TCEL, and TCA increase in a dose-dependent manner, and they accumulate in the body. Upon continuous exposure, TCE reaches equilibrium in adipose tissue in 5 to 7 days.

Trichloroethylene is initially transformed by mixed function oxidase enzymes to an intermediate electrophilic epoxide, trichloroethylene oxide (TCE oxide). It is subsequently metabolized by several different pathways. The predominant pathway begins with a spontaneous rearrangement of TCE oxide to chloral (trichloroacetaldehyde), followed by hydration of chloral to chloral hydrate, and either oxidation of the hydrate to TCA or reduction of the hydrate to trichloroethanol (TCEL). The TCE-oxide is believed to produce some types of TCE-induced toxicity by binding to critical macromolecules in the cell. In humans, after exposure to TCE, TCA and TCEL are found in the urine. Long-term exposure in mice increases TCA levels and decreases TCEL levels in the urine. Data from metabolic studies in mice, as well as from pharmacokinetic models simulating human metabolism, suggest that metabolism of TCE ($\geq$ 98% metabolized) increases as the applied dose approaches zero. Some of the other metabolites found in animals are not easily measurable in humans because they are also products of normal metabolism. In humans, the total amount of urinary metabolites is significantly below the total dose metabolized. This indicates that a significant amount of respired TCE is metabolized to products other than TCA, TCEL, and its conjugates. Thus, there appears to be additional

metabolites of TCE to identify in humans. In mice, metabolic saturation of the limiting enzyme system presumably occurs at an exposure of 600 ppm TCE for 6 hours. In man, metabolic saturation of the TCA pathway may be reached above 100 ppm TCE, and the concentration of total trichloro compounds increases linearly up to 200 ppm TCE, which was the highest concentration tested. Absorbed TCE is eliminated both by pulmonary release of unchanged TCE and by metabolic transformation into other products that are excreted primarily in the urine.

Trichloroethylene is a central nervous system depressant and has been used as an anesthetic and analgesic. Occupational exposure to TCE has resulted in nausea, headache, loss of appetite, weakness, dizziness, ataxia, and tremors. Acute exposure to high concentrations has caused irreversible nerve damage and death. Upon exposure to 110 ppm for two 4-hour periods, human volunteers show reduced performance of various psychophysiological function tests. In addition, alcohol intolerance has been reported after TCE exposure. Simultaneous exposure to TCE and ethanol results in a marked inhibition of TCE metabolism leading to an accumulation of TCE in the blood and subsequently to an increase in CNS depression. Trichloroethylene also directly affects the human brain. Accidental, high-level exposures have damaged the brainstem, the cerebral cortex, and the spinal cord. In rats, CNS-related effects (excitability and changes in learning ability) were seen at exposures of 200 ppm TCE.

In the past, exposure to TCE has produced liver damage. Lethal hepatic failure has occurred following TCE-induced anesthesia. Accidental or occupational exposure to TCE has caused liver disease, necrosis, and hepatic failure. Subchronic exposure of rats and mice to TCE has resulted in biochemical, microscopic, and gross changes indicative of liver damage. Liver toxicity is the most sensitive toxic endpoint following subchronic TCE exposure. The No-Observed-Adverse-Effect-Level (NOAEL) for subchronic exposure to TCE in rats is 30 ppm, based on a continuous 90-day exposure.

Kidney damage has been reported in occupationally exposed individuals and in some, but not all, animal studies.

Trichloroethylene has been implicated in numerous cases of cardiac arrest when used as an anesthetic. The concentration and duration of exposure that causes this effect have not been clearly established. Short-term exposures (7.5 to 60 minutes) to high levels (6,000 to 9,000 ppm) of

TCE have caused tachycardia and other arrhythmias when administered with epinephrine. The effect is enhanced by inhibitors of microsomal enzymes, by benzo(a)pyrene, and by ethanol.

Trichloroethylene causes degreaser's flush, characterized by red blotches on the skin of persons who are exposed to TCE and who subsequently consume alcohol. The flush is caused by a dilation of superficial blood vessels. Exposure to 27 ppm for 4 hours also causes irritation of the mucous membranes of the eyes and throat in humans. (Note that this adverse effect in humans occurs below the subchronic NOAEL of 30 ppm for liver effects in rats.) Trichloroethylene has also been shown to cause effects on other organs and systems, including respiratory irritation, renal toxicity, and immune system depression.

For occupational exposures, the U.S. Occupational Safety and Health Administration (OSHA) has assigned a permissible exposure limit (PEL) of 100 ppm (8-hour time-weighted average) to TCE. The American Conference of Governmental and Industrial Hygienists (ACGIH) recommends a threshold limit value, time weighted average (TLV-TWA) of 50 ppm to "control subjective complaints such as headaches, fatigue and irritability." The National Institute of Occupational Safety and Health (NIOSH) considers TCE to be a carcinogen and recommends an exposure limit of 25 ppm (10-hour time-weighted average), which is considered to be the lowest achievable concentration in many occupational settings. The mean environmental concentration of TCE measured by California Air Resources Board staff in 1986 and 1987 was 0.22 ppb for 20 statewide sampling stations and 0.19 ppb for 5 stations in the South Coast Air Basin. At current ambient levels of trichloroethylene, no acute or noncarcinogenic chronic health effects are expected.

Exposure of rats and mice to 300 ppm TCE for 7 hours per day from day 6 to day 15 of gestation results in an inhibition of maternal weight gain, whereas 100 ppm given 4 hours per day during days 8 through 21 of gestation causes a significant delay in fetal maturation and an increase in the number of resorptions. There are inadequate data to evaluate whether TCE may cause reproductive toxicity in humans.

There is mixed evidence regarding whether TCE is capable of causing genetic damage. Trichloroethylene has produced primarily negative results in bacterial assays of mutagenicity. Two studies have reported weakly positive results that were dependent on microsomal activation. Likewise,

weakly positive results have been reported for genetic activity in yeast and the fungus Aspergillus. Human lymphocytes, obtained from workers occupationally exposed to TCE, show an increased incidence of chromosomal rearrangement. Trichloroethylene also induces a slight increase in unscheduled DNA synthesis (UDS) in cultured human cells without metabolic activation. There is weak evidence indicating that injected radiolabeled TCE can bind covalently to DNA in the presence of activation enzymes.

Metabolites of TCE have also been tested for genotoxicity. Chloral hydrate has consistently demonstrated genotoxic activity in bacteria, fungi, and human lymphocytes. A metabolite produced by the glutathione pathway is mutagenic. The TCE-oxide and trichloroethanol have produced both positive and negative results in various assays. Trichloroacetic acid has been tested in only one mutagenicity test with negative results.

The U.S. Environmental Protection Agency (EPA) considers commercial grade TCE to be a weakly active, indirect mutagen. The data on pure TCE do not allow a conclusion to be drawn about its mutagenic potential.

The U.S. EPA has concluded that there is sufficient evidence for the carcinogenicity of TCE in animals, based on increased incidences of malignant liver tumors in B6C3F1 mice in 2 NCI studies, increased incidences of malignant lymphoma in NMRI mice, and increased incidences of renal tumors in rats. Significant increases in the incidence of hepatocellular carcinomas have also been observed in male B6C3F1 mice exposed to high concentrations of the TCE metabolites, TCA and dichloroacetic acid, in drinking water. The EPA has classified the evidence for carcinogenicity of the TCE metabolites, TCA and dichloroacetic acid, in animals to be "limited", because the studies involve only a single species, strain, and experiment. The EPA considers the epidemiologic data on TCE carcinogenicity to be inconclusive. Based on sufficient evidence for the carcinogenicity of TCE in animals, the EPA has placed TCE in group B2, a probable human carcinogen. Based on the criteria for carcinogenicity of the International Agency for Research on Cancer (IARC), TCE has been classified in Group 3 (i.e., unable to be classified as to its carcinogenicity in humans), based on limited evidence of carcinogenicity in animals and inadequate evidence of carcinogenicity in humans. The DHS staff notes the different conclusions regarding carcinogenicity drawn by the EPA and the IARC. DHS staff disagrees with IARC's conclusions because, based on DHS staff's

interpretation of IARC criteria, TCE has shown positive carcinogenic effects "in two or more species of animals or in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols." There are indications that TCE is genotoxic; no evidence exists for a threshold level for this effect; and, based on the animal data, the EPA has concluded that there is sufficient evidence for carcinogenicity (Group B2-probable human carcinogen). Therefore the DHS considers TCE to be carcinogenic and not to have a threshold for carcinogenicity.

A quantitative risk assessment for cancer was done using dose-response data for carcinogenicity from 4 inhalation studies in mice (Bell et al., 1978; Henschler et al., 1980; Fukuda et al., 1983; Maltoni et al., 1986). The applied dose for TCE was determined using standard procedures. In addition, the metabolized dose for TCE was determined using a physiologically based pharmacokinetic model (PBPK) and used for the calculation of carcinogenic potency. Because absorbed TCE is completely metabolized, metabolized dose mirrors applied dose. The DHS staff decided to include the metabolized dose of TCE because it takes into account uptake and distribution factors. The data obtained for uptake and distribution factors are in good agreement with experimental results obtained with human volunteers.

Interspecies variation was accounted for by utilizing a dose per surface area extrapolation between species. The animals were exposed to 50 to 600 ppm TCE for 6 or 7 hours per day, 5 days per week. These levels correspond to a continuous lifetime exposure of approximately 10 to 110 ppm for mice (ppm x 6/24 x 5/7). The adjustment assumes that, for the endpoint of cancer, the product of concentration and time is a constant. (An additional, small adjustment was also made if the animals were not exposed for their lifetime.) Since the mean environmental concentration of TCE is 0.22 ppb statewide, the low-dose extrapolation from exposure concentrations in animal bioassays to those detected in ambient air, spans approximately 5 orders of magnitude.

Carcinogenic responses in the inhalation studies have included increased incidences of hepatocellular carcinoma and adenoma in male mice and increased incidences of lung adenocarcinomas and malignant lymphomas in female mice. Since most tumors were discovered at the time of sacrifice

rather than at the time of their appearance, the GLOBAL79 and GLOBAL86 computer programs for the linearized multistage model, without a time-to-tumor factor, were used for the low-dose risk assessment. The above adjustments to the animals' exposures results in a lifetime time-weighted average dose, either applied or metabolized. The range of 95% upper confidence limit (UCL) potency estimates (q_1^*) obtained using the human equivalent applied and metabolized doses and the tumor incidences in the 4 inhalation studies noted above is 0.006 to 0.098 $(mg/kg\text{-}d)^{-1}$. The range of excess carcinogenic risk from a 70-year lifetime exposure to 0.22 ppb TCE in ambient air ranges from 1 to 10 cases per million persons exposed. Based on the same data, the individual risk for a 70-year lifetime exposure of a 70 kg person breathing 20 m^3 per day of ambient air containing 1 $\mu g/m^3$ (0.19 ppb) of TCE is 8×10^{-7} to 1×10^{-5}. A best estimate of the unit risk was obtained by taking the geometric mean of the unit risks from the four inhalation studies. From the metabolized dose approach a unit risk of 2×10^{-6} $(\mu g/m^3)^{-1}$ was obtained, while from the applied dose a unit risk of 3×10^{-6} $(\mu g/m^3)^{-1}$ was obtained.

The cancer potency range represents the upper range of plausible excess cancer cases; the actual cancer risk cannot be calculated and may be negligible. The potency values are in the range of potency values calculated by the EPA. Based on the annual average of 0.22 ppb TCE in the ambient air of California and estimating that there are 28 million residents, an excess of up to 28 to 280 additional lifetime cancer cases might result from continuous TCE exposure. Based on these findings, DHS concludes that, at ambient concentrations, trichloroethylene may cause or contribute to an increase in mortality or serious illness and may therefore pose a potential hazard to human health.

2. EVALUATION HIGHLIGHTS

I. Exposure Sources

A. Air levels

1. Ambient levels: The population-weighted mean concentration of TCE, based on measurements at 20 stations around California between October 1986 and September 1987, was estimated to be 0.22 ppb. In the Los Angeles air basin, a population-weighted mean of 0.19 ppb was estimated based on measurements taken during the same time interval.

2. Ambient levels measured in "hot spots:" There are no TCE production facilities in California. (Levels in the range of 0.51 to 0.76 ppb have been measured out-of-state.)

3. Indoor air: TCE concentrations are variable depending on the quantity released, the time since release and the size and ventilation rate of the room. Median indoor air concentrations in U.S. homes ranged from 0.05 to 0.51 ppb. In Los Angeles median indoor air concentrations of 0.06 ppb in spring and 0.21 ppb in winter were reported. The maximum concentration measured in homes was 66.5 ppb for a twelve-hour, time-weighted sample. Indoor air levels of TCE appeared to be independent of outdoor concentrations. Thus, this independence is consistent with the assumption that contamination by TCE originates, for the most part, from sources within the home.

B. Reported levels in water

1. National data: Random sampling conducted by EPA in 1982 showed that 30 out of 446 wells were contaminated with TCE. A sampling

program in several states, in areas with a high potential for groundwater contamination, showed 624 out of 4,228 wells contaminated with TCE. Levels in both surveys ranged from 0.2 to 160 μg/liter. There is evidence that trichloroethylene is broken down in soil and groundwater; breakdown products have been detected in groundwater.

2. California drinking water: Since 1984, 6157 wells have been sampled. Of these, 76 showed a contamination with TCE above 5 ppb. The water in 131 wells was found to contain between 0.5 (limit of detection) and 5 ppb TCE.

C. Reported levels in food: The highest levels in food were reported in meat and in tea. Pig's liver was found to contain 22 μg/kg TCE and packaged tea contained 60 μg/kg.

II. Metabolism: Metabolism in humans and animals appears to be similar. The principal metabolites found in humans and animals are trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid. In humans, at environmentally relevant, low doses at least 98% of TCE is likely to be metabolized. Metabolism of TCE is assumed to be complete within 24 hrs. If uptake and distribution are taken into account, a total of 71% of the TCE inhaled is metabolized. A risk assessment based on the applied dose including uptake and distribution factors results in a risk estimate very similar to one based on the metabolized dose. DHS has used both the applied dose and the metabolized dose for the purpose of risk assessment.

III. Quantitative risk assessment for cancer

A. Shape of the dose-response curve

1. Animal: The carcinogenic dose-response curves for mouse tumors indicate linearity, especially for liver carcinomas and adenomas in male mice, but the studies used have some limitations and thus imply some uncertainty.

2. Human: not applicable for TCE

B. Range of extrapolation for animal to human exposure in air for lifetime daily exposure

1. Experimental to ambient: approximately 10^5

2. Experimental to "hot spots:" Not applicable

C. Range of risks: The unit risks for a continuous, lifetime exposure, based on 4 different inhalation studies in experimental animals, ranged from 8×10^{-7} to 1×10^{-5} $(\mu g/m^3)^{-1}$.

IV. National and International Evaluation

A. U.S. Environmental Protection Agency (EPA)

1. Genotoxicity tests: Commercially available TCE is weakly mutagenic. Metabolic activation is required to obtain positive responses. Inadequate information exists to classify pure TCE as a mutagen. The available data suggest that it would be a very weak, indirect mutagen.

2. Animal carcinogenicity tests: Sufficient evidence of carcinogenicity in animals

3. Human evidence: Insufficient data exist to assess human carcinogenicity

4. Potency index: TCE has a potency index of 1 which places it in the lowest quartile of 53 carcinogens for which EPA has calculated potency indices.

5. Conclusions: Based on EPA's proposed cancer guidelines, the overall evidence for TCE would result in its classification as a possible human carcinogen (group B2). EPA considers the induction of liver cancer in mice as sufficient evidence for carcinogenicity in animals and thus believes that its potential for cancer induction does not have a threshold.

B. International Agency for Research on Cancer (IARC)

1. Animal carcinogenicity assays: Limited evidence of animal carcinogenicity

2. Human evidence: Inadequate evidence of human carcinogenicity

3. Conclusion: According to IARC, evidence for the carcinogenicity of TCE is limited. IARC does not consider the liver tumors in mice as sufficient evidence to classify TCE as an animal carcinogen, because liver tumors in mice could also be induced by epigenetic mechanisms such as peroxisome proliferation and as such have a threshold for induction. TCE, as well as other chlorinated organic compounds, are known to be peroxisome proliferators. Therefore, IARC classifies TCE as a group 3 carcinogen, unable to be classified as to its carcinogenicity in humans.

C. National Institute of Occupational Safety and Health (NIOSH)

TCE is a potential carcinogen for humans; however, the results from animal bioassays and from in vitro tests do not warrant considering TCE to be a potent carcinogen. The current occupational limit and control measures in use were established with no concern for carcinogenic potential and thus may be inadequate to assure worker protection from such a carcinogenic potential. NIOSH believes that existing engineering control technology can readily attain a TWA exposure of 25 ppm.

3. PHARMACOKINETICS AND METABOLISM

Trichloroethylene is rapidly absorbed across the surface of the lungs and gastrointestinal tract. Although some TCE can be absorbed through the skin, the rate and extent of dermal absorption are limited compared to that of more lipophilic xenobiotic compounds.

Very little information exists on the distribution of TCE in humans. Data from animal studies, however, indicate that TCE distributes to all tissues. The concentration of TCE in a given tissue depends on the route of exposure, partition coefficients of TCE in each tissue, and on the rates of metabolism and elimination. Once in the blood, TCE is metabolized to a product believed to be the epoxide, TCE-oxide. This epoxide undergoes additional changes by one of 4 paths, ultimately forming metabolites including trichloroethanol (TCEL), trichloroethanol glucuronide (TCEL-G), and trichloroacetic acid (TCA).

This section presents an overview of published studies on the absorption, distribution, metabolism, and elimination of TCE and emphasizes studies on the rate and extent of each of these processes in humans and rodents. Metabolic pathways are also discussed in some depth because these processes transform TCE into one or more reactive molecular species that appear to cause most forms of TCE-induced chronic toxicity.

The discussion of metabolism and excretion kinetics at the end of this section contains a review of physiologically-based pharmacokinetic models that have been used to simulate and predict the absorption, distribution, metabolism, and excretion of TCE in rodents and humans. A detailed analysis is made of the behavior of one of these models under steady-state conditions that may be relevant to regulatory considerations. The dynamic version of this model is then applied using rodent-bioassay-exposure conditions to establish predicted relationships between applied and metabolized dose for these exposure scenarios, which are relevant to the interpretation and assessment of TCE's carcinogenic potency to rodents undertaken in Section 5. Finally, a version of the model is used to estimate the rate at which TCE is metabolically cleared in humans. The model's predictions are fitted to a set of experimental data on urinary concentrations of TCE metabolites found in Japanese workers exposed to various concentrations of TCE in workplace air.

ABSORPTION

In this section, data on the absorption of TCE following dermal exposure, ingestion, and inhalation in animals and humans are reviewed.

Dermal Exposure

The absorption of TCE across the skin has been studied in mice, guinea pigs, and humans.

Animal Studies

Tsuruta (1978) applied 0.5 mL of pure TCE liquid to the (clipped) abdominal skin of mice for periods of 5 to 15 minutes. In these animals, the TCE was retained on the skin by a small impermeable chamber glued to the skin area studied. The estimated percutaneous absorption rate ranged from 7.82 to 12.1 $\mu g/min\text{-}cm^2$. The corresponding dermal absorption rate measured during *in vitro* experiments using excised mouse abdominal skin was 1.125 $\mu g/min\text{-}cm^2$.

Jakobson *et al.* (1982) monitored the concentration of TCE in the blood following administration of pure TCE liquid to the backs of guinea pigs; again the TCE was retained on the skin by a small impermeable chamber glued to the skin region under study. The TCE was absorbed rapidly and reached a peak concentration in the plasma (0.79 $\mu g/mL$) within 30 minutes. Despite continued exposure, plasma levels of TCE decreased gradually after this time.

Human Studies

Stewart and Dodd (1964) estimated the rate of dermal absorption of TCE in humans by measuring the alveolar concentration of TCE in 3 subjects who immersed their thumbs in liquid TCE (98% pure) for 30 minutes. The mean peak concentration of TCE in alveolar air (0.5 ppm) occurred within 30 minutes of exposure. The relatively small amount of TCE present in exhaled breath indicates that absorption of TCE through the skin is limited. These researchers noted that there was considerable individual variation in the rate of absorption and concluded that the dermal absorption of TCE depended on the thickness and area of skin exposed.

Sato and Nakajima (1978) also studied the characteristics of dermal absorption of liquid TCE in humans. The extent of absorption was evaluated by measuring the concentration of TCE in blood and exhaled air of individuals who had immersed one hand in neat liquid TCE for 30 minutes. Measurements were made at the end of exposure, as well as intermittently over a 10-hour follow-up period. The highest levels of TCE were found immediately after the end of exposure (203.11 μg/mL in blood, 28.66 μg/mL in breath) and decreased thereafter. These researchers noted that the conditions of this experiment did not represent a typical occupational exposure and that under average work conditions dermal contact with TCE would probably not result in the absorption of toxic quantities.

Ingestion

Trichloroethylene is readily absorbed across the gastrointestinal (GI) tract of animals. Data on GI absorption of TCE in humans are limited. However, case reports of poisoning after ingestion of TCE indicate that GI absorption in humans is extensive.

Animal Studies

Animal mass-balance studies with radiolabeled TCE have typically recovered most of the dose in excretion products as unchanged TCE or one of its metabolites. In its review of GI absorption of TCE by animals, the EPA (U.S. EPA, 1985a) equated percent recovery of radiolabel with percent of TCE absorbed. This approach assumes that the radioactivity recovered in the urine, feces, carcass, and cage air has been absorbed through the gut prior to its distribution and/or elimination by a given route. However, it should be pointed out that this assumption may not be entirely correct. For example, the TCE measured in cage air (which presumably came from exhalation) may include TCE retained in material that passed through the digestive tract without systemic absorption. In addition, mass-balance studies generally recover 2.0 to 4.0% of the radioactivity in the carcass. These reports have not provided data on the specific disposition of radioactivity within the carcass (i.e., whether or not radioactivity remained in the gut), and it is possible that some of the carcass radioactivity is due to unabsorbed material.

Mass-balance studies using radiolabeled TCE demonstrate that mice can metabolize 38 to 100% and rats can metabolize 15 to 100% of an oral dose of TCE administered in a corn-oil vehicle (Parchman and Magee, 1982; Dekant et al., 1984; Prout et al., 1985; Mitoma et al., 1985; Buben and O'Flaherty, 1985; Rouisse and Chakrabarti, 1986). For both species, the lower values were obtained after treatment with large doses (>1000 mg/kg) (see discussion in subsection on Metabolism and Excretion Kinetics). In general, these data indicate that absorption of TCE through the GI tract is considerable, and at very low concentrations, nearly complete.

Withey et al. (1983) examined the effect of different vehicles on the rate and extent of absorption of TCE in rats as measured by serial blood samples collected over a 5-hour period. Gastrointestinal absorption of TCE was slowed significantly by the use of corn oil as a vehicle, compared with an aqueous solution. After an 18 mg/kg intragastric dose of TCE in water, TCE reached a peak concentration in blood within 5.6 minutes. When the same dose was given in corn oil, the blood concentration profile of TCE exhibited 2 distinct peaks, one within 5 to 10 minutes and a second peak approximately 100 minutes after dosing. Furthermore, the maximum concentration of TCE in blood was almost 15 times greater when TCE was administered in water (14.7 μg/mL) compared to oil (<1.0 μg/mL). Likewise, the observed area under the blood concentration-time curve was estimated to be 218 times greater with TCE administered in water (241.5 μg-minute/mL) rather than in oil (1.11 μg-minute/mL). Thus, a very significant decrease was observed in the rate and extent of GI uptake for TCE administered as an oil solution compared with an aqueous solution. When TCE is administered in corn oil, it appears that its high solubility in this vehicle slows its diffusion from the corn oil across the GI tract. Conversely, TCE is only sparingly soluble in water and diffuses readily from aqueous solutions. In any event, the results of the mass-balance studies referred to above demonstrate clearly that TCE administered orally in a corn-oil vehicle is readily, though not rapidly, absorbed systemically.

D'Souza et al. (1985) determined that the extent of absorption of TCE across the GI tract was dependent on whether test animals (male Sprague-Dawley rats) were starved or well fed. Following an oral dose of 10 mg/kg TCE as a 50% aqueous suspension in a polyethylene glycol-based dispersant (Pegosperse 400-MS), TCE was rapidly and extensively absorbed from the GI tract of fasted rats. Arterial blood had measurable levels of TCE within 30 seconds of

treatment, and blood concentrations of TCE peaked within 6 to 10 minutes. Although specific data were not given for nonfasted animals, the authors reported that the blood levels of TCE in fasted animals were 2 to 3 times greater than in nonfasted animals. Hobara et al (1987) studied the intestinal absorption and subsequent metabolism of TCE in 60 adult male and female mongrel dogs (8 to 12 kg body weight) with a surgically implanted intestinal circulation system. Solutions containing 0.1, 0.25, or 0.5% TCE (weight/volume) were perfused through the jejunum, ileum, and colon of dogs anesthetized with 25 to 30 mg/kg sodium pentobarbital and placed on respirators. Each TCE solution comprised 500 mL of an aqueous solution of TCE dissolved in Tween 80 (1:1 by volume). The absorption rate of TCE was observed to be 50 to 70% of the administered TCE dose after 2 hours. No significant differences were observed in the absorption rates of TCE and water among the 3 intestinal regions studied. The absorption rate for TCE was constant over time. On the basis of these data, Hobara et al. concluded that TCE was absorbed to a high degree in the dogs they studied.

Human Studies

No experimental studies have been conducted on humans to evaluate the extent of absorption following ingestion of TCE. However, Defalque (1961) cited a number of cases of accidental poisoning from ingestion of TCE. The U.S. EPA (1985a) listed several additional instances of poisoning and observed that these reports suggest that absorption of TCE across the GI tract of humans is extensive.

Pulmonary Uptake

The human blood/air partition coefficient of TCE has been estimated to be between 9.2 and 15. Trichloroethylene readily diffuses across the lungs into the capillaries of the alveoli (Eger and Larson, 1964; Sato et al., 1977; Monster, 1979). For a given concentration of TCE, pulmonary uptake depends on the solubility of TCE in blood and tissue, the volume and rate of perfusion of tissues, the rate of elimination, and the rate of alveolar ventilation, i.e., that fraction of total respiratory ventilation from which volatile organic compounds may be cleared by absorption into alveolar capillary blood (Fernandez et al., 1977; Astrand, 1975).

Under nonsteady-state conditions, the amount of TCE that initially enters arterial blood depends primarily on the concentration difference of TCE between alveolar and venous blood (Fernandez et al., 1977; Monster, 1979). Several studies have found that the uptake of TCE from the lungs is rapid during the first 30 to 60 minutes of exposure, but that the rate of uptake decreases as TCE concentration in body tissues approaches steady state (Monster, 1979; Fernandez et al., 1977).

Under steady-state conditions, the net uptake (the difference between the ambient and alveolar air concentrations, multiplied by the alveolar ventilation rate) is theoretically equal to the amount of TCE removed from the body by metabolism. But experimental human studies of pulmonary uptake of TCE have generally not approximated steady-state conditions. Prior to the attainment of a steady-state condition, net uptake (i.e., retention) varies as a function of the amount already retained in tissues at any given time. Thus, generalizations that can be made from reported values of percent uptake or percent retention are necessarily restricted to the specific exposure regimen used. The applicability of these data for risk assessment is limited because generalizations relating uptake to metabolism cannot be derived from these studies.

Animal Studies

Andersen et al. (1980) exposed rats to TCE for 3 hours at concentrations that ranged from 30 to 8000 ppm. The rate of pulmonary uptake in rats was concentration dependent; at the lowest exposure concentration (30 ppm) the uptake of TCE was linear. Uptake deviated from linearity at concentrations of TCE $\geq$ 100 ppm.

Stott et al. (1982) evaluated the metabolism of TCE in rats and mice exposed to 10 or 600 ppm ^{14}C-labeled TCE for 6 hours. Radiolabel was recovered in expired air (as CO_2 and unchanged TCE), as well as in the urine, feces, and carcass. Pulmonary absorption of TCE was evident in both rats and mice, because radiolabeled CO_2 and urinary metabolites were generated as a result of exposure. However, the net uptake of TCE appeared to differ between species at the higher TCE concentration because the amount of unchanged TCE exhaled after exposure to 600 ppm was 10 times greater than the corresponding percent observed after exposure to 10 ppm TCE (21.1% versus 2.1% exhaled of the total body burden of TCE measured). A similar change was not observed in mice,

which exhaled unchanged between 1 and 2% of the measured TCE body burden at both exposure concentrations (see subsection on Metabolism and Excretion Kinetics).

Raabe (1986) determined the uptake of ^{14}C-TCE by beagles at concentrations of TCE less than 25 ppb, a level 3 orders of magnitude below the concentration used for rats and mice. The steady-state fractional systemic uptake for 3 beagles averaged 48% over a two-hour period.

Human Studies

Fernandez et al. (1977) simulated the uptake, distribution, and elimination of TCE after inhalation exposure. Their model separated the body into a pulmonary compartment, an organ responsible for metabolism (the liver), and 3 tissue types (the muscle group, fat group, and vessel-rich group). Fernandez et al. (1977) calculated the partial pressure of TCE in different tissues during an 8-hour exposure to 100 ppm. In the well-perfused vessel-rich and muscle groups, the concentration of TCE increased rapidly over the first 2 hours of exposure. As the partial pressure of TCE equilibrated between tissues and inspired air, the rate of uptake diminished and became relatively constant by the end of the 8-hour exposure. The concentration of TCE in these 2 tissue types began to decrease as soon as exposure ceased. In contrast, levels of TCE in the poorly perfused adipose tissue increased much more slowly and continued to increase for approximately one hour after the end of exposure (see subsection on Distribution and Bioaccumulation).

Various studies have reported the "percent retention" (i.e., net uptake as a percent of alveolarly ventilated amount) of TCE for experimental inhalation exposures. These studies estimated percent uptake by measuring the TCE concentration in inhaled and alveolar-exhaled air, subtracting the ratio of the latter to the former from one, and multiplying by 100%. As noted previously, under nonsteady-state conditions these values vary with the duration of exposure. This variation is apparent in the broad range of values reported for the percent of TCE retained. For example, Soucek and Vlachova (1960) found that the percent of TCE retained varied from 58 to 70% in individuals exposed to 93 to 158 ppm TCE for 5 hours. Bartonicek (1962) reported 58% retention of TCE after a 5-hour exposure to 194 ppm. Nomiyama and Nomiyama (1971, 1974) found that approximately 35% of a dose of TCE was retained in individuals exposed to 252 to 380 ppm TCE for 2.7 hours. Monster et al. (1976) measured

the pulmonary uptake of TCE in six subjects exposed to 70 or 140 ppm TCE for 4 hours. Uptake was found to be rapid during the first 30 minutes of exposure, but after this time uptake became relatively constant and remained so for the duration of the experiment. Subjects exposed to 70 and 140 ppm TCE retained 5.6 ± 1.1 and 5.4 ± 0.28 mg TCE/ppm TCE in air. Thus, percent retention of TCE was not affected significantly by exposure concentration in this study. Monster et al. (1979) calculated the pulmonary uptake in 5 individuals exposed to 70 ppm TCE, 4 hours/day for 5 days. The average quantity of TCE retained during this exposure scenario was 450 ± 51 mg/day, which was approximately 44% ± 5.5% of the estimated, total, alveolarly-respired dose. Raabe (1988) exposed 4 people to ^{14}C-TCE at concentrations of 13 to 22 ppb and found an average steady-state fractional systemic uptake of 55% over a 2-hour period.

The amount of TCE taken up by the body is affected by differences in alveolar ventilation rate. During exercise or work, the alveolar ventilation rate increases and exposure to TCE under these conditions results in a greater uptake of TCE. When volunteers were exposed to 70 or 140 ppm TCE while under a workload (2 1/2 hours x 100 watts), the uptake of TCE increased to approximately 2.5 times what it was at rest (Monster et al., 1976). Åstrand and Ovrum (1976) also reported that the uptake of TCE doubled in subjects who were exposed to 100 or 200 ppm TCE in air while under a 50- to 150-watt workload compared to resting subjects similarly exposed.

DISTRIBUTION AND BIOACCUMULATION

Trichloroethylene diffuses readily through blood and into body tissues. Studies with experimental animals have measured TCE or its metabolites in most organs and tissues. A combination of experimental and theoretical data indicate that TCE becomes widely distributed within the human body.

Animal Studies

Barrett et al. (1939) exposed dogs and rabbits to water vapor saturated with TCE (1.2 g/L) for 25 to 28 minutes. Trichloroethylene and its metabolites were found in the blood, skeletal muscle, fat, liver, kidney, lungs, and heart of both species. The highest concentrations were measured in the adipose tissue of these animals (44 to 48 mg/100 g tissue). In both rabbits and dogs, the lowest quantities of TCE occurred in the muscle (4 to 7 mg/100 g tissue).

Trichloroethylene was detected in the cerebrum, cerebellum, blood, liver, lungs, and perirenal fat of rats 24 hours after the end of exposure to 200 ppm TCE, 6 hours/day for 4 days. The greatest amounts of TCE were measured in perirenal fat (65.9 to 75.4 nmol/g tissue) and the lowest amounts in liver (2.4 to 5.5 nmol/g tissue) (Savolainen et al., 1977).

Parchman and Magee (1982) found that some of the radioactivity from a dose of 10 to 1000 mg/kg ^{14}C-labeled TCE was covalently bound to liver protein of rats and mice. Low levels of radioactivity were also associated with the DNA fraction from both species. However, Parchman and Magee (1982) were unable to distinguish whether TCE or a metabolite was bound to DNA and whether the radioactivity in the DNA fraction was due to labeled DNA or to contaminating protein (see Section 4, Toxic Effects in Humans and Animals).

Male rats given 10, 100, or 1000 mg/kg of TCE orally 5 days/week for 6 weeks had measurable levels of TCE, TCA, and trichloroethanol in the blood, heart, testes, vas deferens, seminal vesicles, prostate, epididymus, adrenals, fat, liver, kidneys, muscle, lungs, and brain (Zenick et al., 1984). In general, blood and tissue levels increased in a dose-dependent manner, indicating that TCE and its 2 major metabolites accumulate in tissue. For example, animals that received 1000 mg/kg of TCE had 30 to 50% more TCA in their reproductive organs than the 100 mg/kg group. The concentration of trichloroethanol in the epididymus and vas deferens did not change substantially with dose. However, the concentration of trichloroethanol was 3 to 7 times higher in the testes, prostate, and seminal vesicles of high-dose animals than in animals that received 100 mg/kg TCE.

Pfaffenberger et al. (1980) dosed male rats by gavage with 1 to 10 mg/day of TCE for 25 days. The TCE levels were measured in fat and blood at 9 separate times during the treatment period. The TCE was detected at very low levels (<1.0 μg/L) in serum, while the amount of TCE present in adipose tissue depended on dose. Animals given 1.0 mg/day had an average concentration of 280 ng/g TCE in their fat, while those that received 10.0 mg/day had an average of 20,000 ng/g. Measurements were also made 3 and 6 days after dosing was discontinued. Animals from both treatment groups had an average of 1.0 ng/g of TCE in adipose tissue. Serum concentrations of TCE were <1.0 μg/L (1.0 mg/day TCE) and 6.0 μg/L (10.0 mg/day TCE). The values reported by Pfaffenberger et al. (1980) are mean values based on measurements from 12 animals taken over the course of the experiment. The authors noted considerable fluctuation in individual values and no steady increase in the

levels of TCE in blood or fat. However, the relatively large amounts of TCE measured in fat over the 25 days of treatment indicate that some accumulation of TCE occurs with continued exposure.

Human Studies

Trichloroethylene distribution depends on the compound's partition coefficient, volume of distribution, and tissue-specific perfusion rate, as well as on the volume of venous blood in equilibrium with a given tissue type. After accounting for these variables, the model of Fernandez et al. (1977), discussed in the subsection on Pulmonary Uptake, predicted that the body burden of TCE in the muscle and vessel-rich (well-perfused) tissue groups would increase only slightly with repeated inhalation exposure (100 ppm, 6 hours/day, 5 days/week). Because of TCE's high fat solubility and the limited perfusion of adipose tissue, TCE is eliminated slowly from fat and repeated exposure is expected to cause a progressive accumulation of TCE in this tissue. Under these conditions of 100 ppm, 6 hours/day, 5 days/week, TCE concentration is predicted by the Fernandez et al. model to reach equilibrium in adipose tissue in 5 to 7 days. Once adipose tissue becomes saturated, this model predicts that the concentration of TCE in fat will remain relatively constant as long as exposure concentration remains constant.

Bartonicek (1962) exposed 8 volunteers to 194 ppm TCE for 5 hours and measured the excretion of TCE and its metabolites TCA and trichloroethanol (TCEL) in feces, sweat, and saliva on day 3 postexposure and in blood and urine on days 2 to 22 postexposure. The metabolites were measured in feces, sweat, and saliva on the third day after exposure at average concentrations of approximately 18, 4.2, and 0.27 mg TCA + TCEL/100 mL, respectively. The metabolites excreted in feces represented about 8.4% of all measured metabolites excreted in feces and urine on day 3 after exposure. Metabolites in blood and urine decreased exponentially by a factor of about 10 from day 2 to day 22 after exposure. Intervening consumption of alcohol by the subjects appeared to influence the measurements of urinary metabolites by transiently increasing their concentration after consumption, particularly for TCEL.

METABOLIC PATHWAYS

Trichloroethylene is initially transformed by the cytochrome P-450-dependent mixed function oxygenases (MFO) to an intermediate

electrophilic epoxide, trichloroethylene oxide (TCE-oxide) (Byington and Leibman, 1965; Henschler, 1977; Bonse et al., 1975). Considerable speculation exists about this epoxide: it is not clear whether it is an obligate intermediate or if it exists in free form (available to bind to cellular macromolecules) (Henschler, 1977; Henschler et al., 1979; Miller and Guengerich, 1982; Miller and Guengerich, 1983). Most recently, as described below, evidence for a pathway in which TCE may react directly with glutathione has been published.

The TCE-oxide is subsequently metabolized by one of 4 paths (Figure 3-1). The predominant pathway is one in which TCE-oxide rearranges spontaneously to chloral. Chloral is then rapidly hydrated to form chloral hydrate. Chloral hydrate undergoes oxidation to trichloroacetic acid (TCA) and some of the TCA contributes to the formation of carbon dioxide (CO_2). Conjugates of TCA including TCA-glucuronide and TCA-coenzyme A (TCA-CoA) have also been identified. Alternatively, chloral hydrate can be reduced to trichloroethanol (TCEL). Most of the TCEL reacts with glucuronyl transferase to form trichloroethanol glucuronide (urochloralic acid) (Barrett et al., 1939, Powell, 1945; Powell, 1947; Butler, 1948; Butler, 1949; Byington and Leibman, 1965; Nomiyama and Nomiyama, 1971; Müller et al., 1972; Kimmerle and Eben, 1973a; Cole et al., 1975; Ikeda et al., 1980a; Hathaway, 1980; Miller and Guengerich, 1982, 1983; Parchman and Magee, 1982; Stott et al., 1982; Costa and Ivanetich, 1984; Green and Prout, 1985; Dekant et al., 1984; Prout et al., 1985). The metabolism of TCE recently has been reviewed by IARC (1979), WHO (1985), and the U.S. EPA (1985a).

Although TCA, TCEL, and trichloroethanol glucuronide (TCEL-G) are the principal metabolites formed, the isolation of a number of minor metabolites can only be explained by the presence of other metabolic pathways. Under certain conditions described below, TCE-oxide forms dichloroacetyl chloride, which rearranges to dichloroacetic acid (DCA) (Kline and Van Duuren, 1977; Hathaway, 1980; Dekant et al., 1984). The TCE-oxide can also undergo oxidation and hydrolysis to formic acid, glyoxylic acid, oxalic acid, CO_2, and carbon monoxide (CO). Monochloroacetic acid (MCA) has been occasionally recovered, but little is known about its formation (Bonse et al., 1975; Hathaway, 1980; Miller and Guengerich, 1982, 1983; Dekant et al., 1984; Fetz et al., 1978; Traylor et al., 1977; Soucek and Vlachova, 1960; Bartonicek, 1962; Ogata and Saeki, 1974; Green and Prout, 1985).

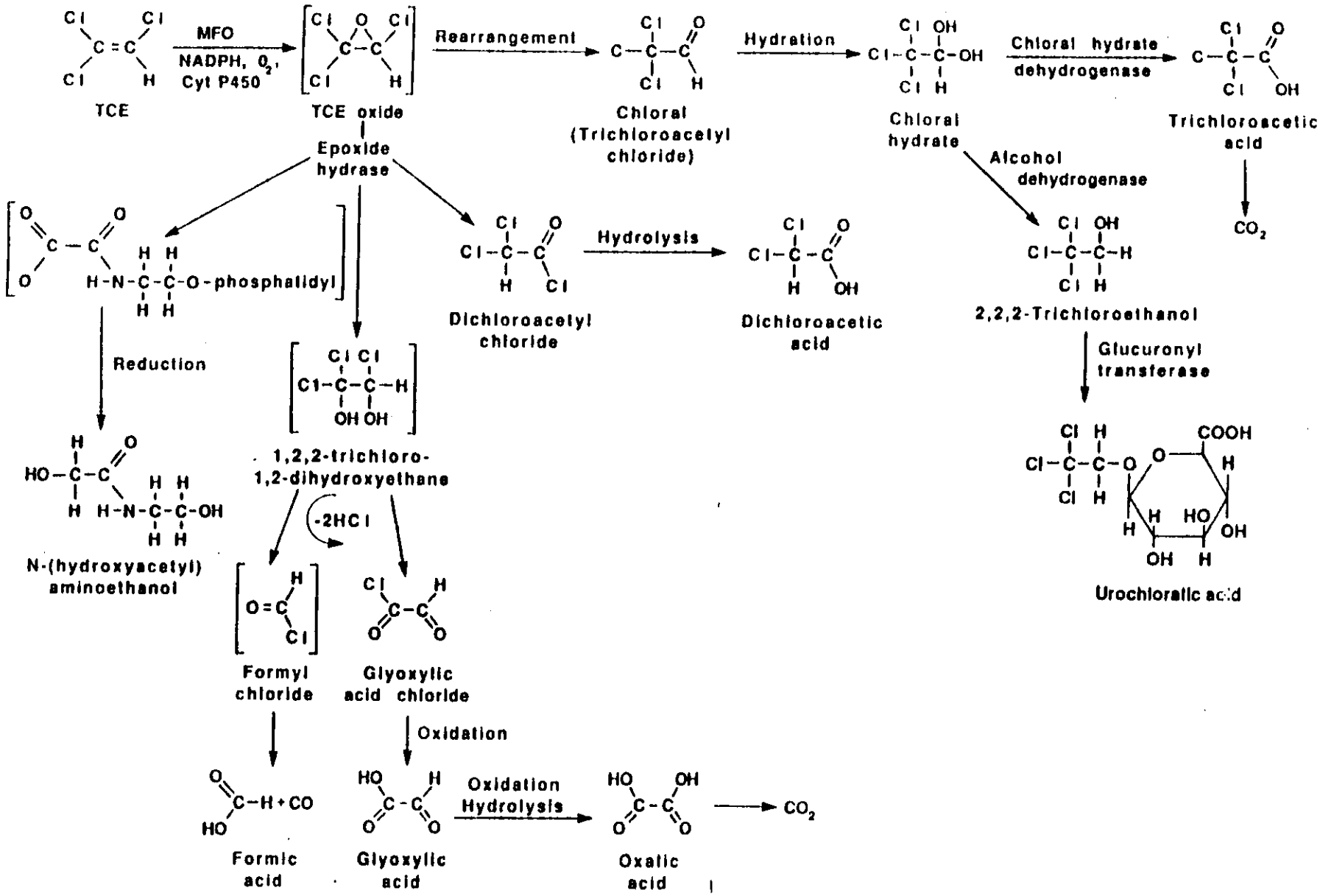


Figure 3-1. Metabolic pathways of TCE (brackets denote compounds that have not been isolated in vivo) (Hathaway, 1980; Miller and Guengerich, 1982; Dekant et al., 1984; Green and Prout, 1985).

Recent isolation of N-(hydroxyacetyl)-aminoethanol (HAAE) as a metabolite of TCE indicates the presence of a fourth and separate pathway for biotransformation of TCE-oxide. No details of HAAE formation have thus far been characterized (Dekant et al., 1984).

Mixed Function Oxygenases and Epoxide Formation

Powell (1945) and Byington and Leibman (1965) proposed that TCE is oxidized to an epoxide, trichloroethylene oxide (TCE-oxide), with the subsequent formation of chloral. This reaction is mediated by the MFO. A number of investigators studying TCE metabolism have used animals pretreated with phenobarbital or Aroclor 1254 (A1254), known inducers of hepatic MFO. If TCE is also a substrate for the MFO, then pretreatment with phenobarbital or A1254 should increase the metabolism of TCE. The involvement of the MFO in TCE metabolism has been confirmed in the studies of Leibman and McAllister (1967), Van Duuren and Banerjee (1976), Moslen et al. (1977a), Allemand et al. (1978), and Reynolds and Moslen (1977). In vitro studies of TCE metabolism have also verified that TCE is initially oxidized by the MFO (Kelley and Brown, 1971; Ikeda et al., 1980a; Miller and Guengerich, 1982, 1983).

Although administration of TCE typically induces hepatic MFO, Rouisse and Chakrabarti (1986) reported a dose-dependent decrease in MFO activity in rats given TCE orally. This decrease became significant ($p < 0.05$) at doses of 1.0 mL/kg. The authors suggested that the decrease in activity may have been due to the reaction of a metabolic intermediate with the MFO, which resulted in the formation of ligand complexes with cytochrome P-450, causing a loss of MFO activity.

Metabolism of a substance by the MFO can also be demonstrated by the binding of the substance to oxidized cytochrome P-450. This binding can be observed as a shift in the light-absorption characteristics of the microsomes known as a "type I spectral change." Pelkonen and Vainio (1975) showed that chlorinated ethanes produce a type I spectral change in rat liver microsomal preparations. Kelley and Brown (1974) and Uehleke et al. (1977) also observed a type I spectral change following the addition of TCE or TCE-oxide to microsomal preparations from rats and rabbits.

While studies of enzyme induction and spectral binding confirm the involvement of the MFO in TCE metabolism, they have not identified the intermediate produced. Chlorinated ethylenes are commonly metabolized to

epoxides and it has been proposed that TCE oxidation produces TCE-oxide (Powell, 1945; Byington and Leibman, 1965; Leibman and Ortiz, 1977; Bonse et al., 1975; Henschler, 1977). While evidence for TCE epoxidation in vivo is largely indirect, initial epoxide formation is believed to offer a plausible explanation for the metabolic products that have been recovered. The chemical reactivity of the postulated in vitro TCE-oxide might be responsible for some types of TCE-induced toxicity, provided that the TCE-oxide exists for a time long enough for it to migrate from its cellular sites of formation, primarily in endoplasmic reticulum and to some extent in mitochondria (<5%) and in the nuclear envelope (<1%) (Pelkonen and Nebert, 1982), to critical macromolecules, such as nuclear DNA, located elsewhere in the cell.

Van Duuren and Banerjee (1976) showed that, when labeled TCE was incubated with liver microsomes, ^{14}C became covalently bound to protein, demonstrating the formation of a reactive intermediate capable of binding with critical macromolecules in this in vitro assay. Furthermore, binding was increased by the addition of an inhibitor of epoxide hydratase, suggesting that the reactive intermediate observed was an epoxide.

Several attempts have been made to demonstrate that chloral, a known metabolite of TCE, can be produced from TCE-oxide. If it could be shown that chemically synthesized TCE-oxide forms chloral, this would provide inferential evidence that TCE-oxide is actually an in vivo intermediate in the formation of chloral. Henschler et al. (1979) and Miller and Guengerich (1982) synthesized TCE-oxide and studied the in vitro degradation products. Both groups recovered DCA, CO, formic acid, and glyoxylic acid, but no chloral. However, Bonse et al. (1975) showed that electrophilic compounds such as $AlCl_3$, BF_3, and $FeCl_3$ can induce rearrangement of TCE-oxide to chloral in vitro. They postulated that, in vivo, the electrophilic iron ion, Fe^{3+}, in cytochrome P-450 may catalyze the transformation of TCE-oxide to chloral, i.e., that TCE-oxide only rearranges to chloral in the presence of metals.

Miller and Guengerich (1982) incubated TCE-oxide in the presence or absence of Fe^{2+} or Fe^{3+} salts, with a number of different heme proteins. Experiments were conducted in both aqueous and nonaqueous solutions. Rearrangement of the epoxide to chloral was observed only in nonaqueous solutions; chloral was not formed in aqueous solutions even when purified cytochrome P-450 was present. However, a subsequent in vitro study found that rat and mouse microsomes were able to metabolize TCE to TCE-oxide, chloral, glyoxylic acid, and CO. The TCE-oxide was identified by trapping with p-nitrobenzyl pyridine (Miller and Guengerich, 1983).

On the basis of their data, Miller and Guengerich (1982; 1983) concluded that formation of TCE-oxide was not an obligatory step in the metabolism of TCE to chloral. They proposed that chlorine migration can occur within a transitional, oxygenated TCE-P-450 intermediate. This transitional complex can rearrange without the formation of free epoxide, leading directly to the production of several different metabolites including chloral. Direct oxidation of TCE to chloral has also been proposed by Van Dyke and co-workers (Van Dyke and Chenoweth, 1965; Van Dyke, 1977).

Chloral and Chloral Hydrate

The formation of the major urinary metabolites TCE, TCEL, and TCEL-G are explained by the production of chloral as an intermediate, following the initial oxidation of TCE (see Figure 3-1). Chloral is rapidly hydrated to form chloral hydrate. Isolation and characterization of chloral hydrate as a metabolite of TCE was hindered by its short half-life of 10 to 40 minutes (Marshall and Owens, 1954; Cole et al., 1975). In 1965, Byington and Leibman were able to demonstrate that chloral hydrate is actually a product of TCE metabolism.

Chloral hydrate has been recovered as a metabolite of TCE in vitro and in vivo; it has been isolated from the urine of humans, rats, mice, and dogs (Butler, 1948; Byington and Leibman, 1965; Leibman and McAllister, 1967; Kimmerle and Eben, 1973a; Cole et al., 1975; Bonse and Henschler, 1976; Ikeda et al., 1980a; Miller and Guengerich, 1982, 1983; Costa and Ivanetich, 1984; Prout et al., 1985). Chloral hydrate is not a final metabolite of TCE, but undergoes oxidation to TCA or reduction to TCEL.

Trichloroethanol and Trichloroethanol Glucuronide

Alcohol dehydrogenase catalyzes the conversion of chloral hydrate to TCEL (Butler, 1948, 1949; Marshall and Owens, 1954; Friedman and Cooper, 1960; Sellers et al., 1972). The TCEL has been isolated in the urine of humans, dogs, rats, and mice exposed to TCE (Butler, 1949; Nomiyama and Nomiyama, 1971; Müller et al., 1972; Ikeda et al., 1980a; Dekant et al., 1984; Green and Prout, 1985; Prout et al., 1985).

Urinary metabolite profiles typically reveal that the majority of TCEL is conjugated to glucuronic acid. The binding is probably mediated by a

glucuronyl transferase (Butler, 1949; Müller et al., 1972; Dekant et al., 1984; Green and Prout, 1985).

Trichloroacetic Acid

The enzyme(s) responsible for the conversion of chloral hydrate to TCA have not been thoroughly characterized. Although structural similarities between chloral and acetaldehyde have led to the proposal that chloral or chloral hydrate is oxidized by acetaldehyde dehydrogenase, the actual role of this enzyme in the formation of TCA is not clear (Cooper and Friedman, 1958; Kraemer and Dietrich, 1968; Blair and Bodley, 1969; Sellers et al., 1972; Tottmar et al., 1973; Grunett, 1973).

Trichloroacetic acid has been identified as a metabolite of TCE in blood and urine of humans, chimpanzees, mice, rats, and dogs (Barrett et al., 1939;Powell, 1945; Powell, 1947; Butler, 1948; Butler, 1949; Nomiyama and Nomiyama, 1971; Müller et al. 1972; Ikeda et al., 1980a; Müller et al., 1982; Dekant et al., 1984; Green and Prout, 1985; Prout et al., 1985). A glucuronide conjugate of TCA has been isolated from the urine of chimpanzees given TCE (Müller et al., 1982). The TCEL, either free or conjugated to glucuronic acid, and TCA are the major metabolites of TCE in all species studied to date.

Data on human production of TCA and TCEL come exclusively from inhalation-exposure experiments. Table 3-1 lists the relative percentage of these 2 compounds in the urine of humans exposed to TCE by inhalation. Although the data in this table are not exhaustive, these values indicate that the relative importance of TCA and TCEL are minimally affected by exposure concentration or duration of exposure (Soucek and Vlachova, 1960; Bartonicek, 1962; Bartonicek and Teisinger, 1962; Nomiyama and Nomiyama, 1971; Ertle et al., 1972; Kimmerle and Eben, 1973b; Monster et al., 1976; Monster et al., 1979).

The percentage of urinary metabolites recovered as TCA and TCEL in rodents exposed to TCE, as well as the relative percentage of these metabolites, are given in Table 3-2. The relative amounts of TCA and TCEL produced as TCE metabolites are fairly consistent in mice and rats, regardless of exposure concentration or route (Dekant and Henschler, 1983; Dekant et al., 1984; Green and Prout, 1985). However, the data of Green and Prout (1985) indicate that the duration of exposure may affect the production of these metabolites. When mice were given a single oral dose of 1000 mg/kg TCE, the relative percentages

Table 3-1. Relative Percentage of Trichloroacetic Acid (TCA) and Trichloroethanol (TCEL) in the Urine of Humans Following Inhalation Exposure to TCE.

Number of Individuals	Exposure Concentration	Exposure Duration	Follow-up Period	Relative Percent TCA	Relative Percent TCEL	References
4	40 ppm	4 h	8 d	37.0	63.0	Kimmerle and Eben, 1973b
4	48 ppm	5 h/d for 5 d	12 d	35.0	65.0	Kimmerle and Eben, 1973b
4	1 mg/L (46.5 ppm)	5 h	16 to 21 d	21.0[a]	79.0[a]	Bartonicek and Teisinger, 1962
4	1 mg/L (46.5 ppm)	5 h	16 to 21 d	39.0	61.0	Bartonicek and Teisinger, 1962
6	50 ppm	6 h/d for 5 d	24 h	33.0	67.0	Ertle et al., 1972
5	70 ppm	4 h/d for 5 d	6 d	36.0	64.0	Monster et al., 1979
4	70 to 140 ppm	4 h	66 h	33.0	67.0	Monster et al., 1976
5	500-850 ug/L (93 to 158 ppm)	5 h	112 h	28.0	72.0	Soucek and Vlachova, 1960
5	100 ppm	5 h	60 h	32.0	68.0	Muller et al., 1974
8	1042 ug/L (194 ppm)	5 h	3 wk	42.0	59.0	Bartonicek, 1962
10	350-380 ppm	160 min	6 d	46.0	54.0	Nomiyama and Nomiyama, 1971

[a] Individuals were pretreated with 3 to 3.5 g disulfiram.

of TCA and TCEL were 7.3 and 92.7%, respectively. When dosing was continued for 180 days, the relative percentage of TCA increased to 20.5%, while TCEL decreased to 79.5%.

Minor Metabolites

The presence of a number of metabolites of TCE cannot be explained by the formation of chloral as an intermediate step. Carbon dioxide (CO_2), CO, MCA, DCA, oxalic acid, and N-(hydroxyacetyl)-aminoethanol (HAAE) have been identified in small amounts from animals and/or humans exposed to TCE (Figure 3-1). As with any substance that is a normal product of human metabolism (such as CO_2, CO, and oxalic acid), there is no practical way to determine what proportion of these metabolites in humans is due to the biotransformation of TCE. Table 3-3 lists the percentage of administered dose recovered as each of these metabolites in rats and mice.

Dichloroacetic acid (DCA) has been identified in the urine of rats and mice by Hathaway (1980), Dekant et al. (1984), and Green and Prout (1985) (Figure 3-1). These investigators proposed that DCA is formed only when the oxidative capacity of the MFO is exceeded. Saturation of this enzyme system would result in the availability of small amounts of the free epoxide. The epoxide could then rearrange, with migration of H^+, to form dichloroacetyl chloride; reaction of the acetyl chloride with water produces DCA.

Dekant et al. (1984) identified oxalic acid as a urinary metabolite of TCE in mice and rats. Oxalic acid may be formed following cleavage of the epoxide ring to form a vicinal diol. This diol is unstable and can spontaneously eliminate water, thereby forming glyoxylic acid chloride. Hydrolysis and oxidation of glyoxylic acid chloride result in the formation of glyoxylic acid. Glyoxylic acid has been produced in vitro from incubation of rat and mouse microsomes with TCE-oxide (Miller and Guengerich, 1982). Subsequent oxidation of glyoxylic acid leads to the formation of oxalic acid (Dekant et al., 1984).

Formic acid and CO have been recovered as metabolites of TCE primarily from in vitro systems (Fetz et al., 1978; Traylor et al., 1977; Miller and Guengerich, 1982). However, Green and Prout (1985) reported traces of CO in the breath of rats and mice after treatment with radiolabeled TCE. Miller and Guengerich (1982) proposed that cleavage of the C-C bond of the vicinal diol formed from TCE-oxide could explain the production of formic acid and CO.

Table 3-2.
Recovery and Relative Percentage of Trichloroacetic Acid (TCA)
and Trichloroethanol (TCEL) in the Urine of Rodents Exposed to TCE.

Species (strain)	Number of Animals	Route of Administration	Dose or Concentration	Dose Regimen	Follow-up Period	Percentage of Urinary metabolites recovered as: TCA (relative percentage)[a]	TCEL[b]	Reference
Mouse (B6C3F1)	4	Gavage	10 mg/kg	Single administration	24 h	12.2 (12.6)	84.5 (87.4)	Green and Prout, 1985
Mouse (NMRI)	3	Gavage	200 mg/kg	Single administration	72 h	0.08 (0.10)	71.8 (99.9)	Dekant et al., 1984
Mouse (B6C3F1)	4	Gavage	500 mg/kg	Single administration	24 h	7.2 (7.3)	91.1 (92.7)	Green and Prout, 1985
Mouse (B6C3F1)	4	Gavage	1000 mg/kg	Single administration	24 h	7.0 (7.3)	88.5 (92.7)	ibid.
Mouse (B6C3F1)	4	Gavage	1000 mg/kg	Daily for 10 days	24 h	15.6 (16.0)	81.9 (84.0)	ibid.
Mouse (B6C3F1)	4	Gavage	1000 mg/kg	Daily for 180 days	24 h	19.9 (20.5)	77.4 (79.5)	ibid.
Mouse (B6C3F1)	4	Gavage	2000 mg/kg	Single administration	24 h	7.2 (7.4)	90.1 (92.6)	ibid.
Rat (OM[c])	4	Gavage	10 mg/kg	Single administration	24 h	5.8 (6.0)	91.1 (94.0)	ibid.
Rat (N/A[c])	N/A[d]	Gavage	200 mg/kg	Single administration	72 h	0.97 (1.9)	50.1 (98.1)	Dekant and Henschler, 1983
Rat (Wistar)	3	Gavage	200 mg/kg	Single administration	72 h	6.3 (17.2)	30.3 (82.8)	Dekant et al., 1984
Rat (OM)[c]	4	Gavage	500 mg/kg	Single administration	24 h	6.3 (6.5)	90.1 (93.5)	Green and Prout, 1985

Table 3-2 (Continued).

Species (strain)	Number of Animals	Route of Administration	Dose or Concentration	Dose Regimen	Follow-up Period	Percentage of Urinary metabolites recovered as: TCA (relative percentage)	TCEL[a] (relative percentage)[b]	Reference
Rat (Wistar)	N/A[d]	Gavage	702 to 1676 mg/kg	Single administration	72 h	1.0 to 5.0 (N/A)	N/A	Daniel, 1963
Rat (OM[c])	4	Gavage	1000 mg/kg	Single administration	24 h	8.3 (8.6)	88.4 (91.4)	Green and Prout, 1985
Rat (SD[e])	6	IP	.25 mL/kg	Single administration	24 h	5.1f (24.6)	15.5 (75.4)	ibid.
Rat (SD[e])	6	IP	2.0 mL/kg	Single administration	24 h	1.9f (18.9)	8.1 (81.1)	ibid.
Rat (OM[c])	4	Gavage	2000 mg/kg	Single administration	24 h	7.0 (7.1)	91.9 (91.9)	ibid.
Rat (N/A)[d]	4	Inhalation	57 to 82 mg/L (10,607 to 15,259 ppm)	37 min	7 d	1.2 to 3.9	N/A	Forssmann and Holmquist, 1953
Rat (N/A)[d]	4	Inhalation	73 to 86 mg/L (13,584 to 16,003 ppm)	60 min	7 d	3.2 to 7.8	N/A	ibid.

[a] Values represent recovery of free TCEL and/or TCEL-glucuronide as a percent of total metabolites measured in urine.
[b] Values in parentheses represent the percentage of TCA and TCEL measured in urine relative to the amount of [TCA + TCEL(bound and free)] measured.
[c] OM = Osborne-Mendel.
[d] N/A = not available.
[e] SD = Sprague-Dawley.
[f] Animals were pretreated with phenobarbital.

Table 3-3. Recovery of Minor Metabolites of TCE in the Urine and Exhaled Air of Rodents.

Species	Strain[a]	Number of Animals	Route of Administration	Dose or Concentration	Dose Regimen[b]	Follow-up Period	Percent of Administered Dose Recovered as:[c] CO_2	CO	DCA	Oxalic Acid	HAAE	MCA	Reference
Mouse	B6C3F1	4	Gavage	10 mg/kg	SA	72 h	11.7						Prout et al., 1985
Mouse	B6C3F1	4	Gavage	10 to 2000 mg/kg	SA	24 h		<0.10	<1.0			<1.0	Green and Prout, 1985
Mouse	NMRI	3	Gavage	200 mg/kg	SA	72 h	6.0		0.80	5.3	3.1		Dekant et al., 1984
Mouse	B6C3F1	4	Gavage	500 mg/kg	SA	72 h	8.9						Prout et al., 1985
Mouse	B6C3F1	4	Gavage	1000 mg/kg	SA	92 h	7.6						ibid.
Mouse	B6C3F1	4	Gavage	1000 mg/kg	daily for 10 d	24 h	6.3						Green and Prout, 1985
Mouse	B6C3F1	4	Gavage	1000 mg/kg	daily for 180 d	24 h	2.3						ibid.
Mouse	B6C3F1	4	Gavage	2000 mg/kg	5 d/wk for 4 wk	48 h	3.9						Mitoma et al., 1985
Mouse	B6C3F1	4	Gavage	2000 mg/kg	SA	72 h	7.8						Prout et al., 1985
Mouse	B6C3F1	4	Inhalation	10 ppm	6 h (SA)	50 h	9.4						Stott et al., 1982
Mouse	B6C3F1	4	Inhalation	600 ppm	6 h (SA)	50 h	9.5						ibid.
Rat	OM	4	Gavage	10 mg/kg	SA	72 h	10.2						Prout et al., 1985

Table 3-3. (Continued).

Table 3-3. Recovery of Minor Metabolites of TCE in the Urine and Exhaled Air of Rodents.

Species	Strain[a]	Number of Animals	Route of Administration	Dose or Concentration	Dose Regimen[b]	Follow-up Period	Percent of Administered Dose Recovered as:[c] CO_2	CO	DCA	Oxalic Acid	HAAE	MCA	Reference
Rat	OM	4	Gavage	10 to 2000 mg/kg	SA	24 h		<0.10	<1.0			<1.0	Green and Prout, 1985
Rat	N/A	N/A	Gavage	200 mg/kg	SA	72	0.5		2.0	0.7	6.8		Dekant and Henschler, 1983
Rat	W	2	Gavage	200 mg/kg	SA	72 h	1.9		0.8	0.5	3.0		Dekant et al., 1984
Rat	OM	4	Gavage	500 mg/kg	SA	72 h	3.2						Prout et al., 1985
Rat	OM	4	Gavage	1000 mg/kg	SA	72 h	2.9						ibid.
Rat	W	N/A	Gavage	702 to 1676 mg/kg	SA	72 h	1.0 to 15.0						Daniel, 1963
Rat	OM	4	Gavage	1300 mg/kg	5 d/wk for 4 wk	48 h	1.8						Mitoma et al., 1985
Rat	OM	4	Gavage	2000 mg/kg	SA	72 h	1.4						Prout et al., 1985
Rat	OM	4	Inhalation	10 ppm	6 h (SA)	50 h	4.8						Stott et al., 1982
Rat	OM	4	Inhalation	600 ppm	6 h (SA)	50 h	2.9						ibid.

[a] OM = Osborne Mendel, N/A = Not available, W = Wistar.
[b] SA = Single administration.
[c] DCA = dichloroacetic acid, HAAE = N-(hydroxyacetyl)-aminoethanol, MCA = monochloroacetic acid.

Balance studies of metabolism of radiolabeled TCE have recovered radiolabeled CO_2 in expired air of rats and mice (Stott et al., 1982; Parchman and Magee, 1982; Dekant et al., 1984; Green and Prout, 1985). Henschler and Hoos (1982) suggested that CO_2 may be formed from the decarboxylation of di- or trichloroacetic acid. An alternate scheme involves the production of CO_2 from formic or glyoxylic acid (Miller and Guengerich, 1982). However, Green and Prout (1985) demonstrated that at least some of the CO_2 recovered was derived from TCA. They suggested that TCA forms an ester with coenzyme A (CoA) and the latter is then degraded to CO_2.

Dekant et al. (1984, 1986a) identified HAAE as a metabolite of TCE in the urine of humans, rats, and mice. The mechanism(s) of its formation are entirely speculative at this point. It may be formed by the reaction of an oxidized intermediate with ethanolamine or phosphatidylethanolamine. This hypothesis is based on the observation of Reichert et al. (1979) and Cohen et al. (1975), who identified ethanolamine adducts as the products of dichloroethylene and halothane metabolism.

Trace amounts of monochloroacetic acid have been recovered in the urine of humans, rats, mice, and rabbits given TCE (Soucek and Vlachova, 1960; Bartonicek, 1962; Ogata and Saeki, 1974; Green and Prout, 1985). Chloroform was measured in tissue and fluid from rats exposed to TCE (Müller et al., 1972; Pfaffenberger et al., 1980). However, the U.S. EPA (1985a) noted that chloroform was probably an artifact of the experimental techniques used.

Trichloroethlyene depletes hepatic glutathione (GSH) (Allemand et al., 1978; Moslen et al., 1977a; Reynolds and Moslen, 1977; Rouisse and Chakrabarti, 1986). This indicates that a separate metabolic path may exist for TCE that involves GSH and GSH-transferases. A GSH metabolite of TCE, S-1,2-dichlorovinyl-N-acetyl-cysteine, has been identified in rat urine collected for 72 hours after a single oral dose of 400 mg/kg ^{14}C-trichloroethylene (Dekant et al., 1986b). Cytosolic enzymes from rat liver and kidney can further metabolize the compound to dichlorovinylcysteine (Vamvakas et al., 1987). The quantitative contribution of this pathway to TCE metabolism has not been established.

METABOLISM AND EXCRETION KINETICS

Absorbed TCE is eliminated both by pulmonary release of unchanged TCE and by metabolic transformation into other products that are primarily excreted in

urine (See subsection on Metabolic Pathways above.) There is a large amount of data on the kinetics of TCE metabolism and excretion in animals and humans, perhaps more than for any other volatile chlorinated organic compound. These data are reviewed extensively in the U.S. EPA (1985a) health-risk assessment document for TCE. A summary, update, and critique of that EPA review of animal and human data follows, supplemented by a description of key data and pharmacokinetic analysis that provide the basis for the calculation of metabolized TCE doses as a function of applied doses to bioassay animals and humans in the context of carcinogenic potency extrapolation for TCE (Section 5).

Animals

Early experiments on rats exposed to TCE by inhalation established that TCA is a major TCE metabolite excreted in urine. These studies showed unmetabolized TCE is excreted by exhalation through the lungs (Forssmann and Holmquest, 1953). Kimmerle and Eben (1973a) studied excretion kinetics of TCE in SPF-Wistar-II rats exposed to TCE by acute and subchronic inhalation. - After rats were exposed to 49, 175, and 330 ppm TCE for 4 hours, they showed an exponential decrease in TCE concentration in expired breath over an 8-hour observation period, as well as in amounts of TCA and trichloroethanol excreted in urine over a 4-day collection period. During subchronic exposure to 55 ppm TCE for 8 hours/day, 5 days/week over a 14-week period, urinary excretion of TCE metabolites averaged approximately 1.5 mg/kg-day. The metabolites TCEL and chloral hydrate were found at fairly constant levels in blood while TCE could not be detected in blood. In Wistar rats given 3 to 15 mg/kg TCE by intravenous injection, Withey and Collins (1980) observed TCE concentrations to decrease in blood and perirenal fat with half-life ($t_{1/2}$) values of about 4 minutes and 3.6 hours, respectively.

Trichloroethylene elimination from blood was compared in mice and rats given a single oral dose of 1000 mg/kg TCE in corn oil (Prout et al., 1985). Peak blood concentrations of TCE occurred at 1 hour and 3 hours for mice and rats, respectively, with corresponding decay ($t_{1/2}$) values of about >1 hour and 3 hours, respectively. While removal of TCE from mouse blood occurred more rapidly than from rat blood, blood concentrations of the TCE metabolite TCA were about 10 times greater in mice than in rats studied over a 48-hour postexposure observation period. Since TCA is cytotoxic and possibly

carcinogenic itself (Elcombe, 1985), the differential ability of mice and rats to convert TCE to TCA has been proposed to explain the differential hepatotoxicity and hepatocarcinogenicity of TCE observed in these species (Stott et al., 1982; Buben and O'Flaherty, 1985; Prout et al., 1985).

The pharmacokinetics of inhaled TCE was investigated in Wistar rats (Filser and Bolt, 1979) and Fischer 344 rats (Andersen et al., 1980). The depletion of various initial concentrations of TCE in a closed chamber was followed to estimate uptake and metabolism. The TCE metabolism in Wistar rats was found to saturate above 65 ppm; the maximum rate (V_{max}) of metabolic elimination was observed to be 28 mg/kg-hour. In F344 rats, TCE metabolism was also found to be dose-dependent, saturating above 500 to 1000 ppm where a V_{max} of 24.3 mg/kg-hour was observed. A close agreement exists for estimates of V_{max}, but not saturation concentrations, for Wistar and F344 rats.

Mass-balance studies using radiolabeled TCE have been used to study the nature and extent of TCE metabolism in rodents and primates. Daniel (1963) dosed rats by gavage with 40 to 60 mg/kg of TCE liquid and observed respiratory elimination of 80% of the dose ($t_{1/2}$ = 5 hours) and excretory elimination of the remainder as urinary metabolites. More recent gavage studies, discussed below, using TCE in a corn oil vehicle demonstrate a much greater degree of TCE metabolism in rodents. Müller et al. (1982) gave intramuscular injections of 50 mg/kg TCE to chimpanzees, baboons, and Rhesus monkeys and observed that urinary plus fecal elimination of the (presumably metabolized) dose amounted to 40 to 60%, 11 to 28%, and 7 to 40%, respectively, of the administered dose.

Other recent studies of TCE metabolism in mice and rats provide further information on the extent of TCE metabolism in these species (Buben and O'Flaherty, 1985; Stott et al., 1982; Parchman and Magee, 1982; Dekant et al., 1984; Mitoma et al., 1985; Prout et al., 1985; Rouisse and Chakrabarti, 1986). The extent of TCE metabolism in mice and rats measured in these studies is summarized in Tables 3-4 and 3-5, respectively, in terms of the total amount of TCE metabolized and the percent of this amount excreted in the form of urinary metabolites. Most of these studies are mass-balance studies, while the studies of Buben and O'Flaherty (1985) and Rouisse and Chakrabarti (1986) reported only urinary metabolites of TCE in mice and rats, respectively.

The results of the studies summarized in Table 3-4 indicate that total TCE metabolism in mice is not saturated at single gavage doses below 2000 mg/kg. Furthermore, the relationship between applied and metabolized dose for mice appears to be approximately linear over most of the dose range below this point

Table 3-4. Trichloroethylene Metabolism in Mice.

Study	Strain Sex	Vehicle	Recovery Period (h)	Applied Dose, D	Total metabolized: Amount, M (mg/kg)	Total metabolized: M as a Percent of D (%)	Percent of M Excreted in Urine (%)
Buben and O'Flaherty, 1985	Swiss-Cox male	Corn oil	24	100 mg/kg[a]	50	50	70[b]
				200 mg/kg	200	100	70
				400 mg/kg	250	62	70
				800 mg/kg	450	56	70
				1600 mg/kg	1000	62	70
				2400 mg/kg	1300	54	70
				3200 mg/kg	1200	38	70
Parchman and Magee, 1982	B6C3F1 male	Corn oil	6	2 mg/kg[c]	1.8	88	25
				10 mg/kg	8.87	88.7	23.2
				250 mg/kg	163	65.2	58.0
Dekant et al., 1984	NMRI female	Corn oil	72	200 mg/kg[d]	178	89	85.6
Mitoma et al., 1985	B6C3F1 male	Corn oil	48	500 mg/kg[e]	454	90.8	--
				2000 mg/kg	1630	81.5	81.7
Prout et al., 1985	B6C3F1 male	Corn oil	72	10 mg/kg[d]	9.32	93.2	63.7
				500 mg/kg	458	91.2	69.8
				1000 mg/kg	777	77.7	59.7
				2000 mg/kg	1566	78.3	60.7
	Swiss-Webster male	Corn oil	72	10 mg/kg[d]	9.51	95.1	62.2
				500 mg/kg	432	86.4	64.5
				1000 mg/kg	792	79.2	70.2

Table 3-4. (Continued)

Study	Strain Sex	Vehicle	Recovery Period (h)	Applied Dose, D	Total metabolized: Amount, M (mg/kg)	Total metabolized: M as a Percent of D (%)	Percent of M Excreted in Urine (%)
Stott et al., 1982	B6C3F1 male	Inhalation	50	10 ppm-6 h	10.2	(52.9[f])	74.8
				600 ppm-6 h	402.3	(34.8[f])	74.4

[a] Administered by gavage, 5 days/week over 6 weeks

[b] Estimated from percent M excreted as urinary metabolites from the other studies listed and applied to urinary metabolite levels (M_U) presented graphically in Buben and O'Flaherty (1985) to derive corresponding values of M, here in equivalent mg/kg, assuming D is applied continuously (i.e., 7 days/week), using the relation $M_U = M \times 0.70(5/7)$.

[c] Administered as single intraperitoneal injection.

[d] Administered as single gavage dose.

[e] Administered as single gavage dose, following similar dosing 5 days/week for 4 weeks.

[f] Assumes a mouse respiratory ventilation rate of $[0.0345(W/25\ g)^{2/3} m^3/day] = 0.033/24\ m^3/hour$, following Anderson et al. (1983), where W = 23 g. Use of a lower, alveolar ventilation rate would produce a proportionally increased value for the percentage of D metabolized. For example, an assumed rate of 1.0 L/hour yields 72.7% and 47.8% as the metabolized percentages of the applied doses 10 and 600 ppm-6 hours, respectively.

Table 3-5. Trichloroethylene Metabolism in Rats.

Study	Strain Sex	Vehicle	Recovery Period (h)	Applied Dose, D	Total metabolized: Amount, M (mg/kg)	Total metabolized: M as a Percent of D (%)	Percent of M Excreted in Urine (%)
Parchman and Magee, 1982	Sprague-Dawley male	Corn oil	6	0.1 mg/kg[a]	0.087	87	49
				10 mg/kg[a,b]	9.7	97	52
				100 mg/kg[a,b]	81	81	65
				500 mg/kg[a,b]	290	58	72
				1000 mg/kg[a,b]	460	46	59
			24	1.16 mg/kg[a,b]	1.02	88.3	90.6
Dekant et al., 1984	Wistar female	Corn oil	72	200 mg/kg[c]	96	48.0	85.8
Mitoma et al., 1985	Osborne-Mendel male	Corn oil	48	325 mg/kg[d]	126	38.9	----
				1300 mg/kg	391	30.1	71.9
Prout et al., 1985	Osborne-Mendel male	Corn oil	72	10 mg/kg[c]	9.63	96.3	72.1
				500 mg/kg	277	55.4	78.7
				1000 mg/kg	395	39.5	78.7
				2000 mg/kg	404	20.2	69.3
	Wistar male	Corn oil	72	10 mg/kg[c]	10	100	58
				500 mg/kg	244	48.8	54.3
				1000 mg/kg	432	43.2	43.3
Rouisse and Chakrabarti, 1986	Sprague-Dawley male	Corn oil	24	370 mg/kg[a,e,f]	120	32	65[f]
				730 mg/kg	230	31	65
				1100 mg/kg	260	23	65

Table 3-5. (Continued)

Study	Strain Sex	Vehicle	Recovery Period (h)	Applied Dose, D	Total metabolized: Amount, M (mg/kg)	Total metabolized: M as a Percent of D (%)	Percent of M Excreted in Urine (%)
Kimmerle and Eben, 1973[a]	SPF-Wistar-II male	Inhalation	96	49 ppm-4 h	5.63[g]	(19.1[f,g])	65.0[g]
				175 ppm-4 h	14.7	(14.0)	65.0
				330 ppm-4 h	23.7	(11.9)	65.0
			16	55 ppm-8 h	18.4	(27.8)	65.0
Stott et al., 1982	Osborne-Mendel male	Inhalation	50	10 ppm-6 h	4.60	(46.0[i])	64.6
				600 ppm-6 h	111	(18.5)	70.4

[a] Administered as single intraperitoneal (ip) injection.

[b] Rats pretreated with one ip injection of 70 mg/kg phenobarbital per day for 5 days.

[c] Administered as single gavage dose.

[d] Administered as single gavage dose, following similar dosing 5 days/week for 4 weeks.

[e] Rats pretreated with one ip injection of 50 mg/kg phenobarbital per day for 7 days.

[f] Estimated from percent excreted as urinary metabolites from the other studies listed, and applied to data of Rouisse and Chakrabarti (1986) on percent applied dose excreted as urinary metabolites in order to derive the metabolized dose values (amount M and percent of D) given here for this study.

[g] Rats assumed to weigh W = 0.30 kg. M in TCE-equivalent mg/kg of total metabolites, assuming 65% of total is excreted in urine.

[h] Assumes a rat respiratory ventilation rate of $[0.105(W/0.113\ kg)^{2/3}\ m^3/day] = 0.164/24\ m^3/hour$, following Anderson et al. (1983), where W = 0.22 kg. Use of a lower, alveolar ventilation rate would produce a proportionally increased value for the percent of D metabolized. For example, an assumed rate of 4.6 L/hour yields 68.2% and 27.4% as the metabolized percents of the applied doses 10 and 600 ppm-6 hours, respectively.

[i] Administered 5 days/week over 14 weeks.

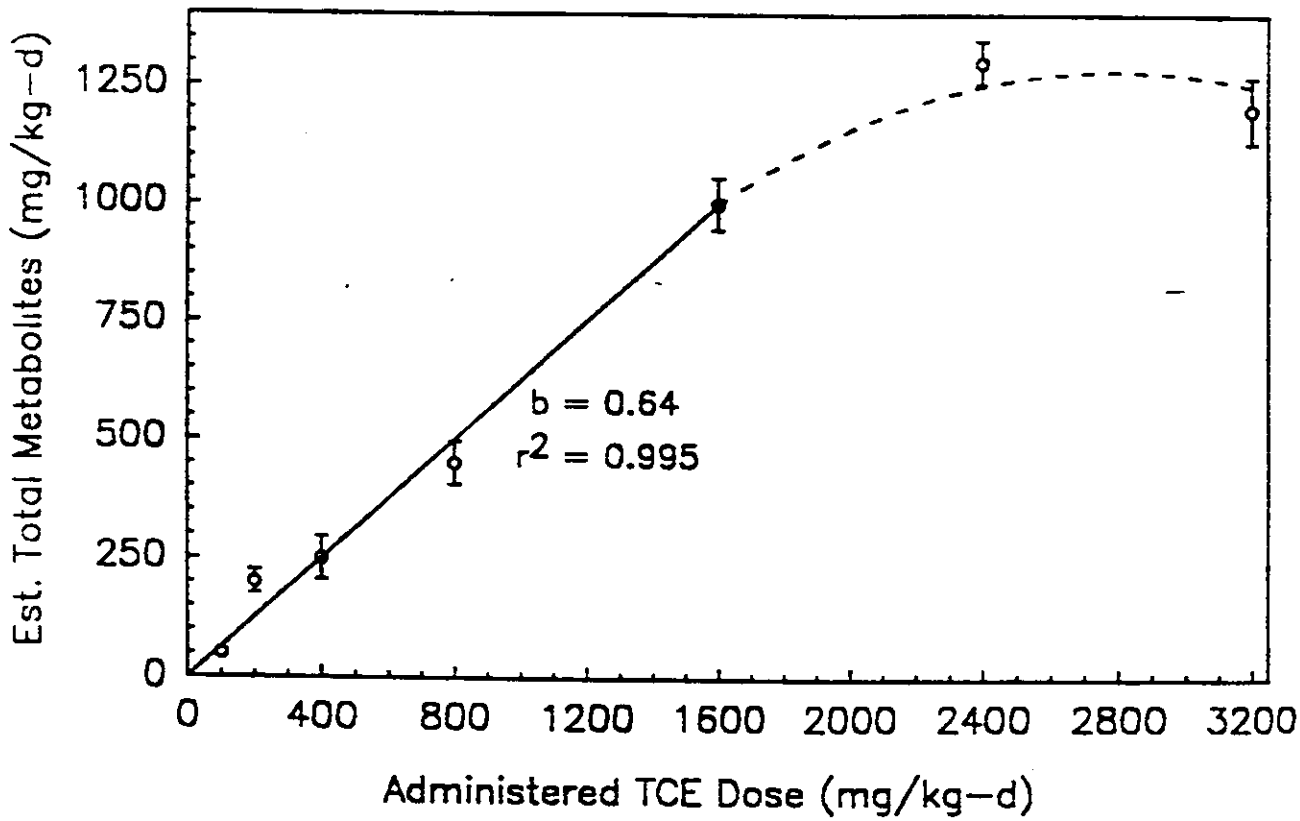


Figure 3-2. Estimated total production of metabolites in male Swiss-Cox mice chronically exposed (5 days/weeks for 6 weeks) to TCE by gavage, based on data of Buben and O'Flaherty (1985).

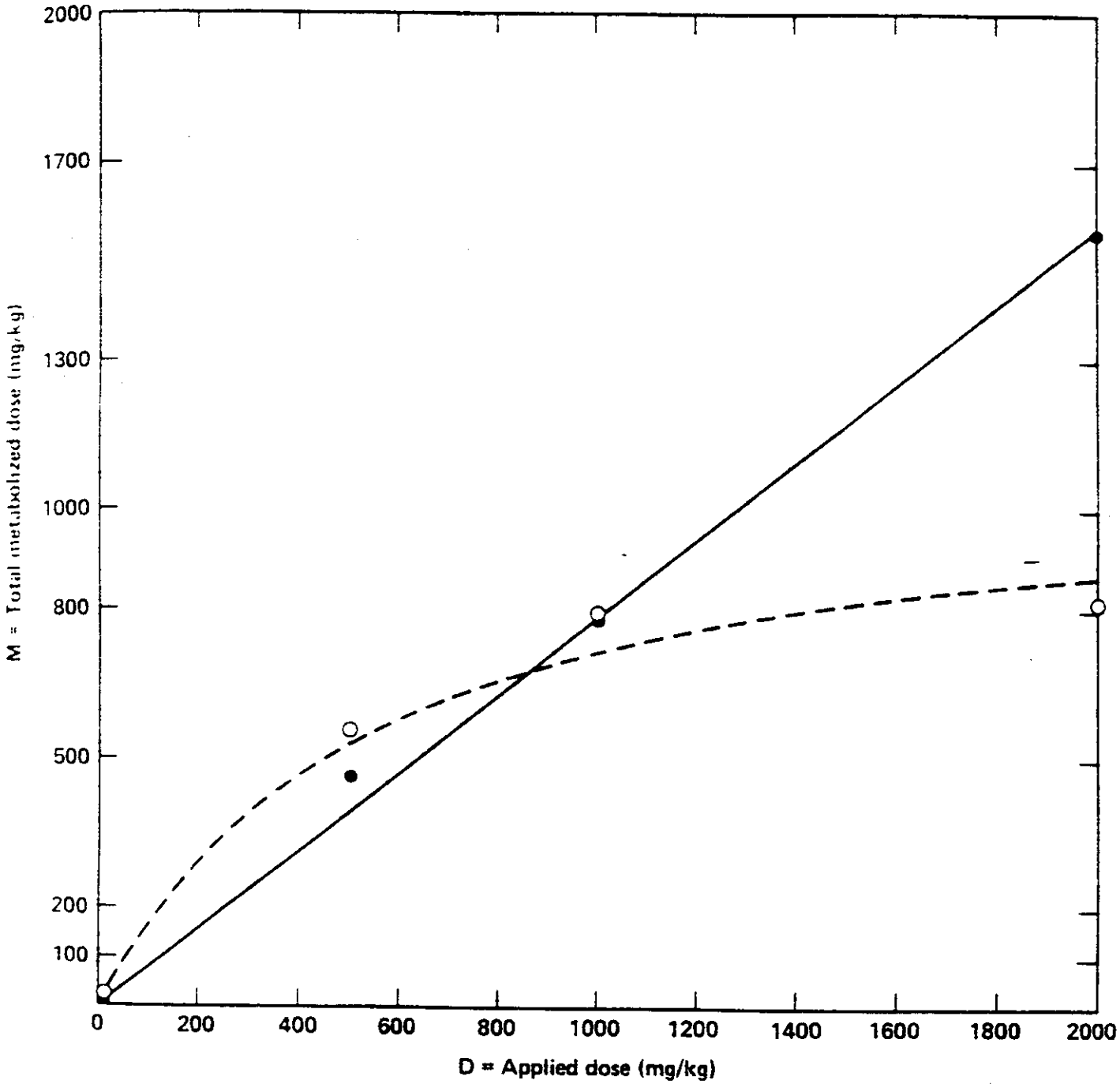


Figure 3-3. Total metabolized amount of single TCE doses administered to male B6C3F1 mice ( ) and Osborne-Mendel rats (O) from data of Prout et al. (1985). The data for mice are consistent with a linear dose-response model with a O-intercept slope of 0.788 (--), while the rat data are described by a Michaelis-Menten model with the parameters $V_{max} = K_m = 555$ mg/kg (--).

of metabolic saturation. Figures 3-2 and 3-3 show this relatinship as observed in the Buben and O'Flaherty (1985) study on Swiss-Cox mice and in the Prout et al. (1985) study on B6C3F1 mice, respectively. The data from Buben and O'Flaherty, based on observed total urinary metabolites excreted per day, are reexpressed in terms of estimated total metabolites in Figure 3-2, using data from Table 3-4. The Prout et al. (1985) mouse data show a fairly linear relationship between applied gavage and total metabolized doses, with slope value estimated by linear regression (R = 0.995) to be 0.788 under the condition of an intercept-parameter value of 0. Thus, the use of a Michaelis-Menten relationship to describe the mouse data may be inappropriate in the applied dose range below 2000 mg/kg, despite the fact that metabolic saturation occurs above this dose level. Saturation kinetics do not necessarily imply Michaelis-Menten (or rectangular hyperbolic) kinetics (see Pang and Rowland, 1977; Andersen, 1981; O'Flaherty, 1985). However, the data of Prout et al. (1985) for both B6C3F1 and Swiss-Webster mice, as well as the B6C3F1 mouse data of Mitoma et al. (1985) and the NMRI mouse data of Dekant (1984), all indicate a deviation from linearity at low doses, implying an increased metabolic efficiency as applied dose approaches zero.

Accordingly, the B6C3F1 mouse data of Prout et al. was interpreted according to the Michaelis-Menten function,

$$M = \frac{V_{max} D}{K_m + D}, \qquad (3\text{-}1)$$

in which M is total metabolized dose and D is applied dose, using an iterative least-squares estimation procedure conditional on $V_{max} \leq K_m$. The resulting, best fit parameter values were: V_{max} = 19860 mg/kg and K_m = 23460 mg/kg (V_{max}/K_m = 0.846). This two-parameter model fits the B6C3F1 mouse data slightly better (R = 0.996) than does the linear, single-parameter model mentioned above. However, the estimated Michaelis-Menten model for those data fails to predict the clear saturation observed at 3200 mg/kg dose in Swiss-Cox mice by Buben and O'Flaherty (1985), and the linear model fails to predict the increased TCE metabolism observed in low doses in several studies. Thus, neither the linear nor the Michaelis-Menten model appears to fully describe the observed data on TCE metabolism in mice, although both models fit the observed data within a restricted dose range.

The data of Stott et al. (1982) on TCE metabolism in mice exposed by inhalation to 10 or 600 ppm TCE for 6 hours appear to indicate that a degree of metabolic saturation takes place at the higher exposure level that is equivalent to a dose of about 840 mg/kg, assuming an alveolar ventilation rate of 1.0 L/hour. This indication is not consistent with the data on TCE metabolism in mice, dosed by gavage as discussed above, which suggest metabolic saturation does not occur unless much higher doses are administered. Relevant factors may include possible changes in pulmonary ventilation rate at high concentrations of TCE in air and the possibility that the apparent reduction in metabolic efficiency observed at the higher respiratory exposure to TCE is a statistical artifact since only 2 data points were taken, each representing the average of values obtained for only 4 mice. (The coefficient of variation for both mean values is about 20%.) From the latter perspective, the data may in fact be consistent with a linear, nonsaturated model relating metabolized to applied dose. The corresponding best estimate for a linear, 0-intercept slope parameter using the 2 data points available is 0.671 mg/kg-ppm-6 hours.

The B6C3F1 mouse data of Prout et al. (1985) contrast with rat data of Prout et al. (1985) in Figure 3-3; rats are shown to have a lesser capacity to metabolize TCE. The Prout et al. (1985) data for Osborne-Mendel rats are clearly nonlinear in the dose range below 2000 mg/kg. To this rat data was fit a Michaelis-Menten function, as described above, yielding the parameter estimates $V_{max} = K_m = 555.4$ mg/kg ($R = 0.961$) (see Figure 3-3). The parameter estimates imply that, as applied dose approaches 0, TCE metabolism in the Osborne-Mendel rats studied approaches 100%, a conclusion also indicated by the Prout et al. data on Wistar rats. These data, in conjunction with the data on TCE metabolism in mice discussed above, indicate that very low, orally applied doses of TCE (in a corn oil vehicle) may be metabolized to an extent approaching 100%. Explicit, detailed data on the extent of low-dose TCE metabolism in animals is currently unavailable. The data available on TCE metabolism in rats exposed to TCE by inhalation (Table 3-5) are generally consistent with the evidence for saturable metabolism of TCE administered by gavage as discussed above. The data of Stott et al. (1982) regarding Osborne-Mendel rats, for example, are predicted by a Michaelis-Menten relationship with the parameters $V_{max} = 183$ mg/kg and $K_m = 387$ ppm-6 hours, where the latter K_m value is equivalent to about 261 mg/kg, assuming an alveolar ventilation rate of 4.6 L/hour

In an additional mass-balance study, Green and Prout (1985) examined daily TCE metabolism in male B6C3F1 mice given subchronic (10- or 180-day doses or a single (1-day) dose of 1000 mg/kg-d of TCE in corn oil by gavage. The singly and subchronically exposed mice metabolized about the same amount (85% and 81%, respectively) of TCE within 24 hours. This study suggests that TCE does not accumulate significantly with daily dosing in mice, and therefore, each daily dose of TCE given in this study was cleared by metabolism and pulmonary excretion within a 24-hour period.

The TCE inhaled by dogs is metabolized significantly at extrahepatic sites. Hobara et al. (1986) exposed groups of 5 dogs, in which extrahepatic circulation sytems were surgically implanted, to 0, 500, 700, or 1500 ppm TCE for 1 hour. Two hours after the exposure, dogs with the hepatic bypass were capable of metabolizing TCE to the following metabolites at the corresponding efficiencies compared to dogs with an unactivated bypass system: 50 to 80% for TCEL, 10% for TCA, and 10 to 20% for conjugated TCEL. From the data on urinary excretion reported in this study, it is clear that the nonbypassed dogs metabolized TCE primarily (>98%) to conjugated TCEL in proportion to applied TCE dose. The hepatic bypassed dogs, however, metabolized a total of only 15 to 50% of that metabolized to urinary metabolites by the nonbypassed dogs (again, primarily to conjugated TCEL). Furthermore, the bypassed dogs' extrahepatic metabolism appeared to be saturated even at the lowest exposure level of 500 ppm-hour, yielding a decreasing fraction metabolized at higher doses. In particular, the bypassed dogs did not appear to be capable of producing significantly increased levels of TCA in response to TCE exposure, indicating that extrahepatic tissues were capable of metabolizing TCE effectively to TCEL and conjugated TCEL, but not to TCA, in the dogs studied.

Humans

Many studies have been conducted using volunteers to assess the extent to which TCE is metabolized in humans. Quantification of metabolism in these studies is done by collection of urinary metabolites and/or by calculating total exhaled TCE subsequent to a known TCE exposure. Unmetabolized TCE is either distributed within body tissues, as discussed above in this section, where it remains available for subsequent excretion or metabolism, or it is excreted via exhalation with kinetics that parallel depletion of TCE concentration in blood after exposure cessation, as shown in Figure 3-4. A

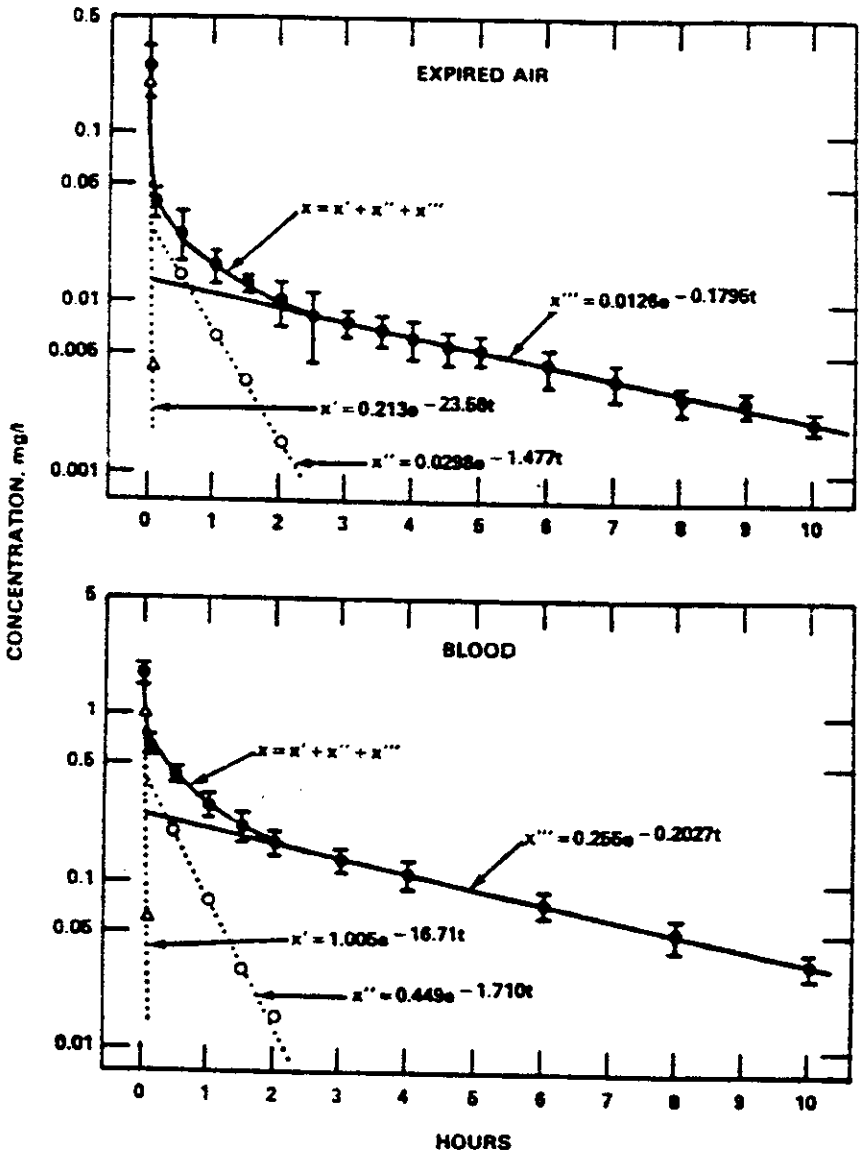


Figure 3-4. Elimination curves for TCE in expired air and blood in 4 Japanese males (with an average weight of 61.6 kg) after inhalation of TCE at 100 ppm for 4 hours (Sato et al., 1977).

small amount of metabolized TCE apparently is excreted in bile (Bartonicek, 1962) and in exhaled air as TCEL (Monster et al., 1976).

Studies have demonstrated that in humans TCE is metabolized primarily to TCA, TCEL, and TCEL-G, which are excreted in urine for the most part, although some TCEL and TCA may be metabolized further as discussed above in this section. Studies of the kinetics of excretion of these urinary TCE metabolites in volunteers have shown that, subsequent to TCE exposure, urinary TCEL is first produced more quickly and in larger amounts than is urinary TCA, but that TCA production persists and eventually surpasses that for TCEL (Soucek and Vlachova, 1960; Nomiyama and Nomiyama, 1971; Müller et al., 1972; Fernandez et al., 1975, 1977; Monster et al., 1979). The time constants for urinary excretion rate decay reported in or calculated from these studies are summarized in Table 3-6. The observed distinction in excretion kinetics between TCEL and TCA after TCE exposure has played an important role in the development of strategies for biological monitoring of occupational exposure to TCE (Guberan, 1977; Sato et al., 1977; Fernandez et al., 1977; Droz and Fernandez, 1977, 1978; Monster and Houtkooper, 1979).

In a number of studies, the extent to which humans metabolize TCE in response to a controlled respiratory exposure has been quantified by measuring total urinary metabolites generated after exposure or by measuring the total amount of unchanged TCE expired after an exposure for which an estimate of total retained TCE dose was available (Soucek and Vlachova, 1960; Ogata et al., 1971; Nomiyama and Nomiyama, 1971; Ertle et al., 1972; Müller et al., 1972; Monster et al., 1976; Fernandez et al., 1975, 1977; Monster et al., 1979). The results of these studies are summarized in Table 3-7. The reported values for average percent of retained dose metabolized (PRDM) range from 81 to 92%. In the most recent studies by Monster et al. and Fernandez et al. involving a total of 18 males given respiratory exposures ranging from 280 to 1440 ppm-hour TCE at TCE concentrations ranging from 54 to 140 ppm, the reported values for average PRDM are remarkably consistent and range from 89 to 92% with a person-weighted average of 90.6 $\pm$ 1.9% (C.V. = 2.1%) and a corresponding individual PRDM range of 87 to 93%. These 18 individual PRDM values are not significantly correlated with either TCE exposure concentration or time-integrated TCE exposure (in ppm-hour).

In contrast to the estimates of PRDM, the values reported for percent of metabolized dose identified as urinary metabolites (PMDU) in humans show a

Table 3-6. Kinetics of Decay in Urinary Metabolite Production After Human Exposure to TCE.

Study	TCE Exposure	Collection Period[a]	Metabolite[b]	Subject Number, Sex	Reported or Calculated Terminal Decay Half-life[c]
Soucek and Vlachova (1960)	93-158 ppm-5 h	10 - 14 d	MCA	3M, 2F	15 h
			TCA	3M, 2F	70 h
			TCEL	3M, 2F	40 h
Nomiyama and Nomiyama (1971)	320 ppm 160 min	6 d	TTC	5M	31 h
				5F	36 h
			TCA	5M	38 h
				5F	36 h
			TCEL	5M	19 h
				5F	26 h
Ikeda and Imamura (1973)	5-200 ppm (intermittent/ occupational)	3 - 8 d	TTC	24M, 6F	41 h
			TCA	6M	40 h
			TCEL	6M	15 h
			TCA	6F	58 h
			TCEL	6F	43 h
Mller et al. (1972)	50 ppm-6 h for 5 d	13 d	TCA	5M	100 h
			TCEL	5M	12 h
Kimmerle and Eben (1973b)	48 ppm-4 h for 5 d	6 d	TCA	3M, 1F	52 h
			TCEL	3M, 1F	20 h
Fernandez et al. (1975, 1977)	54 ppm-8 h	16 d	TCA	2M	60 to 65 h
			TCEL	2M	20 to 25 h
	97 ppm-8 h	21 d	TCA	3M	50 to 100 h
			TCEL	3M	10 to 20 h
Monster et al. (1979)	72 ppm-4 h for 5 d	6 d	TCA	5M	80 to 100 h
			TCEL	5M	40 h

[a] Post-exposure urine collection period.

[b] TCA = trichloroacetic acid, MCA = monochloroacetic acid, TCEL = trichloroethanol, TTC = total trichloro-compounds.

[c] Reported or estimated mean value of (ln 2)/k, where k is the smallest decay-rate constant describing the corresponding metabolism data.

Table 3-7. Extent of TCE Metabolism in People After Experimental Respiratory TCE Exposure.

Study	TCE Exposure	Collection Period[a]	Subject Number Sex	Average % Applied Dose Metabolized[b] = PRDM (range)	Reported % Metabolized Dose Measured as Urinary Metabolites[c]=PMDU (range)
Soucek and Vlachova (1960)	93-158 ppm-5 h	10-14 d	M (3) F (2)	--	≥73 ≥(43-101)
Ogata et al. (1971)	170 ppm-3 h	4.2 d	? (4)	--	>75.9
	170 ppm-7 h	4.2 d	? (5)	--	>62.1
Nomiyama and Nomiyama (1971)	320 ppm-160 min	6 d	M (5) F (5)	80.8 87.3	95 97
Ertle et al. (1972)	50 ppm-6 h for 5 d	7 d	M (5)	--	51[d]
	100 ppm-6 h for 5 d	7 d	M (5)	--	44[d]
Müller et al. (1972)	50 ppm-6 h for 5 d	18 d	M (5)	--	37[d]
Monster et al. (1976)	70 ppm-4 h	9 d	M (4)	90 (87-93)	71 (69-75)
	140 ppm-4 h	9 d	M (4)	92 (91-93)	61 (48-75)
Fernandez et al. (1975, 1977)	54 ppm-8 h	16 d	M (2)	92 (91.7-92.3)	55[e] (54.5-54.7)
	97 ppm-8 h	21 d	M (3)	91 (90.5-93.0)	55 (51.8-57.5)
Monster et al. (1979)	72 ppm-4 h for 5 d	6 d	M (5)	89 (88-91)	75 (58-86)

Footnotes to Table 3-7. (next page)

Footnotes to Table 3-7.

[a] Period of routine urine collection, including exposure period. In the Monster et al. (1976) study, routine urine collection stopped at 66 hours, and a final sample was taken on day 9 postexposure.

[b] Here calculated as 100% minus the reported percent of retained TCE that is expired as TCE subsequent to exposure, for those studies reporting such a value. Retained TCE is defined in these studies as the difference between inspired and expired TCE concentration times the volume of air respired. The retained fraction, fr, of TCE in alveolar air was reported to be about 78% (Monster et al., 1976; assuming an alveolar ventilation rate of 5.9 L/minute), 71 to 78% (Fernandez et al., 1975), and 70% (Monster et al., 1979).

[c] Here calculated as the percent of reported retained dose recovered as urinary metabolites divided by the fraction of applied dose metabolized, where the latter is calculated as explained in note (b).

[d] These values were estimated from reported graphical data, assuming an alveolar ventilation rate of 5.6 L/minute and first-order half-lives for decay in urinary metabolite production of 25 hours and 70 hours for trichlorethanol (TCEL) and trichloroacetic acid (TCA), respectively, where these are weighted average values for males based on the (sex-separable) data presented in Table 3-6. The urinary metabolites include TCA and TCEL (unconjugated, but in later studies also conjugated), and in one case monochloroacetic acid (Soucek and Vlachova, 1960).

[e] Falls outside specified range due to rounding.

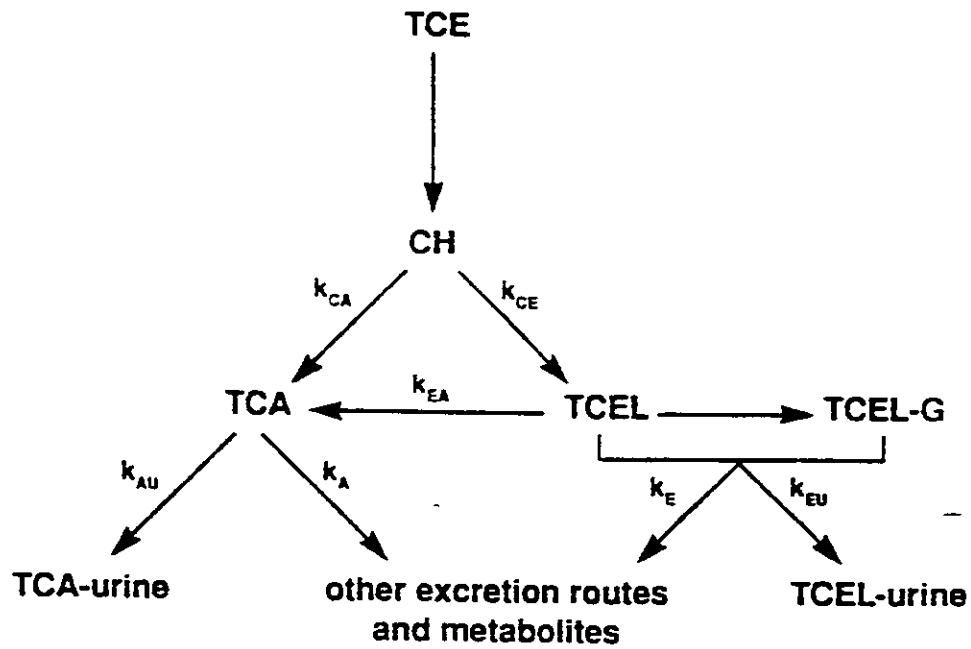
greater degree of inter-individual heterogeneity. The 18 individual PMDU values from the Monster et al. and Fernandez et al. studies average 66.0% ± 10.9% (C.V. = 16.6%). This inter-individual heterogeneity is only partly due to differences in study methodology (e.g., urinary metabolite identification efficiency, specificity, etc.), because the separate Monster et al. (1976), Fernandez et al. (1975, 1977), and Monster et al. (1979) PMDU averages are 67.4% ± 8.5% (C.V. = 12.6%), 54.9% ± 2.1% (C.V. = 3.9%), and 75.0% ± 11.0% (C.V. = 14.7%), respectively. The high PMDU values reported by Nomiyama and Nomiyama (1971) are not consistent with the other reported values, partly because the total urinary metabolite excretion was estimated beyond day 6 using a first-order decay assumption with a half-life of 1.3 to 1.5 days, and also possibly because TCE retention was estimated to be only about 35 to 38%, based apparently on a single postexposure measurement for exposed individuals. The fact that the PMDU values are in general significantly below 100% indicates that respired TCE is metabolized by humans either to products excreted in urine other than those measured in the studies considered, such as HAAE (see Dekant et al., 1984), or to expired products such as CO_2, as discussed above in this section. Comparison of the data in Table 3-7 with that in Tables 3-4 and 3-5 shows that human values for PRDM and PMDU are similar to those obtained from mice and rats given oral (applied) TCE doses below 2000 mg/kg and 500 mg/kg, respectively. This indicates that humans exposed to very low levels of TCE may metabolize almost all of the dose received as dose approaches 0, although, as is the case for animals, detailed human data on this point are entirely lacking. However, results from a study of urinary metabolites excreted by 51 male Japanese workers regularly exposed to TCE in 10 different workshops indicate that certain TCE-metabolism or metabolite-excretion processes may saturate at high exposure levels (Ikeda et al., 1972; Ikeda, 1977). This study showed that, while the concentration of total trichloro-compounds (TTC) and of trichloroethanol (TCEL) in urine increased linearly with increasing exposure levels up to 200 ppm (8 hours/day, 6 days/week), that of trichloroacetic acid (TCA) appeared to saturate at about 200 mg/L in workers exposed to air concentrations of TCE greater than 100 ppm.

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELS

Multicompartment models known as physiologically-based pharmacokinetic (PBPK) models are used increasingly in health-risk assessment for volatile

organic compounds (VOCs). These models predict the uptake, metabolism, and excretion of such chemicals in exposed animals or people. One such model developed by Sato et al. (1977) was used to interpret data gathered on excretion of TCE and metabolites after respiratory exposure of 4 human volunteers to 100 ppm TCE for 4 hours. A first-order PBPK model was used to simulate concentrations in 3 compartments: vessel-rich (richly perfused) tissues, muscle (and other poorly perfused) tissues, and fat (very poorly perfused) tissues. In this model, the compartments are assumed to be interconnected and initially at an equilibrium determined by tissue volumes and blood/air and tissue/blood partition coefficients. The latter coefficients were based on experimental values obtained using rat and human tissues and blood. Richly perfused tissues are assumed to be the source of both metabolic and respiratory excretion of absorbed TCE. In this model, intercompartment exchange of TCE is governed solely by postulated intertissue diffusion. When this model was fitted to experimental human data, a value of 104 L/hour was obtained for metabolic clearance of TCE (Sato et al., 1977). The authors did not explain the basis for their assumption that humans exposed to TCE would have reached compartmental equilibrium after only 4 hours.

A different, more complete PBPK model was used by Fernandez et al. (1977) to simulate the uptake, metabolism, and excretion of TCE in experimentally exposed humans. This model contained the 3 compartments used by Sato et al. (1977), plus a liver compartment and a pulmonary compartment. The model provides for mass balance of TCE in the compartments, for TCE entering and leaving the body through the lung, for TCE metabolized in the liver, and for TCE metabolites excreted in urine. The uptake and excretion kinetics were assumed to be linear and governed by tissue volumes, tissue-specific blood volumes, blood perfusion, and tissue-gas partition coefficients. In the model, TCE metabolism was assumed to take place solely in the liver via the pathways shown in Figure 3-5. The values for the linear kinetic parameters used by Fernandez et al. (1977) were determined from the available literature on TCE metabolism in humans, primarily from Müller et al. (1974). These values are listed in Figure 3-5. In this model, TCE metabolism is assumed to be limited only by blood flow through the liver in such a way that the fraction, f_c, of arterial blood flowing into liver that is metabolically cleared of TCE is a constant equal to 0.86. That is, metabolic clearance is assumed to equal about 86% of hepatic blood flow in this model. The liver-perfusion value of 96.3 L/hour used by Fernandez et al. for a reference 70-kg man yields a metabolic



k_{EU} = 0.0260 h^{-1}
k_{AU} = 0.00685 h^{-1}
k_E = 0.00820 h^{-1}
k_A = 0.00685 h^{-1}
k_{EA} = 0.0191 h^{-1}
$k_{CE} / (k_{CE} + k_{CA})$ = 0.78
$k_{CA} / (k_{CE} + k_{CA})$ = 0.22

Figure 3-5. Pathways and rate constants for TCE-metabolism used in the PBPK model of Fernandez et al. (1977) describing uptake, metabolism, and excretion of TCE in humans. (TCE = trichloroethylene, CH = chloral hydrate, TCEL = trichloroethanol, TCEL-G = TCEL-glucuronide).

clearance rate of $96.3f_c$ = 83 L/hour, which is close to the metabolic clearance rate of 104 L/hour estimated by Sato et al. (1977). The PBPK model of Fernandez et al. (1977) accurately predicted the decay in alveolar-TCE concentration observed over 50 hours and the cumulative urinary excretion of TCEL and TCA observed over 200 hours after volunteers, with an average weight of about 70 kg, were exposed for 8 hours to concentrations of TCE from 54 to 160 ppm.

The Ramsey-Andersen PBPK Model

Recently, the National Research Council (NRC) considered the use of PBPK models to facilitate dose-route extrapolation when using inhalation toxicity data to set safe drinking water limits (NRC, 1986). A range of issues was considered in this study, which included illustrative examples using the PBPK approach for dose-route extrapolation from rats to humans for noncarcinogenic toxicity associated with exposure to TCE and benzene. The pharmacokinetic model used was developed by Ramsey and Andersen (1984) to describe the uptake, metabolism, and excretion of styrene in rats and humans. The structure of the model is shown in Figure 3-6, and its parameter definitions are given in Table 3-8. This type of model has been applied to the study and prediction of animal and human pharmacokinetics for other VOCs, including benzene, methylene chloride, and tetrachloroethylene (NRC, 1986; Gargas et al, 1986; Andersen et al., 1987; U.S. EPA, 1986a; Reitz and Nolan, 1986; Hattis et al., 1987; Ward et al., 1988). The model consists of a set of differential equations that describe the rate of change of the amount of absorbed chemical present in each of 4 physiologically realistic tissue compartments, which are assumed to be ideally well-mixed at any given time. Metabolism is presumed to occur solely in the liver through a saturable enzymatic process with Michaelis-Menten kinetics.

According to the Ramsey-Andersen model, pulmonary uptake of a chemical occurs continuously such that alveolar concentration, C_a, is in instantaneous equilibrium with arterial blood governed by the blood/air partition coefficient, P_b, in accordance with the relation $C_a = B_a/P_b$. Similarly, the concentrations, C_i, of chemical in each tissue compartment are presumed to be in instantaneous equilibrium with the concentrations, B_i, in venous blood exiting the corresponding tissue, governed by the corresponding tissue/blood partition coefficients such that $B_i = C_i/P_i$. The amount of

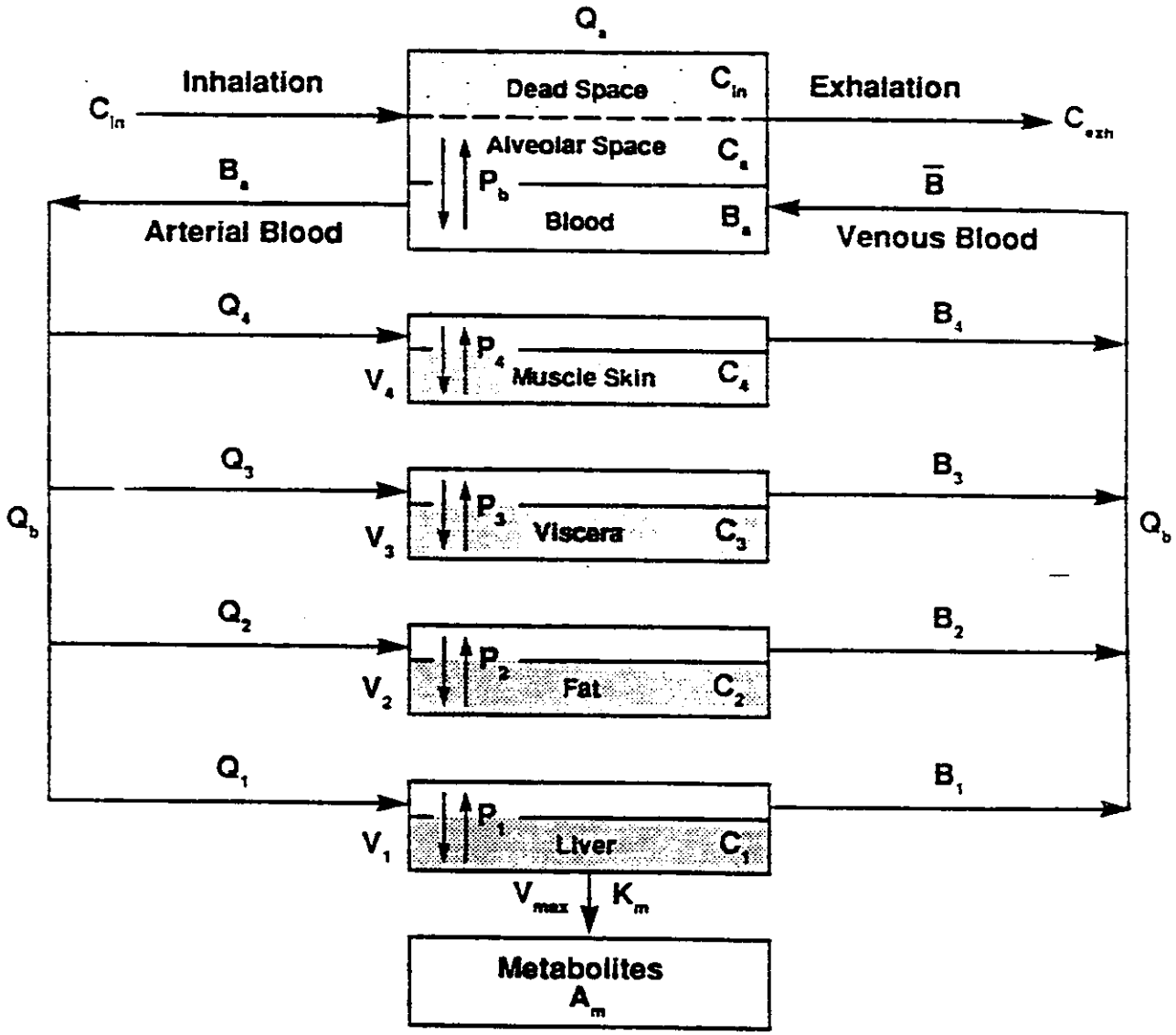


Figure 3-6. Schematic diagram of physiologically based pharmacokinetic (PBPK) model for inhalation of volatile organic compounds. The model assumes that 4 "well-stirred" compartments or tissue groups collect inhaled compound at rates governed by air concentration (C_{in}), air and blood flows (Q), blood concentrations (B), compartment volumes (V), tissue/blood partition coefficients (P), and metabolic parameters (V_{max}, K_m).

Table 3-8. Compartment and Parameter Definitions for the Ramsey-Andersen PBPK Model.

Abbreviation	Definition	Unit
C_{in}	Concentration in air inhaled	mg/L air
C_a	Concentration in alveolar air	mg/L air
C_{exh}	Measured concentration in expired breath	mg/L air
Q_a	Alveolar ventilation rate	L air/h
Q_b	Cardiac output	L blood/h
P_b	Blood/air partition coefficient	L air/L blood
B_a	Arterial blood concentration	mg/L blood
$\bar{B}$	Venous blood concentration	mg/L blood
A_m	Amount metabolized in liver	mg
Q_i	Blood flow rate to compartment i	L blood/h
V_i	Volume of compartment i	L (= kg)
C_i	Concentration in compartment i	mg/L
B_i	Concentration in venous blood leaving compartment i	mg/L blood
A_i	Amount in compartment i	mg
P_i	Tissue/blood partition coefficient for compartment i	L blood/L tissue i
V_{max}	Maximum metabolic rate	mg/h
K_m	Apparent Michaelis constant = ($BldA_m/dt = V_{max}/2$)	mg/L blood
R	Rate of ingestive infusion	mg/h

Compartmental Subscripts:

i = 1 Liver (metabolizing tissue group)
2 Fat tissue (very poorly perfused)
3 Richly perfused tissues (brain, kidney, viscera)
4 Poorly perfused tissues (muscle, skin)

chemical in any given tissue compartment is given by $A_i = C_i V_i$. For notational convenience, the dependence of state variables (C's, B's, and A's) on time t is suppressed.

The model presumes that in any given interval, dt, chemical delivered to the lung via respiratory retention and returning venous blood is balanced by the chemical mass exiting the lung via exhalation and arterial blood, such that

$$(Q_a C_{in} + Q_b \bar{B})dt = (Q_a C_a + Q_b B_a)\, dt$$

or

$$Q_a(C_{in} - C_a) = Q_b(B_a - \bar{B})$$

which, recalling that $C_a = B_a/P_b$, yields

$$B_a = \frac{Q_a C_{in} + Q_b \bar{B}}{(Q_a/P_b) + Q_b} \qquad (3\text{-}2)$$

Note that an experimentally measured concentration in exhaled breath is given by $C_{exh} = F_d C_{in} + (1 - F_d)C_a$, where the dilution factor F_d approaches zero as the efficiency of measuring purely alveolar expired air approaches 100%. Equation (3-2) specifies B_a (and thus C_a and C_{exh}) to be at each instant a flow-weighted average of C_{in} and $\bar{B}$. Similarly, the concentration $\bar{B}$ in venous blood returning from each compartment is presumed to be the instantaneous flow-weighted average:

$$\bar{B} = \frac{1}{Q_b} \sum_{i=1}^{4} Q_i B_i \qquad (3\text{-}3)$$

For the nonmetabolizing tissues, the amount $Q_i B_a dt$ of chemical entering the ith compartment via arterial blood during any given interval, dt, is set equal to the amount dA_i gained by that compartment plus the amount $Q_i B_i dt$ leaving in venous blood, for i = 2,3,4. The chemical concentration in venous blood leaving from each of these compartments is therefore defined by:

$$\dot{B}_i = \frac{Q_i}{V_i P_i} (B_a - B_i) \qquad i = 2,3,4, \qquad (3\text{-}4)$$

where dot notation is used here, and below, to represent differentiation with respect to time (i.e., $\dot{B}_i = dB_i/dt$, etc.). The amount of chemical metabolized in liver is given by the Michaelis-Menten relationship:

$$\dot{A}_m = \frac{V_{max} B_1}{K_m + B_1} \qquad (3\text{-}5)$$

in which K_m is defined as the concentration in venous blood from liver (or, alternatively, $K_m P_l$ is the chemical concentration in liver) at which the liver's metabolic velocity $\dot{A}_m$ is half its maximum value, V_{max}. Thus, the state equation for liver venous blood concentration is given by:

$$\dot{B}_l = \frac{Q_l}{V_l P_l}(B_a - B_l) - \frac{\dot{A}_m}{V_l P_l} \qquad (3\text{-}6)$$

The system of Equations (3-2) to (3-6) represents the PBPK model for inhalation of a volatile chemical, and for any given time its compartmental quantities A_i or corresponding concentrations C_i are found by simultaneous numerical integration of the system.

Exposure by routes other than inhalation is easily incorporated into this model. Because blood draining the stomach, small intestine, and colon passes through the hepatic portal vein, exposure to VOCs via ingestion is modeled simply by assuming a direct introduction of the ingested mass into the liver compartment (NRC, 1986). The latter introduction can be assumed to reflect a first-order infusion process, the approach taken in NRC (1986), or it may be modeled more simply as a constant infusion into the liver at a rate R (mg/hour) by adding the constant $R/(V_l P_l)$ to the right side of Equation (3-6).

Definition of Metabolized Fraction of Applied Dose

Because the chronic toxicity of many halogenated VOCs, and of TCE in particular, is associated with their metabolism or metabolites (see Section 4), the metabolized fraction of the total quantity of chemical potentially available for absorption and metabolism is often of particular interest in the context of regulatory risk assessment. Under steady-state conditions, the corresponding quantity of interest in the context of purely respiratory exposure is the fraction, f_{mr}, of the maximum plausible metabolic rate, or metabolic clearance fraction, given a constant ambient concentration C_{in}, defined as:

$$f_{mr} = \dot{A}_m/(Q_a C_{in}) \qquad (3\text{-}7)$$

The value f_{mr} calculated from the PBPK model may be contrasted with the assumption, sometimes made in the context of carcinogen risk assessment

(Anderson et al., 1983), that 100% of a chemical entering the lungs through total respiratory ventilation (or that contained in approximately 20 m^3/day or a reference 70-kg man) is absorbed and potentially available for metabolism. In the context of purely ingestive exposure, a corresponding, hypothetical quantity may be defined as the fraction f_{mo} of the maximum plausible metabolic rate, given a continuous rate R of ingestive absorption, defined as:

$$f_{mo} = \dot{A}_m/R \ . \qquad (3\text{-}8)$$

Because ingestion is actually a discontinuous process with some average absorption rate R, the maximum plausible metabolic rate will be less than f_{mo}, as defined by Equation (3-8), to the extent that metabolic saturation occurs, allowing more of the parent TCE or "applied" dose to escape via exhalation and thus to avoid being metabolized. For VOCs described by this model, both f_{mr} and f_{mo} are necessarily less than one whenever (finite) metabolism takes place, because any unmetabolized compound is always subject to pulmonary excretion.

Analysis of PBPK System at Steady State.

In the context of environmental regulation, very low-level, continuous exposure scenarios are typically of concern. The following is an analysis of how the PBPK model used by the NRC (1986) to describe TCE pharmacokinetics behaves under steady-state, respiratory exposure conditions. Steady-state ingestive exposure, using the constant infusion model described earlier, is also considered for the purpose of comparison. In addition the mixed exposure case represents a scenario of human environmental exposure that may be of particular regulatory concern.

Because at steady state $B_a = B_i$ for i = 2,3,4, Equation. (3-3) reduces under steady-state conditions to:

$$\bar{B} = \frac{1}{Q_b} \left[Q_1 B_1 + B_a \ (Q_b - Q_1)\right] \ , \qquad (3\text{-}9)$$

so that Equation (3-2) reduces to:

$$B_a = \frac{Q_a C_{in} + Q_1 B_1}{(Q_a/P_b) + Q_1} \ . \qquad (3\text{-}10)$$

Also at steady state, Equation (3-6), modified as described above to reflect constant ingestive infusion, reduces to the form:

$$\frac{V_{max}\, B_1}{K_m + B_1} = Q_1 (B_a - B_1) + R, \tag{3-11}$$

so that the solution for liver venous blood concentration, given input C_{in}, is the quadratic root:

$$B_1 = Y + \sqrt{Y^2 + Z}, \tag{3-12a}$$

in which

$$Y = \frac{1}{2} [C_{in}P_b + (R - V_{max})\, W\text{-}K_m], \tag{3-12b}$$

$$Z = K_m(C_{in}P_b + RW), \text{ and} \tag{3-12c}$$

$$W = \left[\frac{P_b}{Q_a} + \frac{1}{Q_1}\right] \tag{3-12d}$$

Note that the parameters Q_b, V_i, and P_i for $i = 1,\ldots,4$ and Q_i for $i = 1,2,3$ do not appear in this solution. The steady-state metabolic rate is thus given by using Equations (3-12a-d) to evaluate B_1 in Equation (3-5), and likewise in the expressions for the fractions f_{mr} and f_{mo} of maximal metabolic rate for TCE given by Equations (3-7) and (3-8), respectively.

Important in the context of environmental risk management is the limiting value of f_{mr} as $C_{in} \rightarrow 0$, that is, at very low exposure levels that might be typical of nonoccupational, purely respiratory exposure to TCE. At such exposure levels, metabolism is essentially a linear function of applied dose in the Ramsey-Andersen model under consideration. In discussing this low-dose situation, the ratio (V_{max}/K_m) shall be represented by the linear metabolic clearance rate K, which has the units of L/hour. It can be shown that the limiting value referred to is given by:

$$f^*_{mr} = \lim_{C_{in} \rightarrow 0} f_{mr} = \left[1 + \frac{Q_a}{P_b}\left(\frac{1}{K} + \frac{1}{Q_1}\right)\right]^{-1} \tag{3-13}$$

whereas the maximally conservative assumption that complete metabolism is approached as steady-state $C_{in} \rightarrow 0$ yields the corresponding limiting value:

$$f_{mr}^{**} = \lim_{K \to \infty} f_{mr}^{*} = \left[1 + \frac{(Q_a / Q_1)}{P_b} \right]^{-1} , \qquad (3\text{-}14)$$

representing the physiologically determined upper bound on the fraction of intake capable of being metabolized. This value is a function of just 3 parameters, only one of which is influenced by the particular chemical under consideration. Note that f_{mr}* and f_{mr}** would also apply to a VOC dose received via dermal absorption, because such a dose would enter the systemic circulation and thus be subject to pulmonary excretion in the same way that a respired dose would.

The consequences of the Ramsey-Andersen model for purely respiratory exposure indicated by Equations (3-13) and (3-14) are in contrast to the assumption regarding metabolism made in the Fernandez et al. model. Recall that the latter model presumed a purely flow-limited metabolic clearance fraction of $f_c = 0.86$ with a corresponding metabolic rate of $f_c Q_1 B_a$ in the notation used to describe the Ramsey-Andersen model. This assumption is quite different from the implication of Equations (3-13) and (3-14) that metabolic clearance of a respired VOC is directly (but nonlinearly) proportional to the corresponding clearance rate parameter K and only becomes constant as $K \rightarrow \infty$. Indeed, under steady-state conditions of respiratory exposure, Equation (3-6) implies that the relation:

$$f_c = \frac{Q_1 (B_a - B_1)}{Q_1 B_a} = \frac{K}{K + Q_1} \qquad (3\text{-}15)$$

follows from the PBPK model of Ramsey and Andersen. Note that, using Equation 3-15), Equation (3-13) may be rewritten as:

$$f_{mr}^{*} = \left[1 + \frac{(Q_a/Q_1)}{P_b \ f_c} \right]^{-1} , \qquad (3\text{-}16)$$

which, compared with Equation (3-14), clearly shows that f_{mr}* approaches its maximal value, f_{mr}**, only as f_c approaches unity.

The expression for f_{mr}* given in Equation (3-13) may be compared to a similar limiting value of f_{mo} as a hypothetically continuous, purely

ingestive dose approaches zero. It can be shown that this limiting value is given by:

$$f_{mo}^{*} = \lim_{R \to 0} f_{mo} = \left[1 + \frac{1}{K} \left(\frac{P_b}{Q_a} + \frac{1}{Q_1} \right)^{-1} \right]^{-1}, \qquad (3\text{-}17)$$

Therefore, $f_{mo}^{*} > f_{mr}^{*}$, and $f_{mo}^{*} \simeq 1$ under the maximally conservative assumption that K is extremely large. That is, unlike the case of purely respiratory exposure, it is physiologically plausible that close to 100% of a low-level, continuously ingested dose of a VOC can be metabolized. This occurs because of a "first-pass" effect on ingested VOC doses whereby they are collected in blood draining the gastrointestinal tract and transported through the hepatic portal vein directly to the liver before being passed to mixed venous blood where they become subject to respiratory elimination.

In the case of mixed respiratory and ingestive exposure under steady-state conditions, the fraction f_m of the maximum plausible metabolic rate is the weighted average:

$$f_m = \frac{f_{mr} Q_a C_{in} + f_{mo} R}{Q_a C_{in} + R},$$

which, as $C_{in} \to 0$ and $R \to 0$, approaches the limiting value:

$$f_m^{*} = \frac{f_{mr}^{*} Q_a C_{in} + f_{mo}^{*} R}{Q_a C_{in} + R}, \qquad (3\text{-}18)$$

where $f_m < f_m^{*}$. Equation (3-18) remains approximately true when C_{in} and R are replaced by their corresponding time-weighted average values:

$$\bar{C}_{in} = \int_0^T C_{in}(t)dt \quad \text{and} \quad \bar{R} = \int_0^T R(t)\, dt \quad ,$$

provided that temporal discontinuity in $C_{in}(t)$ and R(t) does not result in a significant deviation from linear, nonsaturated metabolism (i.e., provided that $\{(B_1(t) | (C_{in}(t), R(t), t)\} \ll K_m$ for all t where $0 \leq t \leq T$). To the extent that metabolic saturation does take place, the latter approximation will be less than the value f_m^{*} defined by Equation (3-18).

Table 3-9. Parameter Values Used in PBPK Model for TCE.

Parameter		Unit	Reference Rat[a]	Reference Male Human[b]	Japanese Male Worker[c]
W (body weight)		kg	0.30	70	55.2
Q_a		L/h	5.74	353.5	299.3
Q_b		L/h	5.74	371.6	314.7
P_b			22.0	9.92	9.92
Q_i/Q_b	i = 1		0.25	0.25	0.25
	2		0.09	0.05	0.04
	3		0.47	0.51	0.52
	4		0.19	0.19	0.19
V_i/W	i = 1		0.041	0.04	0.027
	2		0.09	0.20	0.15
	3		0.059	0.05	0.10
	4		0.72	0.62	0.61
P_i	i = 1		1.3	4.40	4.40
	2		26	68.0	68.0
	3		1.3	3.28	3.28
	4		0.5	1.64	1.64
V_{max}		mg/h	5.17	--	--
K_m		mg/L	0.25	--	--

[a] Parameter values taken from NRC (1986); metabolic parameters were reported to be determined by gas uptake experiments (NRC, 1986).

[b] Physiological parameter values taken from Ward et al. (1988); values for partition coefficients are based on those listed in U.S. EPA (1985a), derived from Sato et al. (1977).

[c] Parameter values are scaled from those corresponding to reference male human, based on the anatomical model of Kerr (1979) for reference Japanese adults.

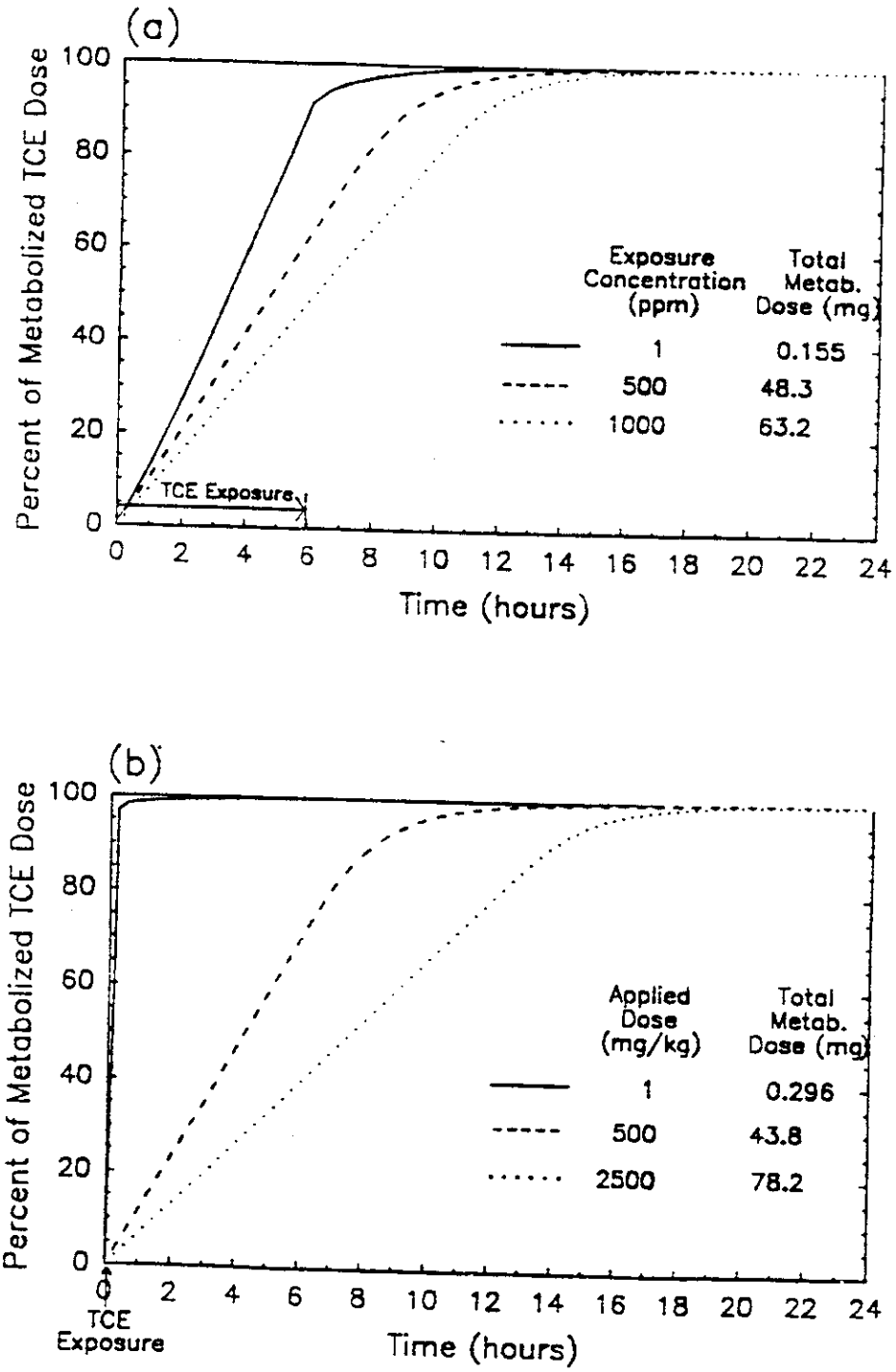


Figure 3-7. Simulation of metabolism of TCE in a 0.30-kg rat exposed via (a) inhalation and (b) ingestion, using a PBPK model with the parameter values listed in Table 3-9.

Note that the quantities f_{mr}*, f_{mr}**, f_{mo}*, f_{mo}**, f_m*, and f_c, defined in the preceding paragraphs, are all invariant with respect to body weight, so long as rates for metabolic processes (e.g., V_{max}, K) and flow rates (e.g., Q_a, Q_b) are all assumed to scale to the same power (e.g., the 0.7 power) of body weight. Such an assumption is indeed reasonable, as pointed out in the following subsection. By the same reasoning, the quantities f_{mr}, f_{mo}, and f_m are also invariant with respect to body weight whenever the pharmacokinetic system involved is entirely linear, i.e., nonsaturable. It also follows that f_{mo}* remains invariant with body weight when both $\dot{A}_m$ and R are in units of mg/kg-d, whereas $\dot{A}_m$ in mg/kg-d will be smaller for the larger of 2 animals exposed to a given very low concentration, C_{in}, of VOC in air by the factor $(w_1/w_2)^{0.3}$, where w_1 and w_2 are the weights of the smaller and larger animals, respectively.

Application of PBPK Model to Rodent-Bioassay Scenarios for TCE Exposure

The PBPK model of Ramsey and Andersen (1984) was applied to typical rodent-bioassay-exposure scenarios, using the same physiologic and metabolic parameter values for TCE-exposed rats as were used in the NRC (1986) study. These values appear in Table 3-9. Numerical integration of the differential equations described above was done on a VAX 11/750 computer using a variable-step Gear method (Hindmarsh, 1983). Results pertinent to the calculation of metabolized dose for the TCE bioassays discussed below are summarized here.

In lifetime bioassays involving rats or mice, typical respiratory exposures are regularly spaced, lasting about 6 hours daily for 5 days/week. Under these exposure conditions and using the PBPK model described, TCE metabolism in rats is predicted to be virtually (>99.9%) complete within 24 hours after each exposure ends (see Figure 3-7a), i.e., no accumulation of TCE in body tissues is expected as the number of daily exposures increases. Since this applies to exposure scenarios involving either low or high (e.g., up to 1000 ppm) concentrations of TCE, the degree of (transient) metabolic saturation occurring does not alter this prediction. As a result, the 24-hour, metabolized dose of TCE to rats is predicted to be virtually identical on the first and last days of exposure during a respiratory bioassay involving typical exposure levels. Consequently, it is reasonable to use the total metabolized

dose measured in a mass-balance study of rats exposed to TCE by inhalation for a single, relatively brief period such as 6 hours as an estimate of daily metabolized dose on exposure days for rats exposed 6 hours/day, 5 days/week over their lifetimes. Furthermore, the model predicts that a small change in the daily exposure duration (e.g., to 7 hours) results in a proportional change in total metabolized dose.

To apply the PBPK model to mice, the parameter values appearing in Table 3-9 for respiratory, blood flow, and metabolic clearance rates (all in units of L/hour) for rats were scaled to the 0.7 power of the ratio of mouse to rat body weight. This approach was taken because, in the context of pharmacokinetic modeling, such physiological parameters are generally assumed to vary with basal metabolic rate in proportion to body surface area (or, approximately, to body weight to the 0.7 power), rather than to body weight per se (Gehring et al., 1978; Dedrick and Bischoff, 1980; Andersen et al., 1980; Andersen, 1981; Ramsey and Andersen, 1984; U.S. EPA, 1985a, 1986a; NRC, 1986). For example, given an estimated V_{max} in mg/hour for an animal of weight w_1, the corresponding predicted values for animals of weight w_2 would be $V_{max}(w_2/w_1)^{0.7}$ (in mg/hour) or, on a weight-normalized -basis, $(V_{max}/w_1)(w_1/w_2)^{0.3}$ (in mg/kg-hour). That is, a heavier animal would be expected to metabolize less per unit body weight than a lighter one in a given amount of time. In contrast, the value of the Michaelis constant, K_m, for a given compound, in the absence of data indicating otherwise, is generally assumed to be independent of body size when this constant is expressed as the reactant concentration (in mg/L blood) at which the metabolic reaction rate is one-half its maximal value (Ramsey and Andersen, 1984; U.S. EPA, 1986a; NRC, 1986). Because blood weight varies with body weight approximately to the first power for animals of widely varying weights (Dedrick and Bischoff, 1980), the independence of K_m and body size should also be expected to hold for K_m values expressed in terms of mg/kg body weight.

If the physiological clearance parameters described above are assumed to be directly proportional to the 0.7 power of body weight, it follows that the corresponding kinetic rate constants governing uptake, metabolism, and excretion, all in units of $hour^{-1}$ or (L/hour)/L compartment volume where 1 L of body volume is assumed to weigh about 1 kg, are inversely proportional to the 0.3 power of body weight. In the Ramsey-Andersen PBPK model for mice, the tissue-compartment volumes are scaled down from the values used for rats in proportion to body weight. Therefore, the rate constants for mice are expected

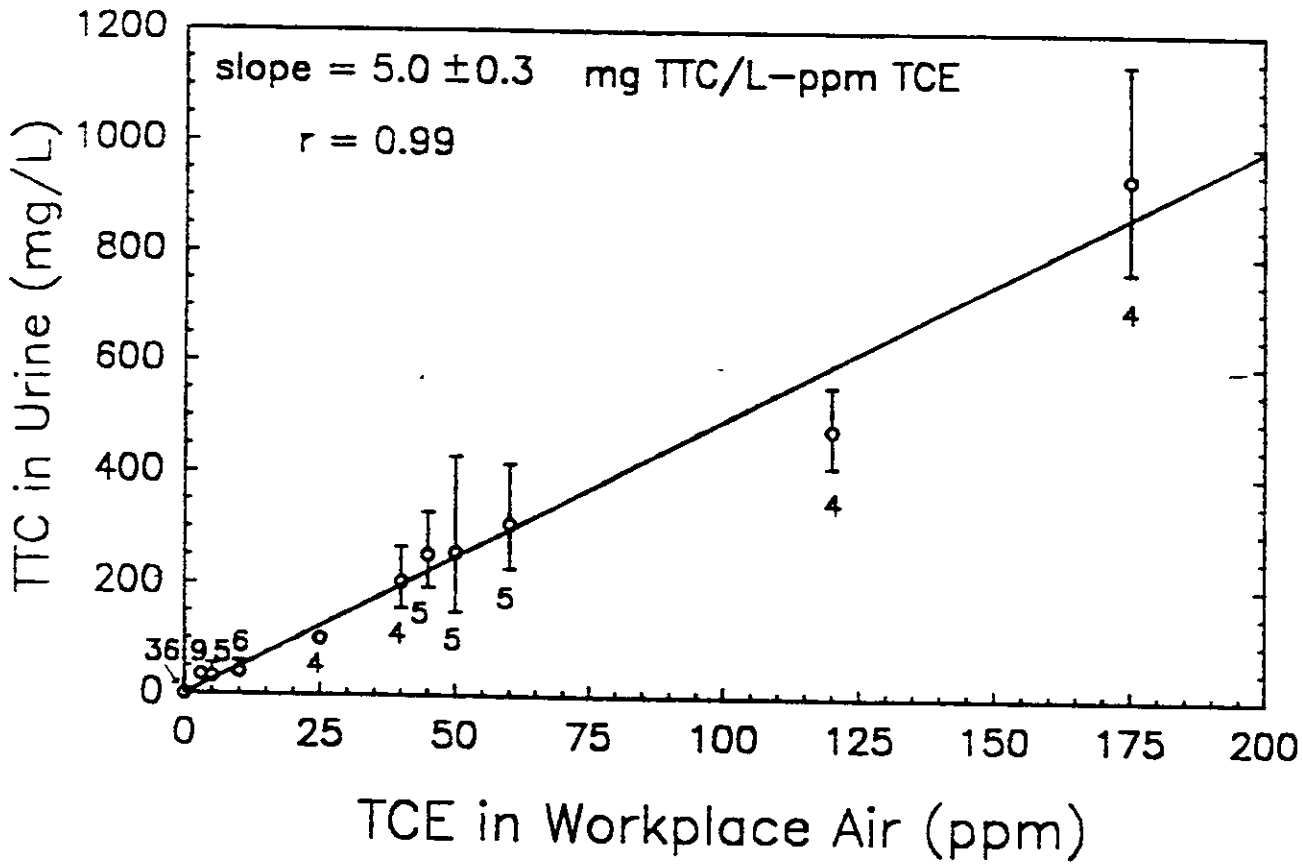


Figure 3-8. Data of Ikeda et al. (1972) on geometric mean levels of total trichloro-compounds (TTC) in urine produced in 51 male Japanese workers exposed 8 hours/day, 6 days/week to different daily time-weighted average concentrations of trichloroethylene (TCE) in air. Numbers next to data points indicate the number of workers monitored at the corresponding concentration level. Error bars represent one geometric standard deviation. Linear, least-squares regression line shown for unweighted data points.

to be greater than for rats by a factor of the 0.3 power of the rat-to-mouse weight ratio. It follows that the pharmacokinetic modeling observations reported above for TCE bioassays with rats are also true for mice. This conclusion is supported by the comparative data of Green and Prout (1985) on TCE metabolism in B6C3F1 mice given single versus subchronic doses of TCE as discussed earlier.

The results described for respiratory bioassay exposures with rats and mice also hold true for gavage (or single-bolus ingestion) exposures, assuming constant ingestive infusion at different rates, R, for a single period of 15 minutes (see Figure 3-7b). Again, completed TCE metabolism within 24 hours is affected neither by increasing the gavage dose to high levels (e.g., up to 2500 mg/kg) at which metabolic saturation occurs, nor by increasing the continuous ingestion period from 15 minutes to several hours.

Finally, in the context of extrapolating the results of quantitative metabolic studies in animals to bioassay exposure conditions, it is sometimes necessary to know how body weight affects quantities such as f_{mo} and f_{mr} in animals exposed to higher dose levels at which metabolic saturation does occur. Unfortunately, a simple mathematical generalization is not possible in this case. However, results from simulations that use the PBPK model for TCE described above and the parameter values for a reference rat listed in Table 3-9 show that small changes in rodent body weight result in only minor deviations from the generalizations about fraction metabolized made at the end of the previous subsection on analysis of PBPK systems at steady state.

Application of PBPK Model to Human Data on TCE Metabolism

Data shown in Figure 3-8 demonstrate the extent to which urinary metabolites are produced in workers exposed to TCE in air at concentrations of 0 to 175 ppm (Ikeda et al., 1972; Ikeda, 1977). The data are derived from surveys of workplace air and urine samples collected from 51 males who worked in 10 TCE workshops in Japan. Workplace air concentrations of TCE were reported to be relatively constant over the common 8-hours/day, 6-days/week occupational schedule of the workers studied. Urine samples were passed at about 1:00 p.m. in the "latter half of a week" (Ikeda et al., 1972). Concentrations of metabolites (TCEL, TCA, and TTC) in urine were measured and plotted against time-weighted average concentrations of TCE in air. The relationship between the reported average TTC levels in urine (adjusted to

a specific gravity of 1.016) and corresponding ambient TCE concentrations ranging from 0 to 175 ppm are well represented by a 0-intercept-regression line ($r = 0.99$) with a slope of 5.0 mg TTC/L urine per ppm TCE (Figure 3).

Based on the Ramsey-Andersen model used by NRC (1986) to perform interspecies exposure-route extrapolations, a PBPK model was used to suggest TCE pharmacokinetics in humans occupationally exposed to ambient TCE at the levels and time pattern experienced by the Japanese workers observed by Ikeda et al. (1972). For the analysis, the Ramsey-Andersen model was extended to include the TCE metabolism model used by Fernandez et al. (1977), discussed above and illustrated in Figure 3-5. An explicit model of human urinary output as well as a urine-sampling scheme similar to that used in the Ikeda et al. study was also included.

Reference values for the parameters listed in Table 3-8 that were used in the PBPK model are given in Table 3-9. The values for the physiological parameters Q_a, Q_b, Q_i, and V_i, pertaining to the reference 70-kg male worker referred to in Table 3-9, are taken from a study by Ward et al. (1988). These researchers examined a PBPK model for humans exposed to tetrachloroethylene and obtained values similar to those used in the NRC-(1986) analysis. The values for the blood/air and fat/blood partition coefficients (P_b and P_2) for TCE were taken from U.S. EPA (1985a), as adapted from Sato et al. (1977). The values used for P_1 and P_4 are based on the liver/blood, muscle/blood, and blood/air partition coefficients for rats (equal to 1.69, 0.63, and 25.82, respectively) reported by Sato et al. (1977). The corresponding tissue/air partition coefficients for rats and humans are assumed to be equal. Supporting this assumption is the fact that the fat/air partition coefficients for rats and humans are 661 and 674, respectively, as reported by Sato et al. (1977). Similarly, the value used for P_3 is based on an average tissue/blood partition coefficient of 1.26 for rat visceral tissues that was calculated from the data of Sato et al. Finally, the metabolic rate-parameter values used by Fernandez et al. (1977) (see Figure 3-5) were used to represent those of a reference 70-kg worker.

A 70-kg male was assumed to produce an average of 0.0583 L urine per hour during the day (urine specific gravity adjusted to 1.016) (ICRP, 1975). It was also assumed that, in the absence of more specific data, urine samples were passed by the workers studied by Ikeda et al. (1972) at 1:00 p.m. on Thursdays, Fridays, and Saturdays of each week, and that a prior urination which emptied the bladder occurred 4 hours before, i.e., at 9:00 a.m., on each collection day for all workers.

For the purpose of modeling the occupational exposure studies by Ikeda et al. (1972), the reference parameter values just reviewed were scaled to approximate those applicable to a typical Japanese male worker. To this end, body weight was set at 55.2 kg and the tissue volumes V_i were adjusted to the new values shown in Table 3-9, based on an anatomical model for reference Japanese adults developed by Kerr (1979). As shown in Table 3-9, the values for the blood-flow fractions Q_2/Q_b and Q_3/Q_b were changed slightly to reflect the altered tissue volumes. The reference flow rates Q_a and Q_b and the flow rate for urinary output were all decreased by the factor $(55.2/70)^{0.7}$, and the reference rate constants for metabolism, which appear in Figure 3-5, were all increased by the factor $(70/55.2)^{0.3}$, for the reasons discussed above concerning rat-to-mouse extrapolation of PBPK-parameter values.

Workers were assumed to be exposed to TCE from 8:00 a.m. to 12:00 noon and 1:00 p.m. to 5:00 p.m., i.e., 8 hours/day with a 1-hour lunch break, on Monday through Saturday of each week.

To approximate conditions of dynamic equilibrium, the occupational exposure scenario was run for a simulated 5-week period. On each of the last 3 urine collection days of the simulation, calculated urinary concentrations of TCA and TCEL (adjusting for the molecular weight difference between these metabolites and TCE) were added and the 3 sums were averaged to yield a predicted concentration of TTC in urine corresponding to any given input value for the metabolic clearance parameter $K = V_{max}/K_m$. The unique value of K was estimated for which the model yields a value of 5.0 mg TTC/L urine, consistent with the data of Ikeda et al. (1972) for workers exposed to TCE in the manner described above. The estimate of K obtained is 47,000 L/hour, which from Equation (3-15) implies that 99.8% of the TCE in blood entering the liver is metabolized, which supports the approach taken by Fernandez et al. (1977) to model TCE metabolism in humans.

On the basis of this analysis of data on TCE metabolism in humans, a good estimate of the metabolized fraction f_{mr}* of TCE inhaled by humans at ambient concentration levels of 0 to 175 ppm, or dermally absorbed at similarly low levels, is therefore provided by Equation (3-14) to be approximately 72% of all the TCE contained in alveolarly ventilated air or dermally absorbed within this low-dose range, regardless of body weight. This value of 72% is somewhat less than the mean value of 91% (range of 87 to 93%) for the "percent of retained dose metabolized" (PRDM) based on 3 studies of TCE metabolism in humans experimentally exposed to TCE by inhalation for relatively brief periods,

discussed earlier and summarized in Table 3-6. This discrepancy is explained by the fact that, by definition, f_{mr} does not equal PRDM because PRDM already takes into account that only a (time-dependent) fraction, f_r, of respired air is cleared of TCE to constitute the "retained" TCE dose referred to by PRDM, such that f_{mr} equals PRDM times f_r. If it is assumed that the average value of f_r was approximately 0.78 for the 3 studies referred to (see Table 3-9, footnote b), then the product PRDM times f_r equals about 71%, which is consistent with the estimate of f_{mr}^* equal to 0.72, based on fitting a PBPK model to the data of Ikeda et al. (1972).

Again using the estimate of K, the fraction f_{mo}^* of ingested TCE that is metabolized by humans as projected by the analysis of the Ikeda et al. (1972) data is given by Equation (3-17) to be approximately 100%. Consequently, for humans subject to low levels of TCE exposure by both respiratory and ingestion pathways, Equation (3-18) may be rewritten as:

$$24A_m = 24(0.72Q_a \bar{C}_{in} + \bar{R}) \quad \text{or} \qquad (3\text{-}19a)$$

$$24A_m = 17Q_a \bar{C}_{in} + 2C_w, \qquad (3\text{-}19b)$$

which represents the daily dose of TCE metabolized (in mg/day), given a daily time-weighted average ambient concentration $\bar{C}_{in}$ (in mg/L) and ingestion rate $\bar{R}$ (in mg/hour) or water concentration C_w (in mg/L = 12 $\bar{R}$, assuming ingestion of 2 L/day).

4. TOXIC EFFECTS IN HUMANS AND ANIMALS

This section reviews the toxic effects of TCE in humans and animals. Previous reviews of the toxicity of TCE include those by Condie (1985), Kimbrough et al. (1985), U.S. EPA (1980, 1984a, 1985a, 1985b), World Health Organization (1985), Lauwerys (1983), National Research Council (1980), Cornish et al. (1977), Infante (1977), Van Duuren (1977), Waters and Black (1976), Waters et al. (1977), Walter et al. (1976), Huff (1971), Smith (1966), Defalque (1961), Atkinson (1960), and von Oettingen (1937, 1964). This review is not exhaustive but presents a comprehensive, current summary and update of the available literature.

TOXIC EFFECTS ON ORGANS AND SYSTEMS

A summary of dose-response information of toxic effects in major body organs and systems for different routes and periods of exposure is presented in Appendix A. In the absence of adequate human toxicity data, the information in Appendix A could serve as the basis of air standards to protect against acute, subchronic, and certain chronic toxicological endpoints (specifically, noncarcinogenic and nonmutagenic effects). Appendix A also includes a summary of TCE's toxicity to aquatic organisms.

Central Nervous System

Trichloroethylene is a potent central nervous system (CNS) depressant and has been used as an anesthetic and analgesic (Striker et al., 1935). Its popularity as an anesthetic declined with the accumulation of evidence that documented severe, and occasionally fatal, neurotoxic effects (Defalque, 1961; Atkinson, 1960; Tomasini and Sartorelli, 1971; Nowill et al., 1954; Thierstein et al., 1960; Nomura, 1962).

Occupational exposure to TCE has resulted in nausea, headache, loss of appetite, weakness, dizziness, ataxia, and tremors (Longley and Jones, 1963; Milby, 1968; Mitchell and Parsons-Smith, 1969; Okawa and Bodner, 1973). Acute exposure to high concentrations of TCE has caused irreversible nerve damage (Mitchell and Parsons-Smith, 1969; Buxton and Hayward, 1967; Barret et al., 1982) and death (Kleinfeld and Tabershaw, 1954; James, 1963; Buxton and

Hayward, 1967). Evidence from experimental human and animal studies indicates that TCE induces a variety of adverse effects on the CNS (Annau, 1981). In a controlled experiment conducted by Salvini et al. (1971), 6 individuals were exposed to 110 ppm of TCE vapor for 2 4-hour periods. Volunteers had a statistically significant ($p < 0.001$) reduction in performance on various psychophysiological function tests, such as perceptiveness, immediate memory, and complex reaction time. In an attempt to remove a source of bias, the experiment was repeated with 6 workers familiar with the odor of TCE. Similar decrements in test performance were also noted in these individuals.

Vernon and Ferguson (1969) evaluated the effects of TCE exposure (100 to 1000 ppm TCE for 2 hours) on the psychophysiologic performance of 8 volunteers. Exposure to 1000 ppm TCE caused a statistically significant ($p < 0.05$) decrease in performance on the Howard-Dolman, steadiness, and pegboard tests. Stopps and McLaughlin (1967) also reported a decline in psychophysiologic test performance following exposure to 500 ppm TCE for approximately 3 hours. Stewart et al. (1970) reported mild fatigue and sleepiness in 5 subjects exposed to 200 ppm TCE for 4 to 5 7-hour workdays.

Triebig et al. (1982) did not detect any change in motor or sensory nerve-conduction velocity in persons occupationally exposed to TCE (400 ppm, 1 month to 36 years). However, other reports have documented trigeminal nerve impairment in workers who were chronically exposed to TCE (Barret et al., 1982; Mitchell and Parsons-Smith, 1969). Exposure concentration and duration were not provided.

Alcohol intolerance associated with exposure to TCE has been described by Defalque (1961) and Müller et al. (1975). The experimental data of Müller et al. (1975) indicate that simultaneous exposure to TCE and ethanol results in a marked inhibition of TCE metabolism. This leads to an accumulation of TCE in blood, which increases the extent of CNS depression.

Several animal studies have demonstrated behavioral changes due to TCE exposure. Grandjean (1960) exposed starved rats to 200 or 800 ppm of TCE for 3 hours and noted the reaction time to a food-motivated, preconditioned response. The average reaction time of the exposed rats was approximately the same as that of the controls. However, the total number of times an animal responded to a test stimulus was significantly greater in treated animals ($p < 0.01$) than in controls. These data indicate that TCE may cause an increase in excitability or loss of inhibition in rats. Khorvat and Formanek (1959) observed similar effects in rats exposed to 400 ppm of TCE for 49 days. Kjellstrand et al. (1985) noted various effects on motor activity in mice after

a 1 hour exposure to TCE concentrations varying from 700 to 2300 ppm.

Goldberg et al. (1964a) found that rats exposed to TCE (200, 560, 1568, or 4380 ppm, 4 hours/day for 10 days) exhibited a specific inhibition of the avoidance response. This effect was not clearly concentration dependent, however. Furthermore, animals exposed to 1568 ppm showed "some evidence" of ataxia, while gross ataxia was observed in animals treated with 4380 ppm TCE. Test results also indicated a slight increase in learning ability following exposure at 200 ppm, while all other concentrations inhibited learning ability.

To develop exposure protocols that were representative of episodes of TCE "sniffing," Utesch et al. (1981) exposed rats to high concentrations of TCE (i.e., 9000, 12,000, 14,000, or 15,000 ppm) for up to 15 minutes. Animals displayed signs of CNS depression, such as difficulty in locomotion and loss of the righting reflex. With the exception of animals exposed to 15,000 ppm, recovery was rapid. In high-dose animals, the effects of exposure persisted for several hours. In a separate experiment, the coupling of ethanol exposures and/or fasting regimens with exposure to TCE had no marked effects on neurological responses.

Baetjer et al. (1970) studied the effect of dehydration on rats exposed to 2500 or 3000 ppm of TCE for 3 days. The CNS function was measured by the "operant-behavior technique" of hypothalamic self stimulation. Rats subjected to dehydration during exposure to TCE exhibited a reduction in hypothalamic self stimulation. The significance of this observation is not known. However, it was suggested by Baetjer et al. (1970) that the implanted electrodes may have been responsible for the measured effect.

Subchronic exposure of rats to TCE at 2600, 5000, or 8000 ppm 30 minutes/day, 6 days/week for 80 days caused a statistically significant ($p < 0.05$) decrease in open-field ambulation performance and response time (Ikeda et al., 1980b). In a study conducted by Battig and Grandjean (1963), rats that were exposed to 400 ppm TCE 8 hours/day, 5 days/week, for 44 weeks showed a decrease in swimming performance, exploratory activity, and learning ability (Hebb test or conditioned-avoidance response test).

Mikiskova and Mikiska (1966) demonstrated that TCE can also cause CNS depression with intraperitoneal (IP) administration. Guinea pigs given a single IP dose of 880 mg/kg developed ataxia, a loss of muscle tone, and hyperalgesia (an excessive sensitiveness to pain).

Liver

Baerg and Kimberg (1970) described 3 cases of intentional intermittent

exposure to TCE-containing products by humans that resulted in drowsiness, headache, vomiting, fever, chills, abdominal pain, jaundice, abnormal hepatic function, and hepatic necrosis. In 2 of the 3 cases, hepatic damage occurred after inhalation of Carbona, a cleaning fluid composed of 44% TCE and 56% petroleum distillates.

Occupational exposure to TCE from heated degreasing tanks was described by Thiele et al. (1982). A worker reportedly became inebriated after periods of exposure to TCE fumes from the tank. In addition to these intermittent exposures, the individual was inadvertently trapped several times in tanks that contained residual TCE. Upon switching jobs, this worker was exposed to 1,1,1-trichloroethane and began to suffer from fatigue, weight loss, anorexia, icterus, and abdominal swelling. Results from liver tests were abnormal and the worker was diagnosed as having hepatic cirrhosis. There was no history of alcohol consumption or hepatitis, and Thiele et al. (1982) attributed the cirrhosis to repeated exposure to chlorinated hydrocarbons.

The U.S. EPA (1985a) and the World Health Organization (WHO) (1985) have reviewed a number of early occupational and medical reports on the hepatotoxicity of TCE. Exposure to TCE has not consistently produced liver damage. Of 248 patients anesthetized with TCE, none had evidence of hepatic injury (Brittain, 1948). However, lethal hepatic failure has occurred in some instances following TCE-induced anesthesia (Ayre, 1945; Dodds, 1945; Elam, 1949; Werch et al., 1955).

Accidental or occupational exposure to TCE has also caused liver disease, necrosis, and hepatic failure. For example, Cotter (1950) found that accidental exposure to TCE induced hyperglobulinemia and hypercalcemia. Secchi et al. (1968) reviewed 7 cases of poisoning caused by ingestion of TCE. Liver damage was documented in 3 of these individuals. The authors noted, however, that this damage may have been due to the presence of contaminants. More recently, Phoon et al. (1984) described jaundice, hepatomegaly, and abnormal liver function in 5 individuals occupationally exposed to TCE (unknown concentration) for 2 to 5 weeks. One of these workers eventually died from liver failure.

Kleinfeld and Tabershaw (1954) reported the death of an individual following accidental ingestion of an unspecified quantity of TCE. Prior to his death, the individual developed jaundice (elevated bilirubin levels); at autopsy, evidence of severe centrilobular necrosis was found. Liver failure following TCE exposure was also implicated in a case reported by Joron et al. (1955). Trichloroethylene caused hepatitis and jaundice in an individual

exposed to 27 to 294 ppm. Liver damage progressed to massive liver necrosis, which was ultimately fatal.

Stott et al. (1982) examined the effects of orally administered TCE on rats and mice. In one group of experiments, mice were given 2400 mg/kg of TCE by gavage daily for 3 days, or 5 days/week for 3 weeks. The livers of animals from both groups exhibited altered hepatocellular morphology, centrilobular hepatocellular swelling, and necrosis. A statistically significant ($p < 0.01$) increase in liver weight and hepatic DNA synthesis was observed, as well as a slight reduction in DNA content per gram of liver. Stott et al. (1982) documented similar effects in mice that received oral doses of 250, 500, 1200, or 2400 mg/kg-day of TCE daily for 3 weeks. Rats given 1100 mg/kg of TCE by gavage 5 days/week for 3 weeks had a statistically significant ($p < 0.01$) increase in liver weight and in hepatic DNA synthesis. However, there were no statistically significant changes in the livers of rats that received 1100 mg/kg of TCE for 3 days.

Tucker et al. (1982) conducted an experiment in which TCE was administered to mice by gavage at 24 and 240 mg/kg per day for 14 days. A statistically significant ($p < 0.05$) increase in liver weight was observed only in animals that received 240 mg/kg-day.

In a recent study by Buben and O'Flaherty (1985), mice were given 100, 200, 400, 800, 1600, 2400, or 3200 mg/kg of TCE by gavage 5 days/week for 6 weeks. All dose levels caused a statistically significant ($p < 0.001$) increase in the liver-weight/body-weight ratio. Glucose-6-phosphatase (G-6-P) activity was significantly lower ($p < 0.01$) in the livers of animals that received doses of 800 mg/kg or more. Liver triglycerides increased significantly ($p < 0.01$) at 2400 mg/kg and serum glutamic pyruvic transaminase (SGPT) activity increased significantly ($p < 0.05$) after treatment with 2400 mg/kg or 3200 mg/kg. Buben and O'Flaherty (1985) also analyzed the DNA content of hepatocytes of mice from 2 treatment groups (400 and 1600 mg/kg). They reported a statistically significant decrease in DNA content in cells from both groups ($p < 0.05$ and $p < 0.001$, respectively).

Kylin et al. (1962, 1963, 1965) conducted a series of experiments in which mice were exposed to TCE by inhalation. Following exposure, the livers of animals were examined histologically for evidence of fatty infiltration and liver damage. After a single 4-hour exposure to 800, 1600, 3200, or 6400 ppm TCE, Kylin et al. (1962) observed only "slight" fatty infiltration of the liver. (However, 2 of 10 animals exposed to 6400 ppm TCE died during the experiment). When part of this study was repeated (4-hour exposure to 1600 or

3200 ppm TCE), no marked difference was seen in the livers of treated mice compared to controls (Kylin et al., 1963). Mice exposed to 1600 ppm TCE for 4 hours/day, 6 days/week for 1, 2, 4, or 8 weeks had a statistically significant increase ($p < 0.01$) in liver fat (Kylin et al., 1965).

Kimmerle and Eben (1973a) evaluated the toxicity of TCE to rats after acute and subchronic inhalation exposure. One group of animals was exposed to 49, 175, or 330 ppm TCE for 4 hours, while a separate group was exposed to 55 ppm of TCE 8 hours/day, 5 days/week for 14 weeks. At the end of treatment, liver- and kidney-function test results were within normal limits. However, treated animals had a significant ($p < 0.01$) increase in relative and absolute liver weight.

Continuous exposure to 150 ppm TCE for 30 days caused a statistically significant increase ($p < 0.05$) in the liver weights of mice, rats, and gerbils (Kjellstrand et al., 1981). Kjellstrand et al. (1983a) used the same protocol to evaluate the effects of TCE on 7 different strains of mice. All strains had a significant increase ($p < 0.001$) in plasma butylcholinesterase activity.

In a subsequent study by Kjellstrand et al. (1983b), mice were exposed to 37, 75, 150, or 300 ppm of TCE vapor continuously for 30 days. Statistically significant ($p < 0.05$) increases in plasma butylcholinesterase activity were found in the males and increases in liver weight were measured in both sexes at concentrations of TCE greater than 75 ppm. In a concurrent experiment, mice were exposed to 225, 450, 900, 1800, or 3600 ppm of TCE for 1, 2, 4, 8, or 16 hours/day, 7 days/week for 30 days. Kjellstrand et al. (1983b) reported that intermittent exposure also caused a significant ($p < 0.05$) increase in plasma butylcholinesterase activity and liver weight.

Cornish and Adefuin (1966) investigated the effects of TCE exposure in rats previously treated with ethanol. Rats were given oral doses of ethanol (5 g/kg) 16 to 18 hours prior to treatment with TCE. Animals were then exposed to 2000 or 5000 ppm TCE for 4 hours. A statistically significant increase was noted in the activity of SGPT ($p < 0.001$), serum isocitric dehydrogenase (SICD) ($p < 0.001$), and in serum glutamic-oxaloacetic transaminase (SGOT) ($p < 0.05$). In a second experiment, rats were exposed to 100 ppm TCE for 8 hours, with or without ethanol. No marked changes in enzyme activity were observed following either treatment.

Moslen et al. (1977a, 1977b) examined the effect of microsomal enzyme induction on TCE-induced liver damage. Hepatic damage occurred in rats anesthetized with TCE following pretreatment with 5 different inducers of microsomal enzymes. Animals were given 400 μmol/kg

3-methycholanthrene (3-MC), phenobarbital, hexachlorobenzene, spironolactone, pregnenolone-16-alpha-carbonitrile, or 150 to 300 μmol/kg of Aroclor 1254 by gavage, daily for 7 days. Rats from all treatment groups were subsequently exposed to 1% TCE for 2 hours. In vehicle control animals (corn oil pretreatment), exposure to TCE did not cause hepatic injury. Moslen et al. (1977a) noted a striking increase in SGOT activity ($p < 0.01$ to $p < 0.001$) accompanied by hepatic necrosis in all other animals, except those pretreated with spironolactone. In a separate study, Moslen et al. (1977b) evaluated the effects of TCE on hepatic enzyme activity in rats pretreated with phenobarbital. Rats were given an oral dose of 400 μmol of phenobarbital or 5 mL/kg of vehicle daily for 7 days followed by a 2-hour exposure to 1% TCE (by volume). Liver homogenates from phenobarbital-treated animals had significantly lower levels of cytochrome P-450 ($p < 0.001$) and cytochrome b_5 ($p < 0.05$) compared to controls. Phenobarbital pretreatment caused hepatic glutathione (GSH) levels to decrease to half of control levels during the exposure, but GSH increased to above normal levels 8 hours after the end of treatment. Moslen et al. (1977b) also reported a marked increase in the rate of NADH-cytochrome c reduction following exposure to phenobarbital and TCE, but did not find any significant changes in the activity of other microsomal enzymes: aminopyrine N-demethylase, ethylmorphine N-demethylase, G-6-P, and zoxazolamine hydroxylase.

Carlson (1974) studied the effects of TCE in rats treated with 3-MC or phenobarbital. In one phase of this study, animals were exposed to 6900, 7800, 10,400, 12,000, or 16,000 ppm TCE for 2 hours. Forty-eight hours later, half of these animals were given an intraperitoneal (IP) injection of 40 mg/kg 3-MC; the remaining animals served as controls. A separate group of rats received 4 IP injections of 50 mg/kg phenobarbital daily for 4 days prior to a 2-hour exposure to 10,400 ppm TCE. Isocitrate dehydrogenase activity was significantly greater ($p < 0.05$) in animals exposed to 7800 ppm TCE, both with and without 3-MC treatment. Carlson (1974) also measured a significant increase ($p < 0.05$) in SGPT and SGOT activity in animals exposed to 6900 or 10,400 ppm TCE. Treatment with 3-MC did not appear to affect the levels of these enzymes. Pretreatment with phenobarbital followed by exposure to 10,400 ppm TCE caused a significant ($p < 0.05$) decrease in G-6-P activity.

Norpoth et al. (1974) measured a statistically significant ($p < 0.05$) increase in cytochrome P-450 content in the liver homogenates of rats exposed to 470 ppm of TCE 3 hours/day for 10 days. Aminopyrine demethylase activity was not significantly affected by this treatment.

Vainio et al. (1978) exposed rats to TCE at 7.9 μmol/L 6 hours/day for 4 or 5 days. No effects were observed after 4 days. However, exposure to TCE for 5 days was associated with an increase in hepatic cytochrome P-450 content. The statistical significance of this increase was not given.

Exposure to 500 to 1000 ppm of TCE 18 hours/day for 3 months produced no measurable effect on the liver function of rats, rabbits, or a dog (Nowill et al., 1954).

A correlation has been established between sleep prolongation after anesthetization and microscopic hepatic derangement. Plaa et al. (1958) attempted to define an ED_{50} for TCE-induced hepatic damage by using prolongation of sleeping time as an index. Mice were injected subcutaneously with 749, 1130, 1314, 1840, or 2627 mg/kg of TCE; these doses were associated with "varying degrees of centrilobular necrosis." Plaa et al. (1958) then measured the duration of the resulting anesthesia and used these data to develop a dose-response curve. An ED_{50} of 1445 mg/kg was estimated from this curve.

Klaassen and Plaa (1966) studied TCE-induced liver dysfunction in mice by measuring SGPT activity and retention of sulfobromophthalein (BSP) after IP injections of TCE. These authors reported an ED_{50} for BSP retention of 2.0 mL/kg and an ED_{50} for elevation of SGPT activity of 1.6 mL/kg. In addition, IP administration of 1.5 mL/kg TCE produced enlargement of hepatocytes, with cellular infiltration, vacuolation, and slight necrosis. When a single oral dose of ethanol (5 g/kg) was given 12 hours or 3 days prior to treatment with TCE, no significant change in BSP retention was observed.

A later study by Klaassen and Plaa (1967) found that the IP ED_{50} in dogs for an increase in SGPT activity was 0.57 mL/kg (828 mg/kg). At this dose, TCE produced moderate neutrophilic infiltration in the sinusoids and portal areas of the liver but did not cause necrosis. A single gavage dose of ethanol (4 g/kg) 24 hours prior to treatment with TCE caused a significant increase in SGPT activity ($p < 0.05$).

Lewis et al. (1984) administered 330 mg/kg of TCE to mice by IP injection every other day over a 5-day period. A second group of mice was exposed to 10,000 ppm TCE for 1 or 4 hours daily for 5 consecutive days. The activity of liver microsomal NADPH-cytochrome c reductase was significantly greater ($p < 0.05$) in animals that were exposed to TCE by inhalation for 4 hours/day or that received TCE by IP injection. Exposure to TCE for 1 hour/day did not cause a significant change in enzyme activity.

The protective effects of sodium diethyldithiocarbamate (DDC) and carbon

disulfide (CS_2) against TCE-induced liver injury in mice were investigated by Masuda and Nakayama (1982). Administration of TCE (2.0 mL IP) produced "moderate increases" in plasma glutamic-pyruvic transaminase (GPT) activity and liver calcium content. These effects were inhibited when oral doses of DDC (10, 30, 100 mg/kg) or CS_2 (3, 10, 30 mg/kg) were given 30 minutes prior to treatment with TCE.

Danni et al. (1981) studied the ability of TCE to induce peroxidation of liver microsomal lipids. Rats were given a single 678 mg/kg dose of TCE by gavage. Hepatic lipids from these animals were analyzed for signs of peroxidation. (Lipid peroxidation in unsaturated fatty acids produces conjugated dienes, which can be measured spectrophotometrically.) However, no evidence of lipid peroxidation was found. In a parallel study, male and female rats received 170 mg/kg TCE 48 hours after an IP injection of 5 or 8 mg/kg of phenobarbital. Statistically significant increases in hepatic triglycerides were measured in male and female rats ($p < 0.025$ and $p < 0.005$, respectively) at both doses of phenobarbital. Rats that received only TCE had no measurable adverse effects. A third group of rats was given 85 or 339 mg/kg TCE orally. No effect was found on hepatic or serum lipoproteins.

Cornish et al. (1973) treated rats with phenobarbital (50 mg/kg IP) for 1 or 2 days prior to a single IP dose of TCE (0.3, 0.5, 1, or 2 mL/kg). A separate group of rats received TCE alone. No significant difference in SGOT activity was found in any of the treatment groups. In contrast to the results of Cornish et al. (1973), Allemand et al. (1978) found that phenobarbital and TCE had a significant effect on hepatic enzymes of rats. Animals that were given 70 mg/kg of phenobarbital by IP injection daily for 5 days followed by a single IP dose of TCE (1.0 mL/kg) had a significant decrease ($p < 0.001$) in hepatic cytochrome P-450 content. The SGPT activity was significantly greater ($p < 0.05$) in rats treated with phenobarbital as above and TCE in single IP doses of 0.25, 1.0, or 2.0 mL/kg. Phenobarbital alone did not significantly affect SGPT levels. Analogous experiments, in which cobaltous chloride (30 mg/kg) was administered subcutaneously twice daily for 3 days prior to treatment with TCE, caused a significant decrease ($p < 0.05$) in SGPT activity. Allemand et al. (1978) also found that hepatic GSH levels decreased by 61% 4 hours after a single IP dose of TCE (2.0 mL/kg). However, 16 hours after treatment, GSH levels were 108% of control.

The biochemical mechanism of TCE-induced hepatocarcinogenicity has been the focus of studies by Elcombe (1985), Elcombe et al. (1985), Mirsalis et al. (1985), and Goldsworthy and Popp (1987). Results from these studies have led

to a new theory of carcinogenesis implicating peroxisome proliferation and the differential induction of peroxisomal enzymes in neoplastic transformation (see review by Reddy and Lalwani (1983). This hypothesis proposes that certain xenobiotic chemicals cause an increase in the number of hepatic peroxisomes as well as certain alterations in peroxisome metabolism, e.g., increased oxidation of fatty acids. According to this theory, these changes lead to an increase in intracellular H_2O_2 or other reactive oxygen species which in turn react with critical cellular macromolecules, such as DNA.

Although peroxisome proliferation has been observed primarily in response to hypolipidemic drugs and phthalate ester plasticizers, Elcombe (1985), Elcombe et al. (1985), and Goldsworthy and Popp (1987) have documented proliferation of peroxisomes and/or changes in peroxisomal enzymes in rodents following treatment with TCE and TCA. Based on the observation that the levels of TCA in mouse blood were approximately 7 times those in rats following administration of TCE (Green and Prout, 1985), Elcombe (1985) and Elcombe et al. (1985) suggested that hepatic peroxisome proliferation is actually induced by TCA rather than by TCE and that the different quantities of TCA in serum may be the basis of the apparent difference in species sensitivity to TCE. Mice that had been treated with 50 to 2000 mg/kg of TCE by gavage for 10 days exhibited a dose-related increase in the volume density of peroxisomes and in peroxisomal oxidation of fatty acids (Elcombe, 1985). No significant effects were noted in the livers of rats that received the same treatment. When TCA was administered to rats and mice at 200 mg/kg for 10 days, the activity of peroxisomal fatty acid oxidation increased 6.5-fold in rats and 4.8-fold in mice (Elcombe, 1985). Elcombe et al. (1985) also observed a dose-related increase, as much as 1100% of controls, in hepatic peroxisomes of mice treated with 500 to 1500 mg/kg TCE. Only slight increases (127 to 133% of controls) were seen in rats.

Goldsworthy and Popp (1987) found that TCA (500 mg/kg) was more effective than TCE (1000 mg/kg) in inducing peroxisomal oxidation of fatty acids in rats (284% versus 180% of controls). In mice, TCE and TCA caused a 625% and 280% increase, respectively, in fatty acid oxidation.

An alternate model of carcinogenesis proposes that cellular proliferation is a crucial component of neoplastic transformation (Moolgavkar, 1983; Thorslund et al., 1987). Mirsalis et al. (1985) and Elcombe et al. (1985) provided evidence that TCE induces proliferation of hepatocytes as measured by an increase in DNA synthesis. It is not clear whether TCE induces a primary mitogenic response or whether the increase in DNA synthesis is due to DNA

repair. However, Elcombe et al. (1985) measured a 500% increase in DNA synthesis in the hepatocytes of mice after animals received 500 to 1500 mg/kg TCE by gavage for 10 days. DNA synthesis was not affected in rats. These doses did not elicit any overt signs of hepatotoxicity. Mirsalis et al. (1985) have also found that TCE-induced DNA synthesis, but not DNA repair, in mouse hepatocytes. Neither synthesis or repair of DNA were observed in hepatocytes from TCE-treated rats.

Kidney

Renal damage following TCE exposure is relatively uncommon. However, Baerg and Kimberg (1970) diagnosed acute tubular necrosis in 2 individuals with a history of repeated inhalation of Carbona, a cleaning fluid composed of 44% TCE and 56% petroleum distillates. These authors cited a number of other case reports that also implicated TCE exposure in renal damage.

Gutch et al. (1965) documented hyperkalemia and elevated blood urea nitrogen levels in an individual occupationally exposed to TCE ($\geq$ 2 hours). A kidney biopsy revealed abnormalities of the glomeruli and tubular degeneration. An acute occupational exposure to TCE of unknown concentration reportedly caused oliguria and hepatorenal insufficiency (Suciu and Olinici, 1983). Kleinfeld and Tabershaw (1954) reported that 1 of 5 deaths they attributed to TCE exposure was caused by acute renal failure.

Several animal studies have found no evidence of renal toxicity in mice or rats exposed to TCE (Nowill et al., 1954; Utesch et al., 1981; Stott et al., 1982) In contrast to these reports, Tucker et al. (1982) found a significant increase ($p < 0.05$) in the kidney weights of mice that received 5.0 mg/mL TCE in drinking water for 4 to 6 months (2.5 and 5.0 mg/mL). In addition, high-dose females (5.0 mg/mL) and males in the 2 highest dosage groups (2.5 and 5.0 mg/mL) excreted greater amounts of protein and ketones in their urine than controls. More specific data were not published.

A series of studies by Kjellstrand et al. (1981, 1983a, 1983b) has documented nonspecific effects of TCE on the kidney. For example, male and female gerbils exposed to 150 ppm TCE continuously for 30 days had a significant ($p < 0.05$) increase in kidney weight (Kjellstrand et al., 1981). Continuous exposure of NMRI mice to TCE for 30 days (37, 75, 150, or 300 ppm) caused a statistically significant ($p < 0.05$) increase in kidney weight at $\geq$ 75 ppm (males) and $\geq$ 150 ppm (females) (Kjellstrand et al., 1983b). No differences in sensitivity to TCE-induced kidney toxicity were observed among 7

strains of mice (Kjellstrand et al., 1983a).

Chakrabarti and Tuchweber (1988) demonstrated significant increases in urinary levels of N-acetyl-B-glucose-D-aminidase and glucose and in serum urea nitrogen in phenobarbital-treated male rats, 24 hours after an intraperitoneal injection of 22 mmoles/kg TCE. These effects were not seen at lower doses of 5.5 and 11 mmoles/kg. After inhalation exposure to 1000 or 2000 ppm TCE for 6 hours, significant increases were seen in urinary levels of glutamyl transpeptidase and proteins as well as in the 3 materials increased after intraperitoneal injection. These studies demonstrate acute nephrotoxic effects of TCE at high levels.

Cardiovascular System

The use of TCE as a surgical anesthetic has provided a number of case reports that have documented its adverse effects on the cardiovascular system. Its cardiotoxicity has been reviewed by Norris and Stuart (1957), Defalque (1961), and the U.S. EPA (1985a). In addition, the World Health Organization (WHO, 1985) published a comprehensive review of foreign-language articles on the cardiovascular effects of TCE in humans. These effects reportedly include cardiac arrest, atrial and ventricular extrasystole, tachycardia, and ventricular fibrillation.

Inhalation or ingestion of TCE has resulted in a number of instances of sudden death, presumably due to heart failure. Cases of nonlethal cardiac arrest due to TCE exposure have also been reported. For the most part, the concentration of TCE and the duration of exposure that caused these effects have not been clearly established (Kleinfeld and Tabershaw, 1954; Bernstine, 1954; Norris and Stuart, 1957; Dhuner et al., 1957; James, 1963; Defalque, 1961; U.S. EPA, 1985a).

Trichloroethylene can cause sensitization of the cardiac muscle to epinephrine. Concurrent administration of TCE and epinephrine has induced arrhythmia and tachycardia that were fatal in some instances (Defalque, 1961; U.S. EPA, 1985a). These phenomena have also been observed in animals.

White and Carlson (1979) studied epinephrine-induced cardiac arrhythmias in rabbits and rats. Animals were pretreated with agents that induced (phenobarbital or Aroclor 1254) or inhibited (SKF 525A or Lilly 18947) microsomal enzymes. Rabbits were exposed to 3000 ppm TCE for 7.5 to 60 minutes, while rats were exposed to 25,000 ppm TCE for 10 to 60 minutes. Increasing doses of epinephrine (0.5 to 4.0 μg/kg) were administered

Cardiac arrhythmias were not observed in rats treated with phenobarbital, Aroclor 1254, or Lilly 18947 and subsequently exposed to TCE and epinephrine. However, rats pretreated with SKF 525A developed arrhythmias in response to TCE, and sensitivity to epinephrine became more pronounced over the course of an hour. In rabbits, arrhythmias were observed in response to epinephrine in all treatment groups. The effective dose of epinephrine varied with the pretreatment regimen and duration of exposure to TCE.

In a later study, White and Carlson (1981) examined the effect of ethanol on epinephrine-induced cardiac arrhythmias in rabbits exposed to TCE by inhalation. A single dose of 1 g/kg ethanol was given either orally or intravenously to rabbits 30 minutes before exposure to 6000 TCE ppm for 1 hour. Epinephrine (0.5 to 3.0 μg/kg) was then injected until arrhythmia developed. Rabbits treated orally with ethanol and TCE developed epinephrine-induced cardiac arrhythmias sooner and at lower doses of epinephrine than control rabbits. Ethanol administered by intravenous injection induced arrhythmias in rabbits more often than orally administered ethanol. No cardiac arrhythmia occurred when TCE was administered alone. The authors commented that concomitant ethanol ingestion and TCE exposure could pose a significant health hazard.

Chloral hydrate administration has been associated with cardiac arrhythmia in people (DiGiovanni, 1969). Fossa et al. (1982) postulated that the arrhythmias associated with TCE exposure could also be due to TCE itself, rather than to one of its metabolites. To evaluate this hypothesis, rabbits were given an oral dose of 1.0 g disulfiram 6 or 24 hours prior to exposure to 6000 ppm TCE for 7.5 to 30 minutes. (Disulfiram inhibits the metabolism of xenobiotic chemicals.) However, rabbits treated with disulfiram failed to develop arrhythmias, even when challenged with 3.0 μg/kg epinephrine. When TCE was increased to 9000 ppm for 7.5 to 60 minutes and disulfiram or its metabolites DDC and CS_2 were administered intraperitoneally, a significant ($p < 0.05$) number of animals developed arrhythmias during the first 30 minutes of exposure to TCE. When exposure to TCE was continued for 30 to 60 minutes, a significant decrease ($p < 0.05$) in arrhythmias was noted in disulfiram- or DDC-treated animals. The authors concluded that disulfiram possesses some cardioprotective activity that is not related to its effects on metabolism.

Carlson and White (1983) examined the effect of benzo(a)pyrene (B(a)P) and 3-methylcholanthrene (3-MC) on the cardiotoxicity of TCE. Rabbits were exposed to 8100 ppm TCE for 1 hour, either 48 or 72 hours after a 40 mg/kg injection of B(a)P or 3-MC. Subsequent treatment with 1.0 to 3.0 μg/kg epinephrine

revealed that B(a)P increased the incidence of arrhythmias compared to controls. No statistical evaluation was provided. Cardiotoxicity of TCE was not potentiated by 3-MC.

Reinhardt et al. (1973) exposed 12 male beagles to 0.5 or 1.0% TCE vapor for 10 minutes. Exposure to TCE was followed by 2 injections of 8 mg/kg epinephrine. No effect was observed at the lower concentration. One dog experienced ventricular fibrillation and cardiac arrest after exposure to 1% TCE and several other dogs developed cardiac arrhythmia.

Lungs

When used as an inhalation anesthetic, TCE vapor was "slightly irritating" to the respiratory tract of patients (Atkinson 1960; U.S. EPA, 1985a). However, the primary respiratory problem associated with the medical use of TCE has been a pronounced increase in the rate of respiration (tachypnea). Although Atkinson (1960) stated that the magnitude of this effect was dose related, the effective concentrations of TCE were not given. The TCE-induced tachypnea has commonly been associated with the onset of shallow respiration.

Coleridge et al. (1968) attempted to determine the physiologic basis for TCE-induced alterations in respiration. Earlier work, cited by Coleridge et al. (1968), had indicated that an increase in excitability of the pulmonary stretch receptors may be responsible for the decrease in depth of respiration during TCE anesthesia. This study found that the frequency of discharge of the pulmonary stretch receptors increased when cats and dogs were exposed to 1.0 or 3.0% TCE for 5 minutes. In concurrent experiments, TCE did not produce any change in the impulse frequency of the pulmonary endings of vagal nerve fibers. Prendergast et al. (1967) conducted an extensive study on dogs, guinea pigs, rats, rabbits, and monkeys, in which the animals were exposed to TCE at 189 mg/m^3 continuously for 90 days or to 3825 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks. No visible signs of toxicity were noted after exposure to 189 mg/m^3. All animals exposed to 3815 mg/m^3 had nonspecific inflammatory changes of the lung; some rats and guinea pigs also developed lung congestion.

Recent studies by Forkert et al. (1985) and Forkert and Troughton (1987) assessed the damage induced by a single intraperitoneal dose of 2000, 2500, or 3000 mg/kg of TCE. The 2000 mg/kg dose caused injury to (nonciliated) Clara cells of the bronchiolar epithelium within 24 hours, as well as a significant decrease ($p < 0.05$) in pulmonary cytochrome P-450 and aryl hydrocarbon

hydroxylase activity. Various degenerative changes including loss of cells were noted in Clara cells following administration of TCE. In addition, high dose animals developed bronchiolar necrosis, pathologic changes in Type II alveolar cells, an increased accumulation of pulmonary calcium, and prolonged anesthesia-recovery time.

Lewis et al. (1984) exposed mice to 10,000 ppm TCE for 1 or 4 hours/day on 5 consecutive days. This treatment regimen caused a statistically significant decrease ($p < 0.01$) in the activity of pulmonary NADPH-cytochrome c reductase. Treated animals also displayed some evidence of pathologic changes in their lungs including thrombus formation and vacuolization of bronchiolar epithelia.

Brain

Buxton and Hayward (1967) described an industrial incident involving 4 men who were exposed to TCE. The actual concentrations were not known. Of the 4 men, 2 developed severe multiple cranial nerve palsies and 1 of them died after 51 days. An autopsy revealed damage to the brainstem, cerebral cortex, and spinal cord.

Haglid et al. (1981) studied the impact of TCE on the levels of the glial brain-specific protein S100 in discrete areas of the brain. Gerbils were exposed to 60 or 320 ppm TCE continuously for 3 months. A pronounced decrease in total soluble protein and a significant ($p < 0.05$) increase in S100 protein were measured in the hippocampus, cerebellar vermis (posterior), and brain stem of exposed animals. The DNA content of cells was significantly ($p < 0.05$ to $p < 0.01$) elevated in the cerebellar vermis and sensory motor cortex in animals exposed to 320 ppm.

Savolainen et al. (1977) and Vainio et al. (1978) collaborated on a series of experiments in which rats were exposed to 200 ppm TCE 6 hours/day for 5 days. Both studies reported a decrease in the brain RNA content of treated animals. Statistical significance was not given. In addition, Savolainen et al. (1977) found that exposure to TCE had no significant effect on brain protein content, but that brain acid proteinase activity was higher in treated rats on the fifth day of exposure to TCE.

Honma et al. (1980) measured levels of neurotransmitters in the brain of rats after exposure to 200, 400, or 800 ppm TCE. Following 1 month of treatment, biochemical effects were observed only in the rats exposed to 800 ppm TCE. Statistically significant ($p < 0.05$) reductions occurred in the acetylcholine content of the striatum and in the norepinephrine content of the

cortex and hippocampus; only slight increases of dopamine were measured in the striatum. In addition, the serotonin content of the cortex and hippocampus was slightly elevated in the treated animals.

Kjellstrand et al. (1982a) evaluated acid phosphatase (ACPase) activity in the brain of animals exposed to TCE. The ACPase is a lysosomal marker enzyme; changes in ACPase activity may be indicative of neuronal damage. Rats, mice, and gerbils were exposed continuously to 150 ppm TCE for 30 days. At the end of treatment, Kjellstrand et al. (1982a) found significant differences in total ACPase activity and in ACPase activity per mg of tissue in the brain stem of mice ($p < 0.05$) and gerbils ($p < 0.01$). Kanje et al. (1981) also found statistically significant differences in the ACPase activity of the brain stem of mice ($p < 0.05$) and gerbils ($p < 0.01$) exposed continuously to 170 ppm TCE for 30 days. The same treatment had no measurable effect on brain acetylcholinesterase, glutamine synthetase, or glutamate dehydrogenase activity.

Bartonicek and Brun (1970) reported a broad spectrum of changes in the nerve cells of rabbits injected repeatedly with TCE. Animals were given intramuscular injections of 3 mL TCE 3 times/week for 29 days or 2 mL TCE 2 times/week for 41 to 247 days. The authors interpreted the neuropathological changes as evidence of anoxic-ischemic damage due to TCE treatment. However, they also noted that it is difficult to distinguish actual damage from that due to sample preparation.

Immune and Hematologic Systems

Trichloroethylene induced a concentration-dependent decrease in the leukocyte count of dogs exposed to 200, 500, 700, 1000, 1500, or 2000 ppm of TCE for 1 hour. In a second experiment, a group of dogs received 50 mg/kg TCE intravenously at the rate of approximately 1 mL/minute through the right femoral vein. No significant differences were noted in erythrocyte counts, thrombocyte counts, or the hematocrit values of treated dogs (Hobara et al., 1984). Sanders et al. (1982) found a dose-related decrease in the cell-mediated immune response of mice given 24 or 240 mg/kg of TCE by gavage daily for 14 days. In a follow-up study, the immune status in mice was evaluated after administration of 0.1, 1.0, 2.5, or 5.0 mg/mL TCE in drinking water for 4 or 6 months. Humoral immunity was inhibited only at 2.5 and 5.0 mg/mL, whereas cell-mediated immunity and bone-marrow-stem cell colonization were inhibited at all 4 concentrations. Lymphocyte proliferation was not

affected when animals were challenged with T-cell mitogens.

Skin

Trichloroethylene is a primary irritant and contact with relatively pure liquid TCE can cause intense itching, subcorneal pustular eruptions, erythema, and generalized dermatitis (Conde-Salazar et al., 1983; Phoon et al., 1984; Bauer and Rabens, 1974; Saihan et al., 1978). In a guinea pig model, Anderson et al. (1986) found that TCE was the most irritating solvent to skin of 14 tested. No evidence has implicated dilute aqueous solutions of TCE as a cause of dermatitis.

Stewart et al. (1974) have also described "degreaser's flush" in TCE workers. This syndrome is characterized by distinct red blotches on the face, neck, shoulders, and backs of individuals who consumed alcohol shortly after exposure to TCE. Degreaser's flush is thought to be caused by transient dilation of superficial blood vessels and has been observed only following exposure to both TCE and alcohol. Exposure to TCE has been associated with the development of scleroderma (Walder, 1983; Lockey et al., 1987).

Eyes

Volunteers suffered from irritation to the mucous membranes of the eyes and throat when exposed to 27, 81, or 201 ppm of TCE for 4 hours (Nomiyama and Nomiyama, 1977). Nomiyama and Nomiyama (1977) also documented complaints of eye, nose, and throat irritation among individuals occupationally exposed to various concentrations of TCE for various periods of time.

REPRODUCTIVE TOXICOLOGY

Human Studies

Tola et al (1980) conducted an epidemologic study on the mortality of Finnish workers exposed to TCE. As part of this study, these investigators consulted the Finnish Registry of Congenital Malformations. No malformed babies had been born to women exposed to TCE during the 13 years covered by this study. Based on the national incidence, 3 malformed infants would have been expected during this time period.

Animal Studies

Zenick et al. (1984) studied the effects of TCE exposure on the reproductive function of male rats. Rats were intubated with 10, 100, or 1000 mg/kg of TCE 5 days/weeks for 6 weeks and then were mated with ovariectomized, hormonally primed females. At the end of 1, 5, and 10 weeks after the start of the experiment, copulatory behavior and semen samples were evaluated. All dose groups exhibited a statistically significant ($p < 0.001$) increase in body weight. No significant effects were found in semen plug weights, sperm count, sperm motility, sperm morphology, or copulatory behavior. As noted in the subsection on Distribution and Bioaccumulation in Section 3, the data of Zenick et al. (1984) indicate that male reproductive organs can accumulate TCE and its metabolites. Although this suggests that these substances may have the potential to interfere with normal reproductive function, Zenick et al. (1984) found little evidence of any direct effect of TCE on the male reproductive system.

Manson et al. (1984) found that TCE had no effect on the reproductive performance of female rats. Rats received 10, 100, or 1000 mg/kg of TCE daily for 6 weeks: 2 weeks before mating, throughout mating (1 week), and through day 21 of gestation. Females in the high-dose group gained significantly ($p < 0.01$) less weight than those in other groups . The litters of these animals had a significantly ($p < 0.001$) greater number of deaths compared to controls and to the other 2 treatment groups. The authors attributed the high pup mortality to maternal TCE-induced toxicity rather than to any direct action of TCE. Gross examination of pups found no "major malformations."

Schwetz et al. (1975) evaluated the teratogenic potential of TCE in rats and mice exposed to 300 ppm of TCE 7 hours/day on days 6 through 15 of gestation. A statistically significant ($p < 0.05$) inhibition of maternal weight gain was observed in treated rats. The exposure had no apparent effect on the average number of implantation sites per litter, litter size, the incidence of fetal resorptions, fetal sex ratios, fetal body measurements, or on the incidence of fetal tissue or skeletal anomalies in either species.

Dorfmueller et al. (1979) examined whether exposure to TCE before mating and during pregnancy was more detrimental to reproductive outcome than exposure either before mating alone or during pregnancy alone in rats. Three treatment groups were used: (1) TCE exposure before mating and during pregnancy, (2) TCE exposure before mating, and (3) TCE exposure during pregnancy. Prior to mating, animals were exposed to 1800 ppm TCE 6 hours/day, 5 days/week for 2 weeks. Exposure during pregnancy was for 6 hours/day, 7 days/week and

continued through day 20 of gestation. Treatment had no significant effect on female rats, although the fetuses of rats exposed to TCE during pregnancy had an increased incidence of skeletal anomalies. The incidence was not significantly different from controls, however.

Healy et al. (1982) exposed rats to 100 ppm TCE for 4 hours/day during days 8 to 21 of gestation. Compared to controls, a significant ($p < 0.05$) delay was seen in fetal maturation as measured by decreased fetal weight and in the number of resorptions in treated animals.

Beliles et al. (1980) conducted a study to determine the teratogenic potential of workplace contaminants, including TCE. Rats and rabbits were exposed to 500 ppm of TCE 7 hours/day, 5 days/week for 3 weeks prior to mating and during gestation. Separate groups of animals were exposed to TCE during gestation only. No adverse effects were noted in the offspring of rats. However, fetuses from 2 litters of rabbits exhibited hydrocephaly. Although the incidence of this defect was not statistically significant, the authors considered it to be of potential biological significance.

Kjellstrand et al. (1982b) studied the growth of neonatal Mongolian gerbils exposed to TCE. The exposure regimens used in the study consisted of continuous exposure to 230 ppm TCE for 7 days (21 to 28 days of age), 14 days (14 to 28 days age), 21 days (7 to 28 days of age), or 28 days (0 to 28 days of age). An age-related incidence of mortality of 2, 13, 8, and 36%, respectively, was observed in the 4 goups.

Slacik-Erben et al. (1980) conducted a dominant lethal assay in mice to detect spermatocyte anomalies induced by TCE. Male mice were exposed to either 50, 202, or 450 ppm of TCE for 24 hours. Each male was then mated to 12 separate unexposed females over a 48-day period. The regimen was designed to sample effects on all stages of spermatogenesis. Females were sacrificed on day 14 of gestation. The following parameters were assessed to determine the effects of the exposure: number of implantations, fertilization rate (percentage of females with implantations in relation to the total number of mated females), postimplantation loss, preimplantation loss, and the frequency of dominant lethal factors. None of these parameters was observed to be significantly different between the exposed and the control group.

Continuous exposure of female mice to 150 ppm TCE for 48 days caused a decrease in the specific gravity of the brain cortex of pups. The differences were statistically significant at birth ($p < 0.01$) as well as in 10-day-old pups ($p < 0.001$). In addition, significant differences in the specific gravity of the occipital cortex ($p < 0.05$) and cerebellum ($p < 0.01$) were measured in

20- to 22-day-old pups (Westergren et al., 1984).

Bross et al. (1983) injected TCE at 1.0, 5.0, 10.0, and 25.0 μmol/egg into the tops of 1- and 2-day-old fertilized chick eggs and examined the morphology of the embryos that developed. TCE was found to cause significant ($p < 0.05$) differences in the crown-rump length, beak length, wing length, and toe length of treated embryos. Leg length was not affected by exposure to TCE. More than 75% of all embryos had malformations at all concentrations. In a similar study by Elovaara et al. (1979), TCE (5.0, 25.0, 50.0, and 100.0 μmol) was injected into the airspace of fertilized chicken eggs on days 2, 3, or 6 of incubation. Toxicity was evaluated by tabulating survival and the number of malformed embryos on day 14 of incubation. Approximately 16% of all embryos exposed to TCE exhibited malformations. These defects included profound edema, exteriorization of viscera, musculoskeletal abnormalities, and the absence of eyes. Treatment on day 6 of development caused the greatest decrease in survival; statistical significance was not given.

In a recent study, Taylor et al. (1985) investigated the exploratory and locomotor (running wheel) activity in the pups of rats exposed to TCE. Female rats were given 312, 625, or 1250 mg/L TCE in drinking water for 14 days prior to breeding and throughout gestation and lactation (21 days after birth). Offspring from the 3 dose groups were observed at 28, 60, and 90 days of age. No difference was noted in the level of exploratory activity between control and exposed groups at 28 days of age; however, at age 60 and 90 days, offspring exhibited an increase in exploratory activity, especially among those whose mothers received TCE exposures of 1250 mg/L.

GENOTOXIC EFFECTS

A range of assays, covering a wide spectrum of genetic endpoints, have been performed to assess possible genotoxic effects produced by TCE or its metabolites. The DNA or chromosome-damaging effects have been evaluated in bacteria, fungi, yeast, plants, insects, rodents, and humans. The genetic endpoints measured by these assays include: forward and reverse mutation, sister chromatid exchanges, gene conversion, chromosomal aberrations, and mitotic recombination. Induction of DNA repair and covalent binding to DNA have also been examined. The U.S. EPA (1985a) health risk assessment document for TCE presents a detailed review of the literature on the genotoxicity of TCE. The EPA document provides an evaluation of procedures employed, experimental designs, and results obtained. The present discussion updates the

EPA review by concentrating on more recent studies of genotoxicity. The studies reviewed in the U.S. EPA document and additional studies reviewed here are summarized in Tables 2-1 and 2-2.

Bacterial Assays

Several different investigations to assess the mutagenicity of TCE in bacterial systems have been conducted and reported in the literature. Salmonella typhimurium is the indicator organism used in most of these experiments (Baden et al., 1979; Bartsch et al., 1979; Beliles et al., 1980; Cerna and Kypenova, 1977; Henschler et al., 1977; Mortelmans et al., 1986; Shimada et al., 1985; Simmon et al., 1977; and Waskell, 1978). The genetic endpoint measured in these assays is reverse mutation by base-pair substitution in strains TA1535 and TA100 or by frameshift mutation in strains TA1537, TA1538, TA98, and TA97 (Maron and Ames, 1983). One report (Greim et al., 1975) provides data on measured forward and reverse mutations in Escherichia coli strain K12. These studies are reviewed in the EPA document with the exception of the studies by Cerna and Kypenova (1977), Shimada et al. (1985), and Mortelmans et al. (1986).

In a study on the role of contaminants in the carcinogenicity of TCE, Henschler et al. (1977) demonstrated the mutagenicity of epichlorohydrin and epoxybutane, contaminants identified in technical grade TCE. A questionable mutagenic response was obtained with TCE of unspecified purity. Independent of microsomal activation, a 1.6-fold increase in revertant colonies was obtained that was not concentration dependent.

Cerna and Kypernova (1977) conducted a study on the mutagenic activity of TCE and several other chloroethylenes, utilizing the Salmonella spot test, the Salmonella host-mediated assay with mice, and cytogenetic analysis of mouse bone marrow cells. The findings of this study were presented in abstract form, with no details on either the procedures followed or the source or purity of the sample and therefore it is not possible to evaluate the results. Because the study is frequently cited in the literature it is included in this review. Cerna and Kypenova (1977) reported that TCE elicited a concentration-dependent response in the spot test with TA1535 and TA1538 at concentrations of 0.01, 0.1, and 1.0 mg/mL. The numbers of revertants scored for the control or treated population were not provided. In the Salmonella host-mediated assay with mice an increase in the number of revertants was obtained with TCE. In this assay, the genetic indicator organism Salmonella is injected into the

Table 4-1. Review of TCE Genotoxicity.

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Escherichia coli preincubation + mouse S9	Forward, reverse mutation	Analytical grade	3.3 mM	+	-	2-fold increase; purity not assayed; no concentration-dependent effects shown	Greim et al., 1975
Salmonella plate incorporation TA 100 + rat S9	Reverse mutation	Technical grade	1.12, 2.8, 11.2, 28.0, 112.0, 280, 1120 mM		?	1.6-fold concentration-independent increase; mutagenicity of TCE contaminants shown	Henschler et al., 1977
Salmonella plate incorporation TA1950, TA1951, TA1952, TA1535, TA1538, TA100, TA98	Reverse mutation	Not reported	0.01, 0.1, 1 mg/mL		+	Abstract; sufficient data for evaluation not provided	Cerna and Kypenova, 1977
Salmonella host-mediated TA1950, TA1951, TA1952 female mice	Reverse mutation	Not reported	LD50, 1/2 LD50		?	No data or concentrations reported; report significant increase but no concentration dependence	ibid.
Salmonella dessicator TA100 ± rat, mouse S9	Reverse mutation	Reagent grade; analyzed	0.5, 1.0, 1.5,2.0, 2.5 mL	+	-	1.7-fold increase	Simmon et al., 1977
Salmonella dessicator TA100, TA98 + rat S9	Reverse mutation	Commercial anesthetic grade	0.5, 1, 2, 5, 10%	-	-		Waskell, 1978
Salmonella preincubation TA98, TA100 + rat S9	Reverse mutation	Commercial anesthetic grade	2 mM	-	-	Single concentration examined; no data presented	ibid.

Table 4-1. (Continued).

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Salmonella preincubation TA100, TA1535 + rat S9	Reverse mutation	Commercial anesthetic grade 99.5%;	0.1, 0.25, 1.0, 3.0, 10.0%	-	-	No trace contaminants	Baden et al., 1978
Salmonella desiccator TA1001 + mouse S9	Reverse mutation	99.5%, no trace contaminants	5, 8, 20%	-	-	Reproducible 1.8-fold increase at 5% TCE using alternate protocol with no controls	Bartsch et al., 1979
Salmonella Host-mediated TA98	Reverse mutation	99.9%	100, 500 ppm	-	-	Some increase at 500 ppm; poor systemic distribution of TCE; TA98 measures frame-shift reversions only	Beliles et al., 1980
Salmonella desiccator TA1537, TA1538, TA98 TA100, TA1535 + rat S9	Reverse mutation	99.8% low stabilized 99.5% stabilized (mutagenic contaminant)	1, 2.5, 5%	- +	- +	Pure TCE negative in all strains; stabilized TCE positive in TA100, TA1535	Shimada et al., 1985
Salmonella preincubation TA1537, TA100, TA1535, TA98 + rat, hamster S9	Reverse mutation	99.9%	10, 33, 100, 333, 1000 ug/plate	-	-	NTP standardized protocol with coded samples	Mortelmans et al., 1986
Saccharomyces suspension test strain: XV185-146 + mouse S9	Reverse mutation	Technical grade	1.11, 5.56, 111.1, 222.2 mM	+	-	Reversion increase only at concentrations yielding extremely low survival (<1%)	Shahin and Von Borstel, 1977

Table 4-1. (Continued).

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Saccharomyces suspension test D4, D7 + mouse S9	Reverse mutation Gene conversion	Reagent grade	10, 20, 30, 40 mM	+	-	3.8-fold concentration-dependent increase in revertants; 2.5-fold increase in convertants	Bronzetti et al., 1978
Saccharomyce host mediated assay D4, D7	Reverse mutation Gene conversion	Reagent grade	400, 3700 mg/kg		+	Increases in both revertants and gene conversion	ibid.
Saccharomyces suspension test D7	Reverse mutation Gene conversion Mitotic recombination	Not reported	15, 22 mM		+	High toxicity at 22 mM, leaves only single concentration; possible P-450 activation; 2-fold increase in reversion, greater increases in conversion and recombination	Callen et al., 1980
Schizosaccharomyces suspension test + mice, rat S9	Forward mutation	Pure grade Technical grade	0.22, 2.2, 22.0 mM	- -	- -	Assay insensitive; Technical grade contains mutagenic stabilizers	Rossi et al., 1983
Schizosaccharomyces host-mediated mice	Forward mutation	Pure grade Technical grade	2 g/kg	-	-	Assay insensitive; technical grade contains mutagenic stabilizers	ibid.
Schizosaccharomyces host-mediated rats or mice	Forward mutation	Not reported	250, 500, 1000, 2000 mg/kg		+	Weak, 2.6-fold increase with mice only	Loprieno and Abbondandolo, 1980
Aspergillus dessicator strains 35, 35 x 17	Forward mutation Mitotic segregation	99%	2500, 5000 ppm		+	Positive response in growing cultures only	Crebelli et al., 1985

Table 4-1. (Continued).

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Tradescantia stamen hair clone 4430	Forward mutation	Not reported	0.5 ppm	+		Negative control not reported; single concentration reported	Schairer et al., 1978
Drosophila chromosome loss, sex-linked recessive lethal mutations	Mutation Chromosome loss	99.9%	100, 500 ppm		-	More sensitive Basc strain not used	Beliles et al., 1980
Mouse dominant-lethal	Chromosome aberration	99.5%	50, 202, 450 ppm		-	Sample free from epichlorohydrin and epoxybutane	Slacik-Erben et al., 1980
Mouse bone marrow	Chromosome aberration	Not reported	1000 mg/kg		-	No details on procedures reported	Loprieno and Abbondandolo, 1980
Rat bone marrow	Chromosome aberration	99.9%	100, 500 ppm		-	No response in acute or subchronic inhalation exposures by males or females; no inhalation positive control.	Beliles et al., 1980
Mouse spot test	Mutation Chromosome aberration	99.5%	140, 350 mg/kg		+	No spots in concurrent controls; positive compared to historical controls.	Fahrig, 1977
Mouse micronucleus test	Chromosome aberration	Analytical 99.5%	375, 750, 1125, 1500, 2250, 3000 mg/kg		+	MCN in mature erthrocytes; response could be artifactual	Duprat and Gradiski, 1980
Human lymphocyte	Chromosome aberration	Unknown	Occupational exposure		-	Unmatched control	Konietzko et al., 1978

Table 4-1. (Continued).

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Human lymphocyte	Hypodiploid cells	Unknown	Occupational exposure		+	Unmatched control	Konietzko et at., 1978
Human lymphocyte	Sister chromatid xchange	Unknown	Occupational exposure		+		Gu et al., 1981
HPC/DNA repair vapor phase	DNA repair	99.8% stabilized 99.5% tabilized	0.1, 1.0, 2.5%	+	- -	No response with liquid or vapor exposure	Shimada et al., 1985
HPC/DNA repair	DNA repair	Not reported	2.8 mM	+	+		Costa and Ivanetich, 1984
HPC/DNA repair Mouse	DNA repair	99.9%	50, 200, 200 mg/kg		-	Induced S-phase synthesis in male mice	Mirsalis et al., 1985
Lymphocyte DNA repair + rat S9	DNA repair	97-99%	2.5, 5.0, 10.0 ul/mL		+	Positive effect could be result of data transformation. No positive control.	Perocco and Prodi, 1981
DNA repair human WI-38 cells ± rat S9	DNA repair	99.9%	0.1, 0.5, 1.0, 5.0 ul/mL		+	Slight increases independent of activation	Beliles et al., 1980
Covalent binding mouse in vivo	DNA binding	99.9%	1200 mg/kg 2.4 mCi/mmol		-	Enhanced DNA synthesis reported	Stott et al., 1982
Covalent binding salmon sperm in vitro + mouse S9	DNA binding	99%	6.7 mmol 1 mCi/mmol	+	-	Requires activation from male mice	Banerjee and Van Duuren, 1978

Table 4-1. (Continued).

Assay	Genetic Endpoint	Sample Purity	Dosage	Results +S9	Results -S9	Comments	References
Covalent binding calf thymus *in vitro* + mouse S9	DNA binding	99%	8.35 mmol 3.4 mCi/mmol	+	-	Requires activation	Bergman, 1983
Covalent binding mouse *in vivo*	DNA binding	99%	33.6 mg/kg 868 uCi/kg		-	Metabolic incorporation for de novo synthesis	*ibid.*
Covalent binding calf thymus *in vitro* + rat S9	DNA binding	unreported	1 mM	+		Adducts not identified	DiRenzo et al., 1982
Covalent binding rat/mouse *in vivo*	DNA binding	99%	10, 100, 500, 1000 mg/kg	?		Weak positive; possible protein contamination; no adducts identified	Parchman and Magee, 1982
Covalent binding calf thymus *in vitro* rat hepatocytes mouse hepatocytes	DNA binding	99%	9.0 mCi/mmol 0.1 mM	+ +	- +	Higher incidence of DNA adducts with mouse than with rat hepatocytes; evidence for stable active metabolite	Miller and Guengerich, 1983

Table 4-2. Genotoxicity of TCE Metabolites.

Metabolite	Assay	Genetic End Point	Sample Purity	Dosage	Results +S9	Results -S9	Comments	Reference
Chloral hydrate	Salmonella preincubation TA100, TA1535, TA98, TA1537 + rat, hamster S9	Reverse mutation	99%	100, 333, 1000, 3333, 4000, 5000, 6,667, 7500, 10,000 ug/ plate	+	+	NTP standardized protocol with coded samples. Positive in TA100	Haworth et al., 1983
Chloral hydrate	Salmonella plate-incorporation TA98, TA100, TA1535 + rat S9	Reverse mutation	Re-crystalized 1-6 times	0.1, 0.5, 1.0, 5.0, 10, 14 mg/plate	+	+	Weakly positive, less than 2-fold increase in TA100 only	Waskell, 1978
Chloral hydrate	Salmonella plate-incorporation TA1535, TA100 + rat S9	Reverse mutation	Not reported	1.0, 2.5, 5.0 mg/plate	+	+	Positive in TA100 only; 4-fold increase without S9. Reduced response +S9. No S9 control	Bignami et al., 1980
Chloral hydrate	Streptomyces Plate-incorporation spot test, hisA1	Forward, reverse mutation	Not reported	2.0, 5.0, 10.0 mg/plate		+	7-fold and 9-fold increase in forward and reverse mutation	ibid.
Chloral hydrate	Aspergillus plate-incorporation spot test, strain 35	Forward mutation	Not reported	1.0, 5.0, 10.0 mg/plate		+	Significant ($p < 0.01$) concentration-dependent increase	Bignami et al., 1980

Table 4-2. (Continued)

Metabolite	Assay	Genetic End Point	Sample Purity	Dosage	Results +S9	Results -S9	Comments	Reference
Chloral hydrate	Aspergillus plate-incorporation strain 35x17	Mitotic segregation	99%	5.0, 10.0 mM		+		Crebelli et al., 1985
Chloral hydrate	Aspergillus suspension test	Mitotic segregation	Not reported	20.0 mM		+	Spindle fibers destroyed	Mercer and Morris, 1975
Chloral hydrate	Aspergillus	Mitotic crossovers Aneuploidy	Reagent grade	10.0 to 40.0 mM		+	No mitotic crossovers	Käfer, 1986
Chloral hydrate	Mouse spermatagonia	Non-disjunction	99%	82.7, 165.4, 413.5 mg/kg		+		Russo et al., 1984
Chloral hydrate	Drosophila sex-linked recessive lethal	Mutation	99%	5,500, 10,000 ppm		-	Questionable results in feeding study; negative response with injection	Yoon et al., 1985
Chloral hydrate	Human lymphocytes	Sister chromatid exchange	Not reported	54.1 mg/L		+	Weak increase	Gu et al., 1981
TCE oxide	Salmonella preincubation TA1535	Reverse mutation	Synthesized	0.5, 1.3, 2.5, 5 mM		-	1.5-min half-life	ibid.
TCE oxide	E. coli pol A-	DNA repair	Synthesized	0.006, 0.01, 0.06, 0.08, 0.11 mM		+	1.5-min half-life	ibid.

Table 4-2. (Continued)

Metabolite	Assay	Genetic End Point	Sample Purity	Dosage	Results +S9	Results -S9	Comments	Reference
TCE oxide	E. coli uvrA WP-2	Reverse mutation	Synthesized	0.25, 0.5, 1.3, 2.5, 5 mM		-	1.5-min half-life	Kline et al., 1982
TCE oxide	Schizosaccharomyces suspension test	Forward mutation	Not reported	0.5 mM		+	Few details on procedures reported; no statistical evaluation	Loprieno and Abbondandolo, 1980
TCE oxide	V79 Chinese hamster cells	Forward mutation	Not reported	.05 mM	+		Not enough information provided to evaluate	Loprieno and Abbondandolo, 1980
Trichloroacetic acid	Salmonella plate-incorporation TA98, TA100 TA1535 + rat S9	Reverse mutation	Not reported	0.45 mg/plate	-	-	Single concentration tested	Waskell, 1978
Trichloroacetic acid	Mouse bone marrow	Chromosome aberration	Analytical grade	125.0, 250.0, 500.0 mg/kg		+		Bhunya and Behera, 1987
Trichloroacetic acid	Mouse micronucleus test	Chromosome aberration	Analytical grade	125.0, 250.0, 500.0 mg/kg		+		ibid.
Trichloroacetic acid	Mouse sperm morphology	Abnormal sperm	Analytical grade	25.0, 50.0, 100.0 mg/kg		+		ibid.

Table 4-2. (Continued)

Metabolite	Assay	Genetic End Point	Sample Purity	Dosage	Results +S9	Results -S9	Comments	Reference
Trichloroethanol	Salmonella plate-incorporation TA98, TA100, TA1535 + rat S9	Reverse mutation	Not reported	7.5 mg/plate	-	-	Single concentration tested	Waskell, 1978
Trichloroethanol	Salmonella repair test TA2322, TA1950 TS24	Differential killing	Not reported	31 mg		-	No increase in killing in repair-deficient strains	ibid.
Trichloroethanol	Salmonella spot test plate-incorporation TA1535, TA100 + rat S9	Reverse mutation	Not reported	1.0, 5.0, 10.0 mL/plate	-	-		Bignami et al., 1980
Trichloroethanol	Streptomyces spot test, plate-test, hisAl	Reverse mutation	Not reported	10.0, 20.0, 40.0 mL/plate	-			Bignami et al., 1980
Trichloroethanol	Aspergillus spot test, plate-incorporation	Forward mutation	Not reported	5.0, 7.5, 10.0 mL/plate		+	Weakly positive in spot test only	ibid.
Trichloroethanol	Aspergillus plate-incorporation strain 35 x 17	Mitotic segregation	95%	5.1, 10.2 mM		+		Crebelli et al., 1985

Table 4-2. (Continued)

Metabolite	Assay	Genetic End Point	Sample Purity	Dosage	Results +S9	Results -S9	Comments	Reference
Trichloroethanol	Human lymphocytes exchange	Sister chromatid	95%	178 mg/L		+	Weak increase	Gu et al., 1981

mouse host where it may interact with the compound tested in vivo under metabolically active conditions. No evidence of a concentration-dependent response was found. No details were provided on the procedures employed, and the concentrations examined were reported only as 1/2 LD_{50} and LD_{50}, with the value of LD_{50} not given.

Two TCE samples with different purities were tested in the Salmonella desiccator assay by Shimada et al. (1985). A TCE sample (99.5% pure) containing stabilizers, including the known mutagenic contaminant butylene oxide, induced a greater than 4-fold increase in revertants with TA100. A positive concentration-dependent response was obtained in TA1535 with vapor exposures from 1.0 to 5.0% TCE. The positive result with both strains was independent of rat microsomal activation. A TCE sample with fewer stabilizers (99.9% pure) did not induce revertants at concentrations from 1.0 to 5.0%. These results strongly indicate that the mutagenic response seen in Salmonella assays is due to the presence of contaminants in the TCE used.

The Salmonella preincubation assay was performed with 270 coded chemicals, including TCE, by three laboratories under contract to the U.S. National Toxicology Program (NTP). The results with TCE are summarized in the NTP Fiscal Year 1983 Annual Plan (National Toxicology Program, 1983) and are reported with data in a review by Mortelmans et al. (1986). The TCE was obtained from Dow Chemical Co. (label purity of 99.9%) and was tested by Microbiological Associates with Salmonella strains TA1535, TA1537, TA98 and TA100. Both rat and hamster Aroclor 1254-induced metabolic activation systems were used with each strain. Standardized conditions included the use of concurrent solvent and positive controls, testing at 5 concentrations (10 to 1000 μg/plate), plating in triplicate, and conducting repeat experiments. No increase in the number of revertants was obtained in the treated groups for any of the strains tested, with or without activation.

Beliles et al. (1980) examined the mutagenic effects of TCE as part of an investigation of workplace contaminants for the U.S. National Institute of Occupational Health and Safety. When strain TA98 was tested in the Salmonella host-mediated assay, no significant increase in the number of revertants was found when mice were exposed over 5 days for 7 hours/day to 100 or 500 ppm TCE. The authors reported a poor systemic distribution of TCE in the mice, which they attributed to the size and reactivity of the molecule. The use of the single strain of Salmonella TA98 precluded detection of base-pair substitution mutations.

Lower Eukaryotes

Induction of reverse mutation, gene conversion, and mitotic recombination by TCE has been evaluated in studies with Saccharomyces cerevisiae by Shahin and Von Borstel (1977), Bronzetti et al. (1978), and Callen et al. (1980). Loprieno and Abbondandolo (1980) and Rossi et al. (1983) utilized Schizosaccharomyces pombe as the indicator organism for evaluating the ability of TCE to induce forward mutations. A review of these investigations is available in the U.S. EPA (1985a) document and will not be repeated here. The weakly positive results with yeast were dependent upon microsomal activation. Results are summarized in Table 4-1.

A TCE sample, determined to be free of epoxide contaminants, was tested by Crebelli et al. (1985) for induction of forward mutation and mitotic nondisjunction in the fungus Aspergillus nidulans. No significant increases in forward supressor mutations were obtained when conidia were incubated in desiccators with 2500 to 20,000 ppm TCE. Cultures exposed in growth phase to 2500 or 5000 ppm TCE showed variable results. However, the nonparametric Mann-Whitney U test showed that the concentration-related increase in mutant frequency was significant. The results of this assay are difficult to assess because the time of induction greatly affects the observed mutation frequency due to clonal expansion in a growing culture. Similar results were obtained when diploid cultures were used to examine induction of chromosomal aberrations. No significant increases were seen when quiescent conidia were exposed to TCE in liquid for 1 hour. However, growing cultures of Aspergillus exposed to TCE for 24 hours in a desiccator had significant frequencies of both haploid and "nondisjunctional" diploids, but not of mitotic crossovers. Crebelli et al. (1985) suggested that this apparent requirement for actively growing cells to obtain a mutagenic response is indicative of an endogenous metabolic conversion of TCE by Aspergillus.

Angiosperms

Schairer et al. (1978) tested TCE of unreported purity in the stamen hair assay with Tradescantia clone 4430. Somatic mutation is measured as a phenotypic change in pigmentation from blue to pink in mature flowers. The mutagenicity data for TCE were presented by these authors in a summary table, along with data on 22 other compounds. Exposure to 0.5 ppm TCE for 6 hours induced a significant ($p < 0.01$) increase in the incidence of somatic mutation.

it is not possible to critically evaluate these weakly positive results because data were reported from only a single exposure concentration and no control values were provided.

Insects

Two TCE studies have been conducted using Drosophila melanogaster as the test organism (Beliles et al., 1980; Abrahamson and Valencia, 1980). Trichloroethylene was not effective at inducing sex-linked recessive-lethal mutations in either study. The experimental design employed in these investigations is described in U.S. EPA (1985a). The feeding protocol employed by Abrahamson and Valencia (1980) was a fixed procedure as part of a screening test and may have been inappropriate for TCE. In addition to testing for sex-linked recessive lethal mutations, Beliles et al. (1980) looked for X or Y chromosome loss. Exposure to TCE did not cause any significant differences compared to negative controls.

Rodents

Several investigations (Fahrig, 1977; Slacik-Erben et al., 1980; Beliles et al., 1980; Duprat and Gradiski, 1980; Loprieno and Abbondandolo, 1980) have been conducted using rodents to assess the genotoxic effects induced by in vivo exposure to TCE.

Fahrig (1977) provided evidence that TCE can cause gene mutation or recombination. In the mouse spot test, concentrations of 140 and 350 mg/kg TCE induced 1.8- and 5-fold increases, respectively, in the incidence of color spots compared to historical controls; no spots were found in concurrent controls.

The mouse dominant lethal test was employed by Slacik-Erben et al. (1980) to detect mutations induced by TCE. Exposure to 50, 202, and 450 ppm TCE did not affect the fertilization rate, pre-implantation loss, or postimplantation loss, which are indices of dominant lethal events. It should be noted, however, that this assay has been criticized for its inability to detect weak mutagens (Russell and Matter, 1980).

A positive response was obtained when TCE was tested in the mouse micronucleus test by Duprat and Gradiski (1980). The U.S. EPA (1985a) suggested that the micronuclei recorded by Duprat and Gradiski (1980) were probably artifacts and did not actually represent TCE-induced damage to erythrocytes.

Acute or subchronic exposure to TCE (100 or 500 ppm for 7 hours, or 7 hours/day for 5 days) did not induce chromosome aberrations in rat bone marrow cells (Beliles et al., 1980). Loprieno and Abbondandolo (1980) observed a slight increase in the incidence of chromosomal aberrations in mouse bone marrow cells after animals received 1000 mg/kg TCE by gavage. The increase was not statistically significant. No description of the methods employed or of the purity of the sample tested was provided.

Beliles et al. (1980) found sperm morphological abnormalities in mice following exposure to 100 or 500 ppm TCE 7 hours/day for 5 days. Negative results were reported after rats were exposed to TCE by the same treatment protocol. The validity of the latter results are questionable because the positive control, triethylenemelamine, also gave negative results.

Nelson and Bull (1988) demonstrated significant increase in single strand breaks in DNA, as measured by DNA unwinding, in both male mice and rats after oral doses of 11.4 and 22.9 mmol/kg, respectively. Pretreatment with the inducer phenobarbital lowered the dose of TCE needed for induction. Metabolites of TCE were also effective at lower doses than TCE. The effects on DNA strand breakage occurred at levels of TCE below those producing the common hepatic effect of liver enzyme release into blood.

In vitro, TCE was weakly positive in an assay for transformation of mouse BALB/c-3T3 cells (Tu et al., 1985) and weakly positive for sister chromatid exchanges (SCE), but not for chromosomal aberrations, in Chinese hamster ovary cells (Galloway et al., 1987).

Humans

Two studies have been conducted that measured chromosomal effects on humans occupationally exposed to TCE. Peripheral lymphocytes were scored for hypodiploid cells and chromosome breaks by Konietzko et al. (1978) and for sister chromatid exchanges (SCE) by Gu et al. (1981). The incidence of hypodiploid cells, cells containing less than the diploid number of chromosomes, was found to be higher in the exposed group of 28 workers compared to an unmatched control group (10.9% $\pm$ 4.4% versus 6.5% $\pm$ 3.2%). Konietzko et al. (1978) also found a greater frequency of chromosome breaks in cells from the exposed group. Although this increase was not considered biologically significant, no statistical evaluation of the data was provided.

Gu et al. (1981) compared an occupationally exposed group of 6 workers to a control group of 9 individuals and reported a statistically significant

($p < 0.01$) increase in SCE in cultured peripheral lymphocytes (9.0 ± 0.4 versus 7.9 ± 0.2, respectively). Exposed workers showed a positive correlation between the average number of SCE/cell and levels of trichloroacetic acid and trichloroethanol in the blood. These studies indicate that TCE or a metabolite may cause chromosomal aberrations or SCE in chronically exposed humans. However, exposure to additional compounds, including TCE contaminants, cannot be ruled out as possible causative agents in these studies.

DNA Repair

Several assays have been conducted that measured unscheduled DNA synthesis (UDS) as an indication of TCE interaction with DNA. Beliles et al. (1980) found that TCE induced a low level of unscheduled DNA synthesis in human WI-38 cells in vitro. Positive results, defined as responses greater than 150% of control values, were obtained for 0.1 and 0.5 uL/mL treatments without S9, and for 0.5 uL/mL treatment with S9.

Perocco and Prodi (1981) reported that TCE elicited UDS in cultured human lymphocytes. These conclusions were based on transformed data; however, the raw data do not indicate that treatment with TCE caused an increase in UDS. No positive controls were used. Trichloroethylene did not include UDS in mice treated with 200 or 1000 mg/kg TCE by gavage (Mirsalis et al., 1985). Negative results were obtained with both male and female mice. The direct-acting mutagen methylmethanesulfonate and the procarcinogen dimethylnitrosamine both elicited UDS when used as positive controls. In a concurrent experiment, Mirsalis et al. (1985) measured increased proliferation of hepatocytes in TCE-treated mice. They suggested that this proliferation may be regenerative hyperplasia that developed in response to TCE-induced liver damage.

Trichloroethylene-induced DNA repair synthesis was measured in isolated hepatocytes from rats pretreated with phenobarbital (Costa and Ivanetich, 1984). Hepatocytes were incubated with 20 μCi deoxy-[5-^{3}H]cytidine (24 Ci/mmol) for 1 hour. DNA repair synthesis was identified by the radioactivity peak banding with parental DNA, coincident with the UV-absorbance peak at 260 nm. A positive response was found with a 2.5-hour exposure to 2.8 mM TCE.

Shimada et al. (1985) exposed rat hepatocytes to TCE (liquid or vapor) and measured induction of DNA repair synthesis. Two samples, one stabilized with butylene oxide and a second containing "low-stabilized" TCE, were tested.

Both TCE samples failed to elicit a response. The positive controls, vinyl chloride (vapor) and 2-acetoaminofluorene (liquid), were both active inducers of UDS.

Covalent Binding

Several studies have been conducted using radiolabeled TCE to measure covalent binding with DNA (e.g., Banerjee and Van Duuren, 1978; Bergman, 1983; DiRenzo et al., 1982; Parchman and Magee, 1982; Miller and Guengerich, 1983; Stott et al., 1982). Banerjee and Van Duuren (1978) tested a sample of ^{14}C-TCE (> 99% pure, 1 mCi/mmol) in an in vitro study with salmon sperm DNA and murine microsomal proteins. No binding was detected in the absence of microsomes. Binding of the radiolabel to DNA increased with increasing concentrations of mouse microsomes. Covalent binding to DNA was 161% higher with microsomes prepared from male mice compared to microsomes from female mice. Miller and Guengerich (1983) measured the covalent binding of radiolabel to intracellular DNA in, as well as to calf thymus DNA incubated with, rat or mouse hepatocytes incubated for 2 hours with 1,2-^{14}C-TCE (>99% purity) in vitro. Binding to extracellular DNA was measured to assess the ability of reactive TCE metabolites to migrate out of hepatocytes and bind irreversibly to a proxy for critical macromolecules of distant cells. Binding to protein and a proxy for RNA was also assessed. Binding to intracellular and extracellular protein, RNA, and DNA was observed in both rat and mouse hepatocytes. Adduct formation in intracellular DNA of rat hepatocytes was dependent on induction of microsomes with phenobarbital; otherwise, the observed binding did not require such induction. Only very low levels of DNA binding could be detected in rat hepatocytes in the absence of phenobarbital pretreatment. A comparison between species showed that DNA adducts were formed at higher levels per mg of DNA in mouse hepatocytes than in rat hepatocytes.

Di Renzo et al. (1982) measured the in vitro formation of DNA adducts in a series of aliphatic halides. Radiolabeled ^{14}C-TCE (1 mM, 1 Ci/mmol) was incubated with phenobarbital-induced rat hepatic microsomes and calf thymus DNA for 1 hour. Binding of radiolabel to DNA was measured as 0.36 $\pm$ 0.14 nmol/mg DNA (mean $\pm$ standard deviation of 7 experiments). Little or no binding to DNA was detected with heat denatured microsomes as a negative control (data not provided).

Bergman (1983) examined the binding of 1,2-^{14}C-TCE (specific activity =

3.4 mCi/mmol) to calf thymus DNA incubated with hepatocytes from phenobarbital-induced male mice. A 1-hour treatment with 8.35 μmol (28.4 μCi) of the labeled TCE resulted in a significant ($p < 0.001$) increase in binding compared to controls (20.7 ± 2.2 pmol/mg versus 13.8 ± 1.8 pmol/mg). No radioactivity was detected in DNA incubated with TCE without hepatic microsomes. Analysis of DNA hydrolysates by ion exchange chromatography revealed that a portion of the radioactivity was associated with a UV-marker for 1,N^6-ethenoadenine, an adduct associated with in vivo experimental exposure to the known human carcinogen, vinyl chloride. No such peak was observed in experiments using hepatocytes that were not preinduced with phenobarbital.

In a separate experiment, Bergman (1983) administered 33.6 mg/kg of 868 μCi/kg ^{14}C-labeled TCE to mice twice daily for 5 days and counted the radioactivity associated with the DNA of different tissues. All radioactivity associated with the DNA of the kidney, testis, lung, pancreas, and spleen was due to metabolic incorporation of C1-fragments used for the de novo synthesis of nucleic acids. Radioactivity associated with hepatic DNA was eluted early in the chromatographic profile and was not structurally identified. No labeled elution peaks associated with 1,N^6-ethenoadenine were observed in these 1 vivo experiments.

Stott et al. (1982) treated mice with 1200 mg/kg ^{14}C-TCE (2.4 mCi/mmol) by gavage and measured the radioactivity associated with purified hepatic DNA. Although low levels of radioactivity were measured in these samples (0.62 ± 0.43 alkylations per 10^6 nucleotides), no specific adducts were identified.

Covalent binding to DNA was also assessed in an in vivo study by Parchman and Magee (1982). No radiolabel was detected in rat liver DNA following intraperitoneal injection of 1.16 mg/kg of ^{14}C-TCE (2.5 μCi) to a single phenobarbital-induced animal. Concentrations of 10, 100, 500, and 1000 mg/kg TCE resulted in some incorporation of radiolabel into the DNA. However, no correlation between specific activity and administered dose was observed. Parchman and Magee (1982) suggested that the radioactivity associated with the DNA may have been the result of contamination of the sample by protein of a high specific activity. Similar results were obtained in experiments with mice. No specific adducts were identified in either species.

Summary of Genotoxic Effects

Trichloroethylene has given primarily negative results in bacterial assays

of mutagenicity. Positive results were obtained with a TCE sample that contained a known mutagenic contaminant, butylene oxide, while purified TCE was negative (Shimada et al., 1985). Two studies reported weakly positive results that were dependent on microsomal activation (Greim et al., 1975; Simmon et al., 1977). Henschler et al. (1977) showed that 2 stabilizers found in commercial TCE samples, epoxybutane and epichlorohydrin, had direct acting mutagenic properties.

Consistent, weakly positive results have been reported for induction of mutation, gene conversions, and mitotic recombination in yeast. Positive responses occurred with metabolic activation. An investigation that utilized the fungus Aspergillus as the test organism confirmed that metabolic activation appears to be necessary to elicit genotoxic effects with TCE. Forward mutations and mitotic nondisjunction were induced only when treatments were administered to cultures in growth phase.

Of 4 rodent assays in which an increase in frequency of chromosome aberrations was the genetic endpoint, 3 had negative results. The positive response reported by Duprat and Gradiski (1980) may have been due to a misinterpretation of experimental results. Trichloroethylene gave a positive response in the mouse spot test, which indicates that TCE can cause forward mutations or possibly chromosome aberrations (Fahrig, 1977).

Two studies with human lymphocytes have been conducted following occupational exposure to TCE (Konietzko et al., 1978; Gu et al., 1981). An increased incidence of hypodiploid cells was reported by Konietzko et al. (1978) and of sister chromatid exchanges by Gu et al. (1981). These results were based on observations from exposed workers and not on data from controlled experiments. Exposure to other chemicals, some of which may have genotoxic capabilities, cannot be ruled out as possible causative agents.

Trichloroethylene elicited mixed responses in DNA repair assays. Beliles et al. (1980) reported that TCE induced a slight increase in UDS in human WI-38 cells independent of activation. Results from studies with primary cultures of hepatocytes have been negative. There is some weak evidence that TCE can covalently bind to DNA. In vitro studies indicate that activation enzymes are required for binding to occur. No actual structural adducts have been identified.

The majority of information indicates that TCE is either not mutagenic or has some very weak mutagenic capability. The weak effects have been observed primarily in systems with microsomal activation, which indicates that TCE metabolites may be the causative agents.

GENOTOXIC EFFECTS OF METABOLITES

A number of metabolites of TCE have been analyzed for their ability to cause genotoxic effects. These compounds include chloral hydrate (CH), 2,2,2-trichloroethanol (TCEL), trichloroacetic acid (TCA), and TCE-oxide. Research conducted by Kline et al. (1982) on TCE-oxide, by Waskell (1978) on TCEL, TCA, and CH, and by Gu et al. (1981) on TCEL and CH has been reviewed by the U.S. EPA (1985a) and will not be reviewed here. The results obtained in these investigations are summarized in Table 4-2.

Haworth et al. (1983) have presented a review, including data, of 250 chemicals tested in Salmonella by 3 laboratories under contract to the U.S. National Toxicology Program. Chloral hydrate (99% pure) was tested by Microbiological Associates with strains TA100, TA1535, TA1537, and TA98 over a CH concentration range from 100 to 10,000 μg/plate. Aroclor-1254-induced microsomal activation systems from both rats and hamsters were used with each strain. Appropriate negative and positive controls were utilized. Chloral hydrate induced concentration-dependent increases in revertants both with and without activation in TA100.

Bignami et al. (1980) evaluated the mutagenicity of CH and TCEL as part of a study on the mutagenicity of 8 halogenated aliphatic hydrocarbons. The genetic endpoints examined were forward mutation in Aspergillus nidulans and Streptomyces coelicolor and reverse mutation in Salmonella typhimurium and S. coelicolor. In Salmonella strain TA100 CH induced a 5-fold increase in revertants without activation. A reduced but positive response was reported with the addition of Aroclor-1254-induced rat liver microsomes. No S9 control was conducted. The data show that CH induced increases in both streptomycin-sensitive forward mutants and in histidine-requiring revertants in the bacterium S. coelicolor at the concentrations examined (2-10 mg/plate). In experiments with A. nidulans, CH treatment induced a significant ($p < 0.05$) number of forward mutations to 8-azaguanine resistance in the plate test at concentrations as low as 1 mg/plate and of methionine suppressors at 5 mg/plate ($p < 0.01$). Trichloroethanol gave negative results in both bacterial systems and in the plate-incorporation assay with A. nidulans. Positive results with TCEL were reported in the A. nidulans spot test at concentrations of 5 μL/plate ($p < 0.01$).

Crebelli et al. (1985) utilized A. nidulans to examine the ability of CH and TCEL to induce mitotic segregation. Both compounds induced haploid and "nondisjunctional" diploid somatic segregants in the plate incorporation

assay. The use of growing cultures of A. nidulans reduces the certainty of the response because the frequency of mutant colonies depends not only on the number of mutagenic events, but also on the time at which they are induced.

Käfer (1986) found that CH induced both aneuploidy and polyploidy in germinating conidia of A. nidulans. It did not induce crossing over. The concentrations of CH ranged from 5 to 40 mM; the duration of exposure varied from 1 to 8 hours.

Chloral hydrate (20 mM) destroyed preexisting mitotic spindle fibers in 2 strains of A. nidulans. Additionally, overnight cultures transferred from normal media to media containing 20 mM CH were unable to form new mitotic spindles (Mercer and Morris, 1975).

Three laboratories under contract to the U.S. National Toxicology Program conducted Drosophila mutagenicity tests on 45 compounds, including CH. The results with data were reported by Yoon et al. (1985). In a coded experiment at Brown University CH was evaluated for its ability to induce sex-linked recessive lethal mutations. Males were fed 5500 ppm CH for 3 days and then mated to 3 consecutive harems of Basc strain females. Questionable results, defined as a borderline response, were reported. Due to the equivocal response obtained in the feeding study, CH was tested at 10,000 ppm by abdominal injection. No difference in the incidence of recessive lethal mutations was found between the treated and control populations.

Loprieno and Abbondandolo (1980) examined the mutagenicity of a number of industrial compounds, including TCE-oxide. The TCE-oxide induced forward mutations in vitro, both with cultured V79 Chinese hamster cells and with Schizosaccharomyces pombe in the suspension test. Only a single concentration was examined in each assay; no details on the procedures utilized or on mutation frequencies in controls were provided. For these reasons, it is not possible to evaluate these results.

Trichloroacetic acid at 25.0, 50.0, or 100.0 mg/kg induced a statistically significant dose-dependent increase in the incidence of sperm head abnormalities in mice (Bhunya and Behera, 1987). The level of significance was not specified. In concurrent experiments, mice were injected with 125.0, 250.0, or 500.0 mg/kg TCA, and bone marrow cells were examined for the presence of chromosome aberrations 6, 24, or 48 hours after treatment. All doses caused a significant increase in the frequency of chromosome aberrations. The frequency was not clearly dose related, however. The same doses of TCA also induced a significant increase in the incidence of micronuclei in polychromatic as well as monochromatic erythrocytes.

The effects of CH on various cell stages of mouse secondary spermatocytes were studied by Russo et al. (1984). Intraperitoneal injections of CH (99% pure) at 82.7, 165.4, or 413.5 mg/kg were administered to groups of 24 mice. Mice were sacrificed at 5, 12, 21, or 42 days following treatment to sample secondary spermatocytes from cells treated in 4 different stages of development. Significant increases in nondisjunction were found at all concentrations examined ($p < 0.005$ for 82.7 and 165.4 mg/kg; $p < 0.05$ for 413.5 mg/kg).

The recently identified (Dekant et al., 1986b) glutathione pathway metabolite of TCE, S-dichlorovinyl-N-acetlcysteine, and its deacylated derivative, dichlorovinylcysteine (DCVC), are both mutagenic in the Ames test (Green and Odum, 1985; Dekant et al., 1986c; Vamvakas et al., 1987). Both mammalian enzymes, present in the S9 supernatant or in kidney cystol, and bacterial enzymes, present in the Salmonella tester strains, can deacylate. Thus, another metabolic pathway exists that can produce mutagenic products from TCE.

In summary, the TCE metabolite CH has consistently demonstrated genotoxic capabilities. These include forward and reverse mutation in bacteria (Haworth et al., 1983; Bignami et al., 1980; Waskell, 1978) and in Aspergillus (Bignami et al., 1980). Chromosomal effects of CH include the induction of aneuploidy (Käfer, 1986) and nondisjunction (Russo et al., 1984) as well as interference with mitotic segregation (Crebelli et al., 1985; Mercer and Morris, 1975). Gu et al. (1981) provided evidence that CH induced SCE in human lymphocytes.

S-dichlorovinyl-N-acetlcysteine, a mercapturic derivative of TCE, is a strong mutagen in the Ames test in the presence of rat kidney cytosol (Vamvakas et al., 1987).

Mixed results have been reported on the genotoxic effects of the TCE metabolites TCEL and TCE-oxide. Trichloroethanol showed negative responses in 4 bacterial assays for induction of reverse mutation or DNA repair (Waskell, 1978; Bignami et al., 1980). Positive results were found in Aspergillus assays of forward mutation (Bignami et al., 1980) and mitotic segregation (Crebelli et al., 1985). Results of an in vitro SCE assay were also positive (Gu et al., 1981). The TCE-oxide did not induce reverse mutations in repair-proficient strains of bacteria, but did induce revertants in a repair-deficient strain (Kline et al., 1982) Positive results were obtained in forward mutation assays by Loprieno and Abbondandolo (1980) with Schizosaccharomyces and with V79 Chinese hamster cells.

Trichloroacetic acid tested negative for reverse mutation in a single experiment by Waskell (1978). No additional information was available to assess possible genotoxic effects of TCA.

CARCINOGENICITY IN ANIMALS

The objective of this subsection is to review the findings and pertinent characteristics of the cancer bioassays that have been completed on TCE and other related substances of possible interest (such as stabilizers, contaminants, and metabolites). These reviews, together with the previous discussions dealing with the pharmacokinetic and genotoxic characteristics of TCE, support a quantitative assessment of the carcinogenic potency of TCE (see Section 5). Studies reported by the National Cancer Institute and the National Toxicology Program are discussed first. The remaining studies are organized chronologically. Table 4-3 lists all bioassays, the route of exposure, and the test species used. In Appendix B (Tables B-11 through B-16) the results of the bioassays are summarized in tabular form by route of exposure and test species. Unless otherwise noted, statistical significance values are from the Fischer Exact test comparing incidence in a dosed group versus incidence in the vehicle control group.

National Cancer Institute, 1976

The National Cancer Institute (NCI, 1976) study was the first major long-term cancer bioassay of TCE and has led to several other investigations. The TCE was administered by gavage 5 days/week for 78 weeks to B6C3F1 mice and Osborne-Mendel rats of both sexes. The industrial grade of TCE used contained 1,2-epoxybutane (0.19%), ethyl acetate (0.04%), epichlorohydrin (0.09%), N-methylpyrrole (0.02%), and diisobutylene (0.03%) as stabilizers.

When the study began, the mice and rats were 5 weeks and 7 weeks of age, respectively. Following the dosing period, the animals were observed for 12 weeks and were sacrificed in the 90th (mice) or 110th (rats) weeks. Dose groups consisted of 50 male and 50 female mice and rats. Twenty animals made up each matched control group. Investigators also reported the tumor incidence in groups of colony controls.

Preliminary acute and subchronic tests were used to determine a maximum tolerated dose (MTD) that could be given to the high-dose groups in chronic studies; the low-dose groups received half of the MTD. Male mice received

Table 4-3. Summary of Cancer Bioassays for TCE and Metabolites.

Study	Species	Route	Table in Appendix B
NCI, 1976	mouse	gavage	B-1
NCI, 1976	rat	gavage	B-2
NTP, 1983	mouse	gavage	B-1
NTP, 1983	rat	gavage	B-2
NTP, 1988	rat	gavage	B-2
Bell et al., 1978	mouse	inhalation	B-3
Bell et al., 1978	rat	inhalation	B-4
Van Duuren et al., 1979	mouse	gavage	B-1
Van Duuren et al., 1979	mouse	skin application	B-5
Van Duuren et al., 1979	mouse	subcutaneous injection	B-5
Henschler et al., 1980	mouse	inhalation	B-3
Henschler et al., 1980	rat	inhalation	B-4
Henschler et al., 1980	hamster	inhalation	B-4
Fukuda et al., 1983	mouse	inhalation	B-3
Fukuda et al., 1983	rat	inhalation	B-4
Henschler et al., 1984	mouse	gavage	B-1
Herren-Freund et al., 1987	mouse	in drinking water	B-5
Herren-Freund et al., 1987	mouse	metabolites (TCA and DCA) in drinking water	B-6
Maltoni et al., 1986			
BT301	rat	gavage	B-2
BT302	rat	inhalation	B-4
BT303	mouse	inhalation	B-3
BT304/304 bis	rat	inhalation	B-4
BT305	mouse	inhalation	B-3
BT306/306 bis	mouse	inhalation	B-3

initial daily doses of 2000 mg/kg of body weight in the high-dose group and 1000 mg/kg in the low-dose group. Dose levels were increased during the course of the study resulting in corresponding experimental time-weighted average (TWA) doses of 2339 and 1169 mg/kg. Initial doses to female mice were 1400 and 700 mg/kg, and the corresponding experimental TWA doses were 1739 and 869 mg/kg. Dose levels to rats were lowered during the study because of poor survival and decreasing body weights. High-dose groups of both sexes initially received 1300 mg/kg, but a lower TWA dose of 1097 mg/kg. The low-dose groups initially received 650 mg/kg, but a lower TWA dose of 549 mg/kg.

Higher incidences of hepatocellular carcinoma in mice were statistically significant in both high- (31/48, $p < 0.001$) and low-dose (26/50, $p = 0.004$) males and high-dose females (11/47, $p = 0.008$) relative to the matched controls (1/20 for males and 0/20 for females). Tests for linear trend on age-adjusted data were highly significant for hepatocellular carcinoma in males ($p < 0.001$) and females ($p < 0.002$). Metastases of the liver cancer to the lung were observed in 4 low-dose and 3 high-dose males. The first hepatocellular carcinoma was observed among the high-dose males at 27 weeks and among the low-dose males at 81 weeks.

In contrast to the positive results in the mouse study, analysis of tumor incidences in rats showed no significant difference in specific or total tumors between treated and control groups. High-dose male rats exhibited significantly ($p = 0.001$) decreased survival relative to that of controls. The response in rats to carbon tetrachloride, the positive control compound, appeared relatively low.

Questions have been raised about the possible impact of the epichlorohydrin (ECH) impurity in the TCE used in the NCI mouse and rat bioassays. The TCE used had a purity of 99.6%. However, ECH was present in the TCE at 0.09% by weight. The presence of this contaminant may have directly contributed to tumor induction observed in the NCI mice. Epichlorohydrin is a direct-acting alkylating agent and a mutagen (Kucerova et al., 1977; Bridges, 1978). Van Duuren et al. (1974) demonstrated that ECH was carcinogenic in mice when injected subcutaneously. A subsequent study by Laskin et al. (1980) showed that ECH induced neoplastic nasal cavity lesions in rats. Most of these tumors were carcinomas of the squamous epithelium. Interestingly, 30-day exposures to 100 ppm ECH produced a much greater incidence of cancer than lifetime exposures of 30 ppm for 6 hours/day, 5 days/week. Results from studies by Konishi et al. (1980) and Kawabata (1981) also showed that ECH fed

discontinuously to rats in drinking water at a concentration of 1500 ppm (a lifetime TWA dose of approximately 40.2 mg/kg-day) induced significantly increased incidences of papillomas and squamous cell carcinomas of the forestomach compared to control animals. The quantity of ECH in the TCE used in the NCI study is estimated to be equivalent to lifetime TWA doses of 1.3 and 0.97 mg/kg-day for high-dose male and female mice, respectively. These doses are only 3.2% and 2.4%, respectively, of the dose that elicited squamous cell carcinomas in rats referred to above. Furthermore, ECH appears to initiate tumors by a localized tumorigenic action at sites in direct contact with tissue, such as nasal or forestomach squamous-cell epithelium (U.S. EPA, 1984b). No animal in the NCI mouse bioassay developed tumors at these sites.

Epichlorohydrin is among the weakest of the more than 50 suspected carcinogens evaluated by the U.S. EPA Carcinogen Assessment Group. Epichlorohydrin has an estimated upper-bound carcinogenic "potency" (i.e., carcinogenic effect per unit dose at low lifetime TWA doses) for humans of 9.9×10^{-3} $(mg/kg\text{-}d)^{-1}$ based on data indicating increased nasal cavity tumor incidence in rats exposed to ECH in drinking water (U.S. EPA, 1984b). Using the methodology of U.S. EPA (1984b), the equivalent potency to mice is estimated to be $9.9 \times 10^{-3} \times (f_m/f_h)$, where f_m and f_h are the fractions of body weight consumed as water per day, equal to 0.17 and 0.029 for mice and humans, respectively. The potency for ECH to mice is, therefore, estimated to be 0.058 $(mg/kg\text{-}d)^{-1}$. Using this potency estimate, the high-dose male and female mice in the NCI (1976) bioassay would incur increased ECH-induced cancer risks of 11% and 8.7%, respectively, using the approximate relation: increased risk = 1 - exp{-(potency) x (lifetime TWA dose)}. Therefore, while it is possible that ECH contributed to the observed increased tumor incidence in TCE-exposed mice in the NCI (1976) bioassay, it is unlikely that ECH was responsible for all or most of the increased incidence observed.

Kimbrough et al. (1985) noted that TCE-treated animals in the NCI (1976) experiments were housed in the same rooms as animals treated with other compounds. Although the bedding was changed weekly, evaporation of volatile compounds may have been a source of exposure to other carcinogenic substances. Ambient levels of test chemicals in the rooms were not measured. The U.S. EPA (1985a) considered it unlikely that other compounds were responsible for the observed response because: (1) controls and treated animals were in the same room; (2) oral TCE doses probably would have greatly exceeded ambient levels of the other volatile compounds; (3) cages were equipped with filters limiting the amount of chemical released; (4) the total room air was exchanged 10 to 15

times per hour; and (5) dosing was performed under a hood in another room. The negligible effect of exposure to other chemicals in mice was substantiated by a repeat study (NTP, 1983), discussed below, in which animals from different experiments did not share the same room.

High rates of early mortality rendered the NCI rat bioassay inadequate for evaluating the carcinogenic potential of the test chemical (IARC, 1979).

National Toxicology Program, 1983

To address the question of contaminant effects on the results of the 1976 NCI mouse study, the National Toxicology Program (NTP, 1983) initiated a repeat series of carcinogenicity studies in mice and rats. Dosing with TCE by gavage began when B6C3F1 mice and F344/N rats of both sexes were 8 weeks of age. The TCE contained no epichlorohydrin and was stabilized with 8 ppm diisopropylamine. Treated mice and high-dose rats received 1000 mg/kg TCE 5 days/week. Low-dose rats received 500 mg/kg TCE 5 days/week. The dosing period lasted 103 weeks. Survivors were killed within 4 weeks after treatment.

The incidences of renal tubular-cell adenocarcinoma in male rats dosed with either 500 mg/kg (0/49) or 1000 mg/kg (3/49) were not significantly different from controls (0/48). However, high-dose male rats that survived until the end of the experiment exhibited a statistically significant higher incidence (3/16) of renal tubular-cell adenocarcinoma than the controls (0/33) ($p = 0.028$ using the "Life Table" or "Incidental Tumor" tests referenced in NTP, 1983). These kidney tumors are considered uncommon occurrences in F344/N rats. Only 3 of 748 (0.4%) male rats from historical vehicle gavage control groups have exhibited such tumors. The incidence of mesotheliomas of the peritoneum among the low dose rats (5/50, 10%) significantly ($p < 0.05$) exceeded concurrent (1/50, 2%) and historical controls (16/752, 2.1%). However, toxic nephrosis that significantly reduced survival among dosed rats produced equivocal results that were considered "inadequate to evaluate the presence or absence of a carcinogenic response" of these rats to TCE (NTP, 1983).

Significantly higher incidences of hepatocellular carcinoma in dosed male mice (30/50, $p < 0.001$) and dosed female mice (13/49, $p < 0.05$) relative to those of their controls (8/48 and 2/48, respectively) confirmed the positive results of the 1976 NCI mouse study. Dosed female mice were also found to have a statistically significant ($p < 0.05$) increase in the incidence of hepatocellular adenomas (8/49) relative to that of the controls (2/48).

This bioassay provided evidence that epichlorohydrin is not needed to induce hepatocarcinogenesis in B6C3F1 mice.

National Toxicology Program, 1988

In another National Toxicology Program study (NTP, 1988), 4 strains of rat (ACI, August, Marshall, and Osborne-Mendel) received high (1000 mg/kg) or low (500 mg/kg) daily doses of TCE in corn oil by gavage 5 days/week for 103 weeks. The TCE used contained no epichlorohydrin. Test groups consisted of 50 animals of each sex. The ACI rats used in the study were 6.5 weeks of age when dosing began; the Marshall rats were 7 weeks; and the Osborne-Mendel and August rats were 8 weeks of age. The experiment was terminated and all the survivors were sacrificed during the 110th and 111th weeks.

All treatment groups showed some reduction in mean body weights relative to vehicle controls. Survival in 7 of the 16 dosed groups was significantly ($p < 0.05$) reduced compared to that of the vehicle controls.

Male Osborne-Mendel rats exhibited a statistically significantly higher incidence of renal tubular-cell adenomas (6/50) at the lower dose relative to that of the controls (0/50) ($p = 0.007$ using "Life Table" or "Incidental Tumor" tests referenced in NTP, 1988).

Male Marshall rats exhibited a statistically significantly higher incidence of testicular interstitial cell tumors at the higher dose (32/48 = 67%) relative to that of the controls (17/46) ($p = 0.002$ using "Life Table" or "Incidental Tumor" tests referenced in NTP, 1988). The incidence of these proliferative testicular lesions was also high in control groups of ACI rats (36/49 = 73%), but dosed ACI rats showed a nonsignificant decrease in incidence (23/49 = 47% in the low-dose rats, 17/49 = 35% in the high-dose rats). Therefore, because of the inconsistency between stains, the biological significance of the dose-related increase observed in male Marshall rats is in question.

Consistent negative dose-response trends were observed in the incidence of adrenal pheochromocytomas in male ACI, female Marshall, and male and female August and Osborne-Mendel rats in this study.

Results of audits conducted in Fall, 1983, and Spring, 1984, revealed that the documentation of animal breeding, animal identity, clinical observations, environmental conditions, and analytical chemistry data were inadequate to support any meaningful interpretation of the reported tumor incidence data (NTP, 1988). The NTP Peer Review Panel met subsequently in August, 1986, and

voted to add a last sentence to the draft affirming the presence of the positive results (NTP, 1988):

> "Despite these limitations [audit results], an increased incidence of renal tubular cell tumors was observed in dosed animals, and an increased incidence of interstitial cell tumors of the testes was observed in dosed Marshall rats."

This additional statement, however, does not refer to the biological significance of the "increased incidence" noted, in light of the inadequate supporting documentation for the study. Uncertainty, therefore, remains regarding the interpretation of the 1988 NTP bioassay results.

Bell et al., 1978

Bell et al. (1978), referenced in U.S. EPA (1985a), reported the results of an audit by the Manufacturing Chemists Association (MCA) of a carcinogenicity study conducted by Industrial Bio-Test (IBT) Laboratories. Charles River rats and B6C3F1 mice were exposed to TCE vapor at concentrations of 100, 300, or 600 ppm for 6 hours/day, 5 days/week, for 104 weeks. Each treatment group consisted of either 120 rats or 140 mice. Animals were sacrificed upon termination of treatment. The test chemical was greater than 99% pure. Impurities included diisobutylene (0.023%), butylene oxide (0.24%), ethyl acetate (0.052%), N-methylpyrrole (0.008%), and epichlorohydrin (0.148%).

The incidences of hepatocellular carcinoma in male mice exposed to TCE in concentrations of 100 ppm (28/95), 300 ppm (31/100), and 600 ppm (43/97) were statistically significant ($p < 0.05$, $p = 0.03$, and $p < 0.001$, respectively) when compared to controls (18/99). The level of significance increased when the incidences of both hepatocellular carcinoma and hepatocellular adenoma combined in treated versus control mice are compared by the Fischer Exact test. Female mice exposed to TCE at a concentration of 600 ppm exhibited a significant ($p < 0.05$) increase in the incidence of hepatocellular adenomas and hepatocellular carcinomas combined (17/99) relative to that of the controls (8/99). No statistically significant increase in the incidence of any other tumor type was observed among the treated rats.

The MCA audit reported marked deficiencies and flaws in both the rat and mouse studies. Actual TCE-exposure levels in each study could not be precisely determined due to large deviations from the nominal concentrations and great variability in the number of daily measurements. The audit also revealed that

animals from the mouse control group were obtained from an earlier shipment and, therefore, were not specifically matched with those of the treatment groups. Histopathologic reexamination of mouse livers resulted in observations that differed from the original findings. In the reexamination, 12 mice were found to have been originally missexed (U.S. EPA, 1985a). According to the U.S. EPA (1985), the usefulness of these bioassays is limited by these deficiencies in their conduct.

Van Duuren et al., 1979

Van Duuren et al. (1979) exposed Ha/ICR mice to purified TCE by 3 different routes: skin application, subcutaneous injection, and gavage. Animals began treatment at 6 to 8 weeks of age. Experimental groups consisted of 30 animals each. The untreated control for these tests consisted of 100 female mice and 60 male mice. Two types of skin application experiments were conducted with female mice. In the 2-stage initiation/promotion experiment, animals received 1.0 mg of TCE in 0.1 mL of acetone followed 2 weeks later by 2.5 μg of phorbol myristate acetate in 0.2 mL acetone 3 times weekly. The duration of the test and the median survival times ranged from 68 to 82 weeks. The median survival time for the positive control was 54 weeks. No significant increase in any tumor was observed. The other skin test revealed no tumorigenic response from the application of 1.0 mg of TCE 3 times weekly for 83 weeks. No significant positive tumorigenic response was obtained.

Subcutaneous injection experiments also revealed no significantly increased tumor incidence in female mice receiving 0.5 mg of TCE once weekly for 89 weeks. Both sexes of mice were exposed to 0.5 mg of TCE once a week for 89 weeks by intragastric feeding tube. This dose is roughly equivalent to a daily dose of 2.8 mg/kg, assuming a 25-g mouse. No significant ($p < 0.05$) increase in any tumor was observed.

Henschler et al., 1980

Henschler et al. (1980) exposed 3 species of rodents (Han:NMRI mice, Han:WIST rats, and Syrian hamsters) to concentrations of pure amine-based TCE at 100 and 500 ppm for 6 hours/day, 5 days/week, for 78 weeks. The ages of the animals when placed on study were not given. Surviving mice and hamsters were sacrificed on the 130th week. Rats were not sacrificed until the 156th week.

Neither rats, hamsters, nor male mice had significantly increased tumor

incidence. Dosed female mice, however, exhibited significantly ($p < 0.05$) higher incidences of malignant lymphoma relative to that of the controls (100 ppm, 17/30; 500 ppm, 18/28; controls, 9/29). The time-to-tumor occurrence also decreased in a dose-related fashion.

Henschler et al. (1980) cited 3 studies that describe a high spontaneous incidence of malignant lymphoma in female NMRI mice. The authors also referenced several studies that attribute the development of murine lymphoma to immunosuppressive agents that allow lymphoma induction by specific inborn viruses. In its review of this study, the U.S. EPA also suggested that immunosuppression by TCE or some other nonspecific agent provides a possible interpretation of the positive results of this study (U.S. EPA, 1985a).

Fukuda et al., 1983

In a study by Fukuda et al. (1983), female Sprague-Dawley rats and female ICR mice were exposed to concentrations of 50, 150, and 450 ppm of reagent grade TCE for 7 hours/day, 5 days/week, for 104 weeks. The surviving animals were killed in the 107th week. Animals of both species were 7 weeks old when placed on the study. Size of the test groups varied between 49 and 51 animals. Chemical analysis revealed the test sample to contain TCE (99.824%), carbon tetrachloride (0.128%), benzene (0.019%), epichlorohydrin (0.019%), and 1,1,2-trichloroethane (0.010%) in the vapor phase. The incidence of lung adenocarcinomas among mice in the 2 higher exposure groups (150 ppm, 8/50; 450 ppm, 7/46) was significantly ($p < 0.05$) higher in both groups than that of the controls (1/49), but the incidence was not dose-related. The incidence of total lung tumors (adenomas and adenocarcinomas combined) in exposed mice was not significantly different from that of the controls. Statistical analysis of the tumor incidences among rats showed no significant increases or trends.

Henschler et al., 1984

Henschler et al. (1984) tested different samples of TCE with or without epichlorohydrin (ECH) and/or 1,2-epoxybutane, traditional stabilizers that had been implicated earlier as carcinogenic contaminants, for carcinogenicity in groups of 50 5-week-old male or female ICR/Ha-Swiss mice. Treated animals received 1 large dose of TCE, with or without epoxides, by corn oil gavage 5 days/week for 18 months. Males received 2400 mg/kg, while females received 1800 mg/kg. Dosing was interrupted during weeks 35 to 40, 65, and 69 to 78.

All doses were reduced to half the initial amount after the 40th week. After the dosing period (61 out of 78 weeks), the mice were observed for 26 weeks and then sacrificed during the 104th week. Experimental TWA daily doses of 1900 mg/kg for males and 1400 mg/kg for females have been calculated by summing the products from number of days dosed times the administered daily dose and dividing the sum by the number of days dosed.

Mice dosed with purified, amine-stabilized TCE did not exhibit a statistically significant increase in the incidence of any tumor type. The administration of TCE with 0.8% ECH equivalent to lifetime TWA ECH doses of 8.1 and 6.0 mg/kg-day for males and females, respectively, or both 0.25% ECH and 0.25% 1,2-epoxybutane was associated with a significant ($p < 0.05$) increase in forestomach papillomas or carcinomas in both sexes. In particular, the incidence of these tumors in the control mice versus that in the mice exposed to ECH-stabilized TCE was 1/50 versus 8/50 in males and 1/50 versus 12/50 in females. The latter response in dosed females was the most highly significant ($p = 0.0002$, by an age-adjusted Chi-squared test) increase in tumor incidence observed in the study. The administration of TCE with 0.8% 1,2-epoxybutane was associated with a significant ($p < 0.05$) increase in squamous cell carcinomas in males.

If a cancer potency of 0.058 $(mg/kg\text{-}d)^{-1}$ for ECH in mice is assumed (see previous discussion), the ECH contained in the doses of ECH-stabilized TCE (8.1 and 6.0 mg/kg-day for males and females, respectively) used in this study are expected to have induced increased tumor risks of 37% in the dosed male and 29% in the dosed female mice. These predicted increased risks more than account for the observed increased incidence of forestomach tumors cited above. Thus, results from this study support the hypothesis that ECH may be the proximate cause of increased tumor incidence observed in some studies of rodents exposed to ECH-stabilized TCE and in light of the negative results of pure TCE observed in this study.

Wester et al., 1985

Wester et al. (1985) administered a mixture of volatile, halogenated hydrocarbons to Wistar rats in their drinking water The drinking water solutions were prepared by adding 0.22, 2.2, or 22 mg of a mixture containing equal quantities of 11 volatile halogenated hydrocarbons (trichloroethylene, trichloromethane, tetrachloromethane, monobromodichloromethane, tetrachloroethylene, 3 isomers of dichlorobenzene, and 3 isomers of

trichlorobenzene) to 1 mL of ethanol for each liter of drinking water. Dose groups consisted of 60 rats of each sex. Exposure duration lasted 25 months. Surviving animals were sacrificed upon termination of treatment. If it is assumed that a 0.3 kg rat drinks 0.030 liters of water per day and that the concentration of TCE in the most highly contaminated water was 2 mg/L (derived by dividing the greatest amount of mixture, 22 mg, by the number, 11, of different chemicals used), then a rat in the most highly exposed group received approximately 0.2 mg TCE/kg body weight per day.

When the experimental data were corrected for age, no significant differences in incidence or time of appearance of any tumor between groups were found. This bioassay may not be sufficiently sensitive at these low levels to reflect a carcinogenic effect of TCE. The potential confounding effects of 10 other chemicals severely limit the value of the results of this study in determining the carcinogenic risk of TCE alone.

Herren-Freund et al., 1987

Unlike the bioassays discussed above, the study by Herren-Freund et al. (1987) dealt with the tumorigenic response of mouse liver to the TCE metabolites trichloroacetic acid (TCA) and dichloroacetic acid (DCA), as well as TCE, with and without initiation. The TCE metabolites TCA and DCA have not been shown to be genotoxic but have been shown to induce liver peroxisome proliferation (see discussions above in subsections on liver toxicity and genotoxicity). Pretreated male B6C3F1 mice received an intraperitoneal injection of 2.5 or 10 mg/kg ethylnitrosurea (ENU), an initiator, at 2 weeks of age. Two weeks later the mice were given drinking water with either TCE at 3 or 40 mg/L, TCA at 2 or 5 g/L, or DCA at 2 or 5 g/L. The TCE concentration was limited by its low solubility in water. The treatment period lasted 61 weeks, after which the animals were sacrificed. The number of animals in each treatment group that were examined ranged from 19 to 31 animals.

Animals exposed to TCE-treated drinking water with or without ENU pretreatment exhibited no significant ($p < 0.01$) increase in the incidence of adenomas or hepatocellular carcinomas relative to that of control groups. The tumorigenic response to ENU treatment alone was only significant ($p < 0.01$) at the highest ENU dose level (10 μg/g). Exposures to both levels of TCA- or DCA-treated drinking water, irrespective of initiation with ENU, were associated with statistically significant ($p < 0.01$) increases in numbers of animals with hepatic adenomas and hepatocellular carcinomas.

Dose-response data for mice that were dosed with TCE, TCA, or DCA in this study, but which were not pretreated with ENU or phenobarbital, are given in Tables B-5 (TCE) and B-6 (TCA and DCA). The bioassay for carcinogenicity of TCE alone in this study was not particularly sensitive due to the low solubility of TCE in water, the relatively short exposure period, and the small number of animals used. Use of a larger sample size might have increased the significance of the difference between the incidence of hepatocellular carcinomas in the TCE-only group (3/31, 10%) compared to the control group (0/22, 0%).

Maltoni et al., 1986

Maltoni et al. (1986) reported the results of a series of 8 TCE carcinogenicity experiments performed between 1976 and 1983 at the Bentivoglio (BT) Research Laboratories of the Bologna Institute of Oncology. This project used nearly 4,000 mice and rats that were observed until spontaneous death. Inhalation was the primary route of administration. The statistically and biologically significant results of these bioassays (BT301, 302, 303, 304, 304-bis, 305, 306, and 306-bis) are presented in the following text and in Tables B-2 through B-4.

Bioassay BT301 was the only noninhalation experiment of the project. Trichloroethylene was administered by stomach tube to Sprague-Dawley rats at dose levels of 50 or 250 mg/kg, 4 to 5 days/weeks, for 52 weeks. Dosing began when the rats were 13 weeks old. Thirty rats of each sex were in each dosing group. The TCE was free of epoxide and contained 50 ppm or less each of 1,2-dichloroethylene, chloroform, carbon tetrachloride, and 1,1,2-trichloroethane. A dose-related higher frequency of leukemia was observed in treated males, but this increase was not statistically significant.

The U.S. EPA (1985a) reviewed this particular bioassay and remarked that the dosing period of 52 weeks was below potential lifetime exposures. They also stated that the older rats (13 weeks old) would give no indication of the carcinogenic potential of TCE in developing animals.

Maltoni et al. (1986) conducted 2 short-term inhalation bioassays with Sprague-Dawley rats (BT302) and Swiss mice (BT303). The animals were exposed to 100 or 600 ppm TCE for 7 hours/day, 5 day/weeks, for 8 weeks. No statistically significant effect was observed. Treated male mice exhibited an increase in the incidence of hepatomas over that of the controls, but the increase was not statistically significant at the 95% confidence level.

Bioassays BT304-bis were both similar long-term inhalation experiments whose results were combined and evaluated together. Sprague-Dawley rats were exposed to either 100, 300, or 600 ppm TCE for 7 hours/day, 5 days/week, for 104 weeks. A statistically significant, exposure-related increase in the incidence of Leydig cell tumors of the testes was observed in treated rats: 31/130 (23.8%) at 600 ppm ($p < 0.01$), 30/130 (23.1%) at 300 ppm ($p < 0.01$), 16/130 (12.3%) at 100 ppm ($p < 0.05$), and 6/135 (4.4%) in the control group. Only 1 animal in the group exposed to TCE at 600 ppm had a Leydig cell tumor of the testes that was malignant. A higher incidence of leukemia was observed in treated rats but was neither significant ($p > 0.05$) nor dose-related. Five rats (4 males, 1 female) of 260 exposed to 600 ppm TCE developed kidney adenocarcinomas that, although lacking statistical significance, must be considered biologically significant due to their rarity. In male rats, these adenocarcinomas arose from tubular cells. This renal tumor had not been observed in any of the nearly 50,000 Sprague-Dawley rats (untreated, vehicle-treated, or treated with different chemicals) examined in these laboratories.

In experiment BT305, Swiss mice were exposed to TCE at a concentration of 100, 300, or 600 ppm for 7 hours/day, 5 days/week, for 78 weeks. Males exposed to the 2 higher levels showed statistically significant increases in the incidence of pulmonary tumors (27/90 at 600 ppm, $p < 0.01$; 23/90 at 300 ppm, $p < 0.05$) relative to that of the control group (11/90). The increased incidence of pulmonary tumors included a slight increase in adenomas and adenocarcinomas, but the statistical significance of the increase in pulmonary tumors was clearly due to an increase in the number of animals with adenomatous hyperplasia or early (i.e., borderline) adenomas. Males exposed to 600 ppm TCE also had a higher frequency of hepatomas (13/90, $p < 0.05$) than that of controls (4/90). Females did not show any significant response to TCE exposure in this bioassay.

Bioassays BT306 and BT306-bis were both conducted with B6C3F1 mice under similar conditions and, therefore, have been evaluated together in the present analysis. Animals were exposed to 100, 300, or 600 ppm TCE for 7 hours/day, 5 days/week, for 78 weeks. The increase in the total numbers of malignant tumors in female mice was significant at all three dose levels: 64.4% at 600 ppm ($p < 0.01$), 58.9% at 300 ppm ($p < 0.01$), and 57.8% at 100 ppm ($p < 0.05$), versus 46.7% in the controls. A dose-related increase in the incidence of pulmonary tumors was observed in females but was significant ($p < 0.05$) only at 600 ppm (15/90) relative to the control group (4/90). The pulmonary tumors

included no adenocarcinomas but consisted primarily of adenomas. When males and females were considered together, a slight increase in the incidence of hepatomas was observed in treated animals and was significant ($p < 0.01$) at 600 ppm TCE (15/180) versus controls (4/180). Males alone showed no statistically significant response to exposure to TCE under these conditions.

Maltoni et al. (1986) reported statistically significant increases in pulmonary tumors (benign and malignant combined) and hepatomas (malignant) in treated mice and testicular tumors (benign and malignant combined) in treated rats. An increased incidence of renal tubular cell adenocarcinoma was also observed in treated rats. Although the incidence of this neoplasia was not statistically significant, Maltoni et al. (1986) considered the appearance of these tumors to be biologically significant because of their rarity in control animals (0/460).

Summary of Evidence of Carcinogenicity in Animals

Like many other chlorinated aliphatic compounds, TCE gives positive carcinogenic responses in animals (Chu and Milman, 1981). The results of the NCI (1976) bioassay of TCE administered by gavage show that oral exposure was associated with a statistically significant ($p < 0.01$) increased incidence of hepatocellular carcinoma in both sexes of B6C3F1 mice. Factors such as dose-related increases of hepatocellular carcinoma in both sexes, a decrease in the time to tumor among males, and the occurrence of metastases to the lung in dosed mice support the NCI conclusion that TCE was hepatocarcinogenic in this strain of mouse under the conditions of the experiment.

In the repeat experiment (NTP, 1983), oral administration of epoxide-free TCE was observed to be associated with increased incidences of hepatocellular carcinoma in both sexes and of hepatocellular adenomas in females, confirming the results of the mouse bioassay by NCI (1976). Bell et al. (1978) reported significant ($p < 0.05$) increases in the incidences of hepatocellular carcinoma among male B6C3F1 mice exposed to technical grade TCE by inhalation. However, serious flaws occurred in the conduct of this study, as noted earlier in this report.

An increased incidence of malignant lymphoma was observed in TCE-exposed female Han:NMRI mice (Henschler et al., 1980). The EPA (U.S. EPA, 1985a) concluded that the biological significance of this observation is unclear because these tumors have a high rate of spontaneous occurrence, which may have

increased as an indirect result of TCE dosing, possibly mediated by immunological sensitivity to the exposure regimen.

The rat bioassays reported by the NTP (1983, 1987) are considered by the NTP to be "inadequate" studies of carcinogenicity. However, under the conditions of these studies, the administration of TCE was deemed by NTP to be "associated" with an increased incidence of renal tubular cell adenocarcinoma in the male F344/N rat (NTP, 1983), renal tubular cell adenoma in the male Osborne-Mendel rat, and testicular interstitial cell tumor in the male Marshall rat (NTP, 1988).

"Sufficient" evidence of carcinogenicity in animals is described by the U.S. EPA as evidence "which indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: (a) in multiple species or strains; or (b) in multiple experiments, e.g., with different routes of administration or using different dose levels; or (c) to an unusual degree in a single experiment with regard to high incidence, unusual site or tumor type, or early age at onset" (U.S. EPA, 1986b). The U.S. EPA Guidelines for Carcinogen Risk Assessment, as well as the State of California's corresponding 1985 guidelines, consider the mouse-liver tumor and other neoplasms that occur with high spontaneous background occurrence as responses that may constitute "sufficient" evidence of carcinogenicity in animals, although such a classification may be weakened to "limited" if adequate supporting evidence is not observed (U.S. EPA, 1986b; CDHS, 1985). These guidelines are based primarily on similar guidelines established by the International Agency for Research on Cancer (IARC), wherein "limited" evidence for carcinogenicity in animals is defined to consist of a response in only a single species, strain, or experiment.

Based only on the positive results of the NCI (1976) bioassay, IARC (1979) determined that there was "limited evidence" that TCE is carcinogenic in animals. Upon additional review of the data of Henschler et al. (1980), IARC's classification of evidence for carcinogenicity of TCE to animals remained "limited" (IARC, 1984). However, based on the increased incidences of malignant liver tumors in B6C3F1 mice in 2 studies (NCI, 1976; NTP, 1983), the increased incidence of malignant lymphoma in NMRI mice (Henschler et al., 1980), and the increased incidence of renal tumors in rats (NTP, 1983), the U.S. EPA (1985a) concluded that the above results constitute "sufficient" evidence for carcinogenicity in animals.

The results reported by Fukuda et al. (1983) and Maltoni et al. (1986) provide support for the U.S. EPA classification of "sufficient"

evidence of carcinogenicity in animals because they showed statistically significant increases in malignant pulmonary tumors and liver tumors in treated mice. The evidence is enhanced by the addition of a tumor type, lung adenocarcinomas (Fukuda et al., 1983), distinct from those observed in previous studies. Results from both studies further strengthen the classification by introducing the inhalation route of exposure and by using different strains of mice. An increase in renal tubular cell adenocarcinomas in treated Sprague-Dawley rats that was not statistically significant (Maltoni et al., 1986) may support the biological significance of the similar results observed in the NTP (1983) study.

Significant carcinogenic responses were observed in male B6C3F1 mice exposed to high concentrations of the TCE metabolites trichloroacetic acid (TCA) or dichloroacetic acid (DCA) in drinking water (Herren-Freund et al., 1987). Based on the EPA guidelines (U.S. EPA, 1986b), the evidence for the carcinogenicity in animals of the TCE metabolites is "limited," because the studies involved only a single species, strain, and experiment.

CARCINOGENICITY IN HUMANS

Epidemiological study designs can be divided roughly into 2 types: retrospective (or case control) and prospective (or cohort). In the attempt to find an association between exposure to TCE and the incidence of cancer in humans, both designs have been employed. In retrospective studies, the exposure history of people with cancer (cases) are compared with that of a control group whereas, in prospective studies, cancer rates in an exposed group (cohort) are compared with those in a nonexposed group. Prospective studies can be further divided as to their method into the true prospective cohort study that identifies a cohort and follows it over time and the historical cohort study that identifies a cohort with past exposure and then checks present medical records. A study that follows a cohort with well-defined exposure prospectively over time is the most valuable type of epidemiological study, but is, unfortunately, very costly and time consuming. The studies presented here are of the retrospective and historical cohort designs.

Retrospective Studies

Hardell et al. (1981) conducted a retrospective study of 169 men, aged 25 to 85 years, who were admitted to the Department of Oncology in Umea, Sweden,

between 1974 and 1978 for histologically confirmed malignant lymphoma. Sixty cases had Hodgkin's disease and 109 had nonHodgkin lymphoma. The investigators selected 338 controls that matched the cases as closely as possible with respect to sex, age, place of residence, and year of death, if applicable. Sixty-two cases and their respective controls were deceased. Cases and controls had completed self-administered questionnaires that addressed type, place, and duration of employment, leisure time activities, exposure to various chemicals, and drug-use and smoking status. Exposure to certain chemicals was classified into high grade and low grade. High grade was defined as continuous exposure for a week or more, or repeated brief exposures for a month or more.

Seven cases and 3 controls reported high-grade exposure to TCE. The EPA (1985a) calculated an odds ratio of 7.88 (95% confidence interval: 2.30 to 27.04) for these exposed cases compared with the 60 cases and 222 controls with no exposure history to organic solvents, chlorophenols, or phenoxy acids. When compared with 162 cases and 335 controls that were not exposed to high-grade levels of TCE, but including those persons exposed to other chemicals and low-grade levels of TCE, the odds ratio dropped to 4.8, but remained significant ($p < 0.05$ by a Chi-squared test).

The usefulness of these odds ratios as estimates of the relative risk is questionable. The U.S. EPA (1986a) cited the potential effects of recall bias and the lack of age adjustment on the results as limitations to the evaluation of the carcinogenicity of TCE. Nonetheless, this study yielded an estimate of the relative risk of developing malignant lymphoma that is more than seven times greater for those who recall a high-grade exposure to TCE compared with those that report no exposure to phenoxy acids, chlorophenols, or organic solvents.

Imperial Chemical Industries conducted a retrospective study of 95 primary liver cancer cases diagnosed between 1951 and 1977 (Paddle, 1983). All cases were selected from the Mersey Regional Cancer Registry and limited to those with an address near the Runcorn TCE manufacturing plant in England. A comparison of the cases with the "tens of thousands" of past employees of the Runcorn plant from 1936 to 1976 revealed no matches. Paddle calculated the expected number of cases of primary liver cancer among Runcorn workers from 1951 to 1977 to be about 0.3.

The U.S. EPA (1985a) reported a retrospective study by Novotna et al. (1979) of 63 histologically confirmed cases of liver cancer identified in Prague in 1972 and 1974. Employment histories dating back to January 1, 1957, revealed that 56 cases had never been occupationally exposed to TCE.

Occupational histories for the 7 other cases did not exist. No controls were defined. At the time of the study, only 544 workers out of a population of 1 million were exposed to TCE at 63 workplaces. This study is of little value in the evaluation of the potential carcinogenicity of TCE.

Historical Cohort Studies

The Swedish TCE manufacturer provides routine laboratory analyses of the metabolite trichloroacetic acid (TCA) in urine of TCE-exposed workers as part of an exposure-control program (Axelson et al., 1978). From the laboratory files, Axelson et al. (1978) established a cohort of 518 men who had been occupationally exposed to TCE earlier than 1970. A subcohort was defined as those men given a follow-up time of greater than or equal to 10 years. Exposure for those men whose urine exceeded 100 mg TCA/L, corresponding to an estimated 8-hour average exposure to 30 ppm TCE, was considered high and values below this level were considered low.

A mortality analysis of the cohort for 1955 to 1975 revealed 49 deaths from all causes, whereas 62 were expected using Swedish national death-rates. No significant elevated risk of tumor-related deaths was observed in the total cohort or the subcohort. No analysis of site-specific tumor deaths was reported because their numbers were too low. Two deaths from leukemia and 2 from stomach cancer occurred but were not compared to any expected value. The EPA found it "interesting" that 2 of the 11 deaths from cancer in this cohort were due to leukemia (18%), whereas leukemia deaths only account for 4% of the cancer deaths among Swedish men.

Axelson et al. (1978) suggested that the deficit of the number of deaths in the cohort relative to the expected number might be somewhat attributable to the "healthy worker effect." This phenomenon explains a relatively low death rate from cancer in working populations, particularly when an adequate latency period is not taken into account. Only a large increase in liver-cancer deaths could have been detected in this cohort. A single case would give a risk ratio 3.4 in the total cohort and 6.7 and 25.0 ($p < 0.04$) in the low-and high-exposure cohorts, respectively.

The duration of exposure of the workers was not taken into account when identifying and subdividing the cohort in the Axelson et al. (1978) study. These investigators did not report the size of the subcohort observed for a latency period greater than 10 years but did report 3643 person-years of observation relative to 7688 person-years for the entire cohort. They also

observed that the study size was probably too small, particularly with respect to the subcohort, to detect a positive association between exposure to TCE and specific cancer deaths. Therefore, an upper bound on potential cancer risk of TCE to humans cannot be estimated on the basis of data from this study.

The U.S. EPA (1985a) reported an historical cohort study by Malek et al. (1979) of 57 dry cleaners who used TCE as a cleaning solvent. Exposure to TCE was confirmed by urine analyses of the metabolite TCA. The cohort supposedly represented 86% of all men in Prague who had spent a year or more as dry cleaners since the 1950s. The follow-up time ranged from 5 to 50 years with a median greater than 20 years. The 6 cases of cancer observed were not significantly ($p < 0.05$) different from the expected number. The small size of the cohort severely limited the power of the study to detect a significant increase in cancer incidence.

Tola et al. (1980) established a cohort of 2117 workers (1148 men, 969 women) who had been occupationally exposed to TCE at some time between 1963 and 1976. The files of the biochemical laboratory of the Institute of Occupational Health in Finland provided 2004 names of workers who had undergone urine analysis for TCA. This laboratory performed at least 90% of the nation-wide routine analyses to monitor exposure to TCE in workplaces. Most (91%) of the urinary TCA values were below 100 mg/L. The remainder of the cohort consisted of 80 TCE poisoning cases from the Occupational Disease Register of Finland and 33 additional names from employers. The Population Data Register of the Central Statistical Office of Finland and the Finnish Cancer Registry provided information concerning vital status and cause of death.

The age-, sex-, and cause-specific mortality rates were compared to expected rates calculated from the Finnish mortality statistics for 1971. The observed number of deaths (58) was lower than those expected (84.3). The cohort exhibited 11 cancer deaths (gall bladder, 1; lung, 4; breast, 1; uterus, 3; testis, 1; multiple myeloma, 1), a number not significantly different from the expected value of 14.3. However, the percentage of deaths attributable to cancer among the workers (11/58 = 19%) was slightly greater than expected (14.3/84.3 = 17%), but the difference was not significant ($p > 0.05$). The numbers of site-specific tumors were not compared to expected values. A subcohort included those workers exposed before 1970. No statistically significant differences in mortality due to tumors were observed in the subcohort. The number of tumor-related deaths observed (3) among those members of the subcohort with high exposure was above expected (1.8), but the difference was not significant (relative risk = 1.7; 95% confidence interval:

0.3 to 4.9).

The results from this study did not demonstrate an increased tumor incidence among workers exposed to TCE relative to that of the general Finnish population. Several limitations, however, prevent a firm conclusion. The duration of exposure to TCE was unknown. The cohort may possibly have included persons exposed to other organic solvents instead of TCE, due to improper testing by the attending physician (U.S. EPA, 1985a). The lower total mortality is expected in a population of workers not given an adequate latency period. The short follow-up time of 6 to 13 years for the subcohort and the youth of the workers (60% of the men and 40% of the women were younger than 40 years in 1976) limit the use of this study in evaluating the carcinogenicity of TCE. Mortality statistics from a nonexposed worker population might have given a more accurate comparison.

Shindell and Ulrich (1985) studied a cohort of 2646 people who had worked at least 3 months between 1957 and 1983 at a facility that used TCE as a degreasing agent. The cohort showed a healthy worker effect (Standard Mortality Ratio = 0.79 for all causes of death) and much lower levels of heart disease and hypertension than the general population. Cancer was classified only into respiratory and nonrespiratory cancer. The observed number of cases of nonrespiratory cancer (n=12) in white males was significantly different from the expected (n=20.9); other observations on cancer were not significantly different from expected. No information was given on exposure levels.

Other Cohort Studies

Use of TCE as a dry-cleaning solvent began in the 1930's and waned in the 1960's (Waters et al., 1977). Cohort studies of dry-cleaning workers have been reviewed in the past (IARC, 1979; Apfeldorf and Infante, 1981) but were not mentioned by the U.S. EPA (1985a). The value of these studies is greatly limited by an undefined exposure to TCE and is confounded by exposure to other dry-cleaning agents such as tetrachloroethylene, carbon tetrachloride, and petroleum solvents.

Significant ($p < 0.05$) increases in the incidence of cancers of the lung, cervix, and skin contributed to an overall significant excess of cancer deaths among 330 deceased laundry and dry-cleaning workers (Blair et al., 1979). In this cohort, employees who had a mean union membership of 13 years also showed a slight increase in leukemia, liver, and kidney cancer, and a deficit of breast cancer compared to that expected. The authors warn that the cohort

mortality pattern may reflect inherent biases, such as socioeconomic status and smoking, and should be interpreted cautiously.

Katz and Jowett (1981) reported a significant elevated risk for cancers of the kidney ($p < 0.05$) and genitals ($p < 0.01$) in a cohort of 671 deceased white female laundry and dry-cleaning workers. The cohort also exhibited smaller excesses of lymphosarcoma, bladder cancer, and skin cancer. An increase in cervical cancer disappeared when compared to low-wage controls.

Most (82-95%) of the TCE produced in the U.S. is used as a solvent for degreasing metal parts (Waters et al., 1977; Kimbrough et al., 1985). A mortality analysis of a cohort of metal platers and polishers revealed significantly ($p < 0.05$) higher proportionate mortality ratios for esophageal and liver cancer deaths relative to a general white male population (Blair, 1980). The lack of increased mortality from lung cancer or cirrhosis of the liver indicates that tobacco usage and alcohol consumption did not contribute significantly to these particular cancer deaths. The positive results are, however, confounded by occupational exposure to known carcinogens, including chromium, nickel, and other metals, along with acids and other solvents.

Summary of Evidence of Carcinogenicity in Humans

Evaluation of the carcinogenic potential of a chemical is based on the results of short-term assays of mutagenesis, pharmacological studies (e.g., uptake, distribution, metabolism), lifetime animal bioassays, and epidemiological evidence. Several agencies and groups have developed systems of classification for evaluating evidence of the carcinogenic activity of a substance. The International Agency for Research on Cancer (IARC, 1984) separates strength of evidence of carcinogenic activity into 4 groups: sufficient evidence, limited evidence, inadequate evidence, and no evidence of carcinogenicity. Inclusion in any of these categories is based on data from short-term assays as well as animal and human studies, if available. The U.S. Environmental Protection Agency uses the same groupings but places chemicals in one or the other category solely on the basis of animal bioassay data (U.S. EPA, 1986b). To assess overall evidence of carcinogenic potential to humans, 6 additional categories are used: Group A - Human Carcinogen, Group B - Probable Human Carcinogen (further separated into B1 and B2), Group C - Possible Human Carcinogen, Group D - Not Classified (due to inadequate animal evidence), and Group E - No Evidence of Carcinogenicity for Humans (U.S. EPA, 1986b).

In the absence of adequate epidemiological data, the greatest weight of evidence in a carcinogen assessment is typically given to the results of lifetime animal bioassays. The criteria employed in analysis of bioassay data include an increase in the incidence of tumors in treated animals over that noted in controls, a decrease in latency as indicated by a shorter time to tumor development, the development of rare tumors, and an increase in the number of tumors in individual animals.

Based on their designation of "limited evidence" of carcinogenicity in animals and "inadequate evidence" of carcinogenicity in humans, IARC (1984) determined that TCE cannot be classified as to its carcinogenicity to humans. The U.S. EPA Health Assessment Document for Trichloroethylene (U.S. EPA, 1985a) analyzed further evidence of carcinogenicity of TCE. This evaluation included an extensive review of short-term test results, data from animal tests, and several epidemiological studies. The U.S. EPA concluded that evidence for the carcinogenicity of TCE in animals was "sufficient," and that the epidemiological data were inconclusive. Trichloroethylene was, therefore, placed in Group B2, a probable human carcinogen (U.S. EPA, 1985a). It has also recently been referred to by the U.S. EPA as a probable human carcinogen in the context of regulating suspected human carcinogens in drinking water and in air (U.S. EPA, 1985b, 1985c).

5. CARCINOGENIC POTENCY

The term carcinogenic "potency" refers to the quantitative expression of increased tumorigenic response per unit dose rate. Responses were predicted using the "linearized" multistage dose-response extrapolation model adopted for regulatory purposes by the California Department of Health Services (CDHS, 1985) and the U.S. Environmental Protection Agency (U.S. EPA, 1980, 1986b; Anderson et al., 1983). The following carcinogenic potency assessment proceeds in 3 steps: (1) selection of bioassay data sets indicative of TCE carcinogenicity which are suitable for dose-response assessment; (2) derivation or adjustment of the appropriate response and dose information to use in dose-response assessment for the animal bioassay data; and (3) quantification of potency in terms of human applied dose based on the latter data taking TCE metabolism into account.

A carcinogenic potency assessment for TCE was included in a recent health risk assessment document on TCE prepared by the U.S. EPA (1985a). The U.S. EPA's potency assessment was based on a smaller set of animal bioassay data than is considered in the present analysis, and the method used to convert applied TCE doses to equivalent metabolized doses was different from the physiologically based pharmacokinetic (PBPK) approach used here. For comparative purposes, these differences are pointed out in the following subsections.

SELECTION OF BIOASSAY DATA INDICATIVE OF TCE CARCINOGENICITY

The criteria used for selecting the most appropriate bioassay data sets, listed in Tables B-1 through B-5 in Appendix B, are contained in regulatory guidelines available for this purpose (CDHS, 1985; U.S. EPA, 1986b). Specifically, to determine tumor-incidence-rates, data sets were used that showed a statistically significant increase in the incidence of malignant tumors of a given histologically defined type within animals of a given sex from a given study. In addition, data sets were used that, along with such malignant tumors, included benign tumors that were histologically related to, and had the potential to become like, the corresponding observed malignant tumors. The use of these selection criteria resulted in a total of 13 data sets on which carcinogenic potency analyses could be based. These data sets consist

of: data on hepatocellular carcinomas in male and female B6C3F1 mice given TCE by gavage (NCI, 1976); data on hepatocellular carcinomas, and carcinomas or adenomas, in male and female B6C3F1 mice given TCE by gavage (NTP, 1983); data on renal tubular cell adenocarcinomas, and adenocarcinomas or adenomas, in male Fischer 344/N rats given TCE by gavage (NTP, 1983); data on hepatocellular carcinomas, and carcinomas or adenomas, in male B6C3F1 mice exposed to TCE by inhalation (Bell et al., 1978); data on malignant lymphomas in female Han:NMRI mice exposed to TCE by inhalation (Henschler et al., 1980); data on lung adenocarcinomas in female ICR mice exposed to TCE by inhalation (Fukuda et al., 1983); and data on malignant hepatomas in male Swiss mice exposed to TCE by inhalation (Maltoni et al., 1986). These data sets are summarized in Table 5-1, which appears at the end of this section, along with corresponding dose, tumor-incidence, and potency information explained below. These bioassay data sets are used here for potency assessment because they represent the only lifetime-exposure studies with well-defined, exposure-response data that indicate a positive carcinogenic response for TCE in animals and because comparable human epidemiological data are not available.

The inclusion of all 13 data sets in this analysis is not intended to imply that all reflect studies that were conducted with equal scientific merit or that yielded results of equal biological significance. The 2 NTP data sets on kidney tumors in male Fischer rats, in particular, are included largely for comparative purposes. The authors of the NTP report concluded that these data are "considered to be inadequate to evaluate the presence or absence of a carcinogenic response" in light of the toxic nephrosis and reduced survival observed in the dosed male rats (NTP, 1983). Similarly, the data on carcinogenicity of inhaled TCE in B6C3F1 mice are based on a study noted to have serious flaws in its records of dosing, animal sexes, and tumor incidence (Bell et al., 1978; U.S. EPA, 1985a).

In its quantitative potency assessment for TCE, the U.S. EPA (1985a) relied only on the NCI (1976) and NTP (1983) data on hepatocellular carcinoma incidence in orally exposed male and female B6C3F1 mice. In the U.S. EPA assessment, the male rat-kidney-tumor data (NTP, 1983) and male mouse-liver-tumor data (Bell et al., 1978) referred to above were excluded because of questions regarding the quality of the data (U.S. EPA, 1985a). The data of Maltoni (1986) on malignant hepatomas in Swiss mice and the data of Fukuda et al. (1983) on lung tumors in female ICR mice exposed to TCE by inhalation were not available for review when the U.S. EPA report was in

preparation. The data of Henschler et al. (1980) on malignant lymphomas in female Han:NMRI mice dosed by inhalation with TCE were also excluded as a basis for potency assessment, apparently for the reason that these tumors had a high rate of spontaneous occurrence that, it was hypothesized by the U.S. EPA, might have increased as an indirect consequence of TCE dosing that was possibly mediated by immunological suppression arising from the TCE-exposure regimen (U.S. EPA, 1985a).

Included among the data sets in Table 5-1 are the results of recent carcinogenicity bioassays (Table B-6 in Appendix B) for 2 TCE metabolites, trichloroacetic acid (TCA) and dichloroacetic acid (DCA), administered chronically in drinking water to B6C3F1 mice over their lifetimes (Herren-Freund et al., 1987). Potency assessments based on the bioassay data for these metabolites are undertaken to compare the calculated potencies for the metabolites to those calculated for TCE itself to test the hypothesis that the carcinogenicity of the metabolites of TCE may account for the carcinogenic response elicited by the parent compound.

ADJUSTMENT OF ANIMAL BIOASSAY DOSE-RESPONSE DATA

Brief reviews are given below of the administered dose and tumor-response information for the NCI gavage study with mice and for the NTP inhalation studies with rats and mice. This information is summarized in Table 5-1, which appears at the end of this section. From the administered doses D used in those bioassays, corresponding lifetime, time-weighted-average (TWA) metabolized doses were derived using pharmacokinetic assumptions and relationships discussed in Section 3. This approach is taken because TCE is a volatile, lipophilic compound and because extensive evidence, reviewed in Section 3, exists that demonstrates TCE metabolism in mammals. The products or the process of this metabolism, rather than the presence of TCE itself, are thought to be responsible for most forms of TCE's subchronic and chronic toxicity and in particular for its carcinogenicity in laboratory animals (Stott et al., 1982; Elcombe, 1985; Elcombe et al., 1985; Green and Prout, 1985; Goldsworthy and Popp, 1987).

In regulatory risk analysis for this type of compound, carcinogenic potencies can be estimated on the basis of metabolized dose, in addition to the administered or applied dose, since adequate metabolic data are available (U.S. EPA, 1985a, 1985d, 1985e, 1986b; CDHS, 1985). The following rationale was used

by the U.S. EPA for calculating the dose-response relationship for TCE-induced tumorigenesis based on the total amount of TCE metabolized as the effective dose (U.S. EPA, 1985a, p. 8-124):

> The use of this surrogate effective dose may not eliminate the uncertainty associated with the low-dose extrapolation because the dose actually reaching the target sites may not be linearly proportional to the total amount metabolized, and the shape of the dose-response relationship is still unknown. However, it seems reasonable to expect that the uncertainty with regard to the low-dose extrapolation would be somewhat reduced by the use of the metabolized dose because the metabolized dose better reflects the dose-response relationship, particularly within the high-dose region.

The available experimental data and modeling techniques, reviewed in Section 3, are sufficient to calculate a metabolized dose using a PBPK approach. Thus, tumor-incidence data from bioassay studies are also modeled in the following assessment as a function of metabolized dose (rate) of TCE, which will hereafter be denoted M and is equal to the quantity $\dot{A}_m$ defined in Section 3, but is here expressed in mg/kg-day.

The use of metabolized, rather than applied, dose as the basis for potency assessment adds new sources of uncertainty to the dose-response analysis. These include: (1) uncertainty in parameter estimates based on animal or human experimental data; (2) uncertainty involved in extrapolating kinetic constants between species (note, however, that certain key constants were estimated, in Section 3, for humans based directly on human metabolic data); and (3) uncertainty regarding the extent to which interindividual variability pertains to pharmacokinetic parameters, particularly for humans. The latter source of uncertainty is, of course, common to many problems in predictive regulatory toxicology, not just pharmacokinetic analysis. For toxic endpoints other than mutation and carcinogenesis, this uncertainty is generally dealt with by incorporating a safety factor in a final risk prediction, but the same approach could easily be adopted for these particular endpoints as well, to the extent that interindividual variability in mutation- or cancer-related metabolic capacity is of particular regulatory concern. Thus, even accounting for uncertainty in interspecies extrapolation of metabolic and other pharmacokinetic parameters, uncertainty remains that is difficult to quantify in the absence of relevant exploratory data.

In addition to using estimates based on metabolized TCE dose in this dose-response analysis, the report also relies on the amount of TCE that is

potentially available for uptake and metabolism, that is, the applied dose. The latter measure is not expected to correspond to the TCE dose capable of covalently binding to critical cellular macromolecules in the "independent hit" fashion presumed to be necessary by the "linearized," multistage, dose-response, extrapolation model that has been adopted for use in this potency assessment. However, use of an applied dose assumes that such metabolism is similar across species. Thus, the decision of whether or not to account for TCE metabolism involves a trade-off between the additional parameter uncertainties associated with a pharmacokinetic model and the probable systematic error associated with some alternative model that does not consider TCE metabolism is similar across species. The application of these approaches, are described below for each bioassay data set considered for the assessment of TCE's carcinogenic potency in animals.

NCI (1976) Study of Mice Dosed with TCE by Gavage

In this study, male and female B6C3F1 mice were dosed with TCE by gavage 5 days/week for 78 weeks. Surviving mice were sacrificed at week 90. The incidence of hepatocellular carcinoma (HCC) was significantly increased in dosed animals of both sexes; the first malignant liver tumors appeared in weeks 27 and 76 for the males and females, respectively. Time-of-death information was available, so the incidence-rate denominators for this study appearing in Table 5-1 represent animals surviving at least until the appearance of the first observed malignant tumor to adjust for the effect of competing mortality risks on observed tumor incidence.

The estimation of metabolized TCE dose for this study was based on the data of Prout et al. (1985), which showed an approximately linear relationship between applied gavage dose and total metabolized dose with a 0-intercept-slope parameter equal to 0.788, as discussed in Section 3. Therefore, the relationship $M = 0.788D$ is assumed for each day on which exposure occurred in this study. However, to represent M in terms of a lifetime TWA equivalent dose of the type used as input for cancer-risk extrapolation, this relationship needs to be modified to account for the interrupted (5 days/week for 78 of 90 weeks) and partial lifetime (approximately 90 weeks out of a theoretical 2-year mouse lifespan) exposure pattern of this bioassay. For regulatory purposes, an adjustment is typically made whenever bioassay duration is less than an animal's lifespan. This adjustment is made by assuming that cumulative

age-specific cancer rates increase as the third power of age (U.S. EPA, 1980; Anderson et al., 1983; CDHS, 1985). Thus, given a bioassay duration of L_e and a natural test-species lifespan of L, the low-dose potency, i.e., dose-response slope value, calculated using a multistage-risk-extrapolation model is increased by the factor $(L/L_e)^3$ to yield the potency expected if the bioassay had been continued for the full lifespan L at the same TWA dose level. Equivalently, the lifetime TWA dose that would yield the corrected potency value, based on risk extrapolation with the same model and observed tumor-incidence data, would be the actual bioassay TWA dose multiplied by the factor $(L_e/L)^3$. The latter approach is used in the potency assessment.

The bioassay data of Prout et al. (1985) on TCE metabolism in male B6C3F1 mice (Section 3) served as the basis for calculation of metabolized doses for mice used in the NCI (1976) bioassay because they constitute the largest experimental data set that involved B6C3F1 mice dosed by gavage and that used a mass-balance approach for the measurement of metabolites. The extrapolation of the Prout et al. data on metabolism after single gavage dosing to the chronic exposure conditions of the NCI (1976) bioassay appears justified because the TCE doses in both exposure contexts were virtually completely metabolized within 24 hours after exposure, as discussed in Section 3. Such an assumption, however, dose not take into account any effects that may result from chronic exposure that could influence metabolism (e.g. inducing P-450 destruction). Based on the PBPK analysis presented in Section 3, the fact that the mice used in the Prout et al. study differed somewhat in weight from those used in the NCI bioassay does not alter the slope parameter expected to apply to the NCI mice.

Combining the adjustments referred to above, lifetime TWA-equivalent values were derived for total metabolized TCE dose, M, in the NCI (1976) study from corresponding values of applied dose, D, using the following relationship:

$$M = 0.788D \times 5/7 \times 78/90 \times (90/104)^3 = 0.316D, \qquad (5\text{-}1)$$

in which D represents an experimental applied TWA dose listed in Table 5-1. Values of M thus calculated appear in Table 5-1.

In contrast to the approach summarized by Equation 5-1, the approach taken by the U.S. EPA (1985a) involved a calculation of metabolized dose for the NCI-bioassay mice based on applying a Michaelis-Menten model to the Prout et al. (1985) data. However, the U.S. EPA assessment pointed out that experimental data on TCE metabolism in mice are also consistent with an

assumption that the metabolized fraction of any applied oral dose to mice from 800 to 1600 mg/kg is equal to about 78% of the administered dose, which is approximately the same as the value (78.8%) used in the present assessment.

NTP (1983) Study of Mice Dosed with TCE by Gavage

In this study, male and female B6C3F1 mice were dosed with TCE by gavage 5 days/week for their expected lifetimes, with terminal sacrifice at 104 weeks. In dosed animals of both sexes, the incidences of HCC and of the combination of hepatocellular adenoma (HCA) or HCC were significantly increased; the first of such tumors was observed during week 57 and week 85 for males and females, respectively. Time-of-death information was available for this study. To adjust for the effect of competing mortality risks on observed tumor incidence, the incidence-rate denominators appearing in Table 5-1 for this study represent animals surviving at least until the appearance of the first observed malignant tumor. Adjustment of tumor-incidence data to remove animals dying prior to the occurrence of the first tumor observed was necessary only for the control and exposed groups of female mice.

Calculation of metabolized dose for the 4 sets of bioassay data associated with this study followed the approach taken with the NCI (1976) dose data as discussed above, except that the adjustments relating to truncated dosing and observation periods were not applicable to the NTP (1983) dosing data. Thus, the lifetime TWA metabolized doses given in Table 5-1 for this study were calculated using the relationship $M = 0.788D \times 5/7 = 0.563D$, where D is the corresponding experimental applied dose listed in Table 5-1.

In its calculation of metabolized doses for the NTP-bioassay mice, the U.S. EPA (1985a) used the same approach described above for the NCI-bioassay mice. Again, this approach yielded values of M that are within 5% of those predicted by a linear approach assuming 78.8% metabolism.

NTP (1983) Study of Rats Dosed with TCE by Gavage

This study involved male and female Fischer 344/N rats dosed with TCE for 5 days/week over their lifetimes with terminal sacrifice at 104 weeks. The incidence of renal tubular cell adenocarcinoma (RTAC), and the combination of renal tubular cell adenoma (RTA) or RTAC, was significantly increased in dosed male rats as shown by an incidental tumor test that adjusts for early mortality

unrelated to RTAC or RTA incidence in the dosed males (NTP, 1983). Time-of-death information was available for this study, so incidence-rate denominators appearing in Table 5-1 for this study represent animals surviving at least until the appearance of the first observed malignant tumor to adjust for the effect of competing mortality risks on observed tumor incidence. The first RTAC and RTA observed in the male rats occurred in weeks 103 and 80, respectively. Therefore, the adjusted tumor-incidence-rate denominators appearing in Table 5-1 associated with this study are much lower than the corresponding unadjusted figures appearing in Table B-12.

For this study, calculation of metabolized TCE dose was based on the data of Prout et al. (1985) on TCE metabolism in male Osborne-Mendel rats given single TCE doses by gavage. This metabolic study was selected from among those studies listed in Table 3-5 because it represents the largest relevant data set involving mass-balance measurement of TCE metabolites produced in rats dosed only with TCE by gavage. A similar data set involving Fischer rats is not available. A choice between the linear and Michaelis-Menten approaches to modeling metabolite yield, as a function of applied TCE dose, was unnecessary in this case by virtue of the coincidence in applied dose levels actually used in both the Prout et al. study and the NTP rat bioassay; the 0, 500, and 1000 mg/kg doses were the same in both studies. For the purpose of estimating metabolized doses for the male F344/N rats in the NTP (1983) study, it was assumed that these rats metabolized the same amount as that measured in similarly dosed male Osborne-Mendel rats in the Prout et al. (1985) study. That is, experimental data were used to estimate metabolized doses in this case, rather than using a mathematical model for this purpose. An additional correction was made to account for the interrupted 5 days/week dosing regimen of the NTP bioassay. Thus, for both the male and female rats in the NTP bioassay, the lifetime TWA metabolized doses given in Table 5-1 were calculated using the relation $M = M_e(5/7)$, where M_e represents the metabolized dose experimentally observed in the Prout et al. (1985) study on Osborne-Mendel rats; i.e., M_e is equal to 277 and 395 mg/kg for administered doses of 500 and 1000 mg/kg, respectively (from Table 3-5).

It was assumed that the differences in weight between the male Osborne-Mendel rats used in the Prout et al. study and the male and female F344/N rats used in the NTP bioassay would not affect the expected fraction of applied dose metabolized, as predicted from the Prout et al. data, even though metabolic saturation, i.e., nonlinear metabolic kinetics, may have occurred at

the doses given to rats in the NTP bioassay. The basis for this assumption was explained in Section 3.

Bell et al. (1978) Study of Mice Dosed with TCE by Inhalation

In this study, B6C3F1 mice were exposed to TCE by inhalation for 6 hours/day, 5 days/week over their lifetimes, with terminal sacrifice at 104 weeks. Exposed male mice showed a significantly ($p < 0.05$ and $p < 0.01$, respectively) increased incidence of HCC and of the combination of HCC or HCA. Time-to-tumor information for this study was unavailable, so unadjusted tumor-incidence data were used for potency assessment.

Metabolized doses for the mice in this bioassay were calculated based on the data of Stott et al. (1982) on metabolism of TCE in male B6C3F1 mice exposed by inhalation to 10 and 600 ppm TCE for 6 hours. In Section 3, the Stott et al. mass-balance data were described as being consistent with a linear relationship between applied and metabolized dose, with a 0-intercept slope of 0.671 mg/kg-ppm-6 hours. The mice in this metabolism study were exposed to TCE for the same period of 6 hours as those in the Bell et al. bioassay, although the bioassay mice were exposed repeatedly over a 2-year period. As explained in Section 3, pharmacokinetic considerations imply that the repeated exposure pattern of the bioassay should not affect the calculation of metabolized dose for the mice in the bioassay. However, the difference in body weight between the mice used in the Stott et al. and Bell et al. studies would be expected to change the calculated values of M for the bioassay mice by the factor $(w_1/w_2)^{0.3}$, where w_1 and w_2 are the weights of the mice in the Stott et al. and Bell et al. studies, respectively, as explained in Section 3. The average weight of the mice used by Stott et al. is estimated to be 23 g. Similar mass-balance data on female Han:NMRI mice were not available. In the absence of specific data on animal weights from the Bell et al. study, it is assumed that the dosed male B6C3F1 mice used in this study had an average terminal weight equal to that of the 3 groups of dosed male B6C3F1 mice from the NCI (1976) and NTP (1983) studies, that is, 35 g. Thus, with the addition of a correction factor to account for intermittent (5 days/week) exposure, the lifetime TWA metabolized doses were calculated for mice in this study, appearing in Table 5-1, using the relationship:

$$M = 0.671D \times 5/7 \times (23/35)^{0.3} = 0.423D, \qquad (5\text{-}2)$$

in which, again, D is a corresponding experimental applied dose appearing in

Table 5-1.

Henschler et al. (1980) Study of Mice Dosed with TCE by Inhalation

This study involved Han:NMRI mice exposed to TCE by inhalation for 6 hours/day, 5 days/week over 78 weeks, with terminal sacrifice at 130 weeks, although most animals were dead after 2 years. Exposed female mice showed a significantly ($p < 0.05$) increased incidence of malignant lymphoma. Time-to-tumor information for this study was unavailable, so unadjusted tumor-incidence data were used for potency assessment.

Metabolized doses for the mice in this bioassay were calculated based on the data of Stott et al. (1982) on metabolism of inhaled TCE in male B6C3F1 mice, as described above with regard to the Bell et al. (1978) bioassay data, with modifications to account for the truncated, 78-week exposure duration and different animal weights involved in the Henschler et al. bioassay. Similar mass-balance data on female Han:NMRI mice were not available. In the absence of specific information on animal weights in this bioassay, the dosed female mice were assumed to have an average terminal weight of 30 g, which is the average terminal weight of the 3 groups of dosed female mice in the NCI (1976) and NTP (1983) bioassays. Thus, the DHS calculated the lifetime TWA metabolized doses, appearing in Table 5-1, for the female mice of the Henschler et al. bioassay using the relationship:

$$M = 0.671D \times 5/7 \times 78/104 \times (23/30)^{0.3} = 0.332D, \qquad (5\text{-}3)$$

in which D represents a corresponding applied dose listed in Table 5-1.

Fukuda et al. (1983) Study of Mice Dosed with TCE by Inhalation

In this study, female B6C3F1 mice were exposed to TCE by inhalation for 7 hours/day, 5 days/week over their lifetimes, with terminal sacrifice at about 2 years. Exposed mice showed a significantly ($p < 0.05$) increased incidence of lung adenocarcinoma. Time-to-tumor information for this study was unavailable, so unadjusted tumor-incidence data were used for potency assessment. Metabolized doses for the mice in this bioassay were calculated based on the data of Stott et al. (1982) on metabolism of inhaled TCE in male B6C3F1 mice, as described above with regard to the Bell et al. (1978) bioassay data with modifications to account for the longer daily exposure period (7 hours/day) and different mouse weights involved in the Fukuda et al.

bioassay. Similar mass-balance data on female B6C3F1 mice were not available. The longer daily exposure was assumed to increase the daily metabolized dose over that predicted by the Stott et al. data from mice exposed to TCE for only 6 hours, by the factor (7/6), in accordance with the PBPK-model results described in Section 3. For the same reasons discussed in the context of the Henschler et al. (1980) study, the dosed female mice in the Fukuda et al. bioassay were assumed to have an average terminal weight of 30 g. Thus, the lifetime TWA metabolized doses, appearing in Table 5-1, for the female mice of the Fukuda et al. bioassay were calculated using a relationship identical to Equation (5-3), except that the factor (78/104) in that equation was replaced by the factor (7/6). For this study,

$$M = 0.671D \times 5/7 \times 7/6 \times (23/30)^{0.3} = 0.516D. \quad (5\text{-}4)$$

Maltoni et al. (1986) Study of Mice Dosed with TCE by Inhalation

In this study, Swiss mice were exposed to TCE by inhalation for 7 hours/day, 5 days/week over 78 weeks, with terminal sacrifice at week 145, although most mice were dead by week 104. Exposed male mice showed a significantly ($p < 0.05$) increased incidence of malignant hepatoma. Time-to-tumor information for this study was unavailable, so unadjusted tumor-incidence data were used for potency assessment.

Metabolized doses for the mice in this bioassay were calculated based on the data of Stott et al. (1982) on metabolism of TCE in male B6C3F1 mice, as described above with regard to the Fukuda et al. (1983) bioassay data, with modifications to account for the truncated (78-week) exposure period and different mouse weights involved in the positive Maltoni bioassay considered here. The dosed male mice in this bioassay had an average terminal weight of about 41 g. Thus, the lifetime TWA metabolized doses, appearing in Table 5-1, for the male mice in this bioassay were calculated using the relationship:

$$M = 0.671D \times 5/7 \times 7/6 \times 78/104 \times (23/41)^{0.3} = 0.353D, \quad (5\text{-}5)$$

in which D is a corresponding applied dose listed in Table 5-1.

Herren-Freund et al. (1987) Study of Mice Dosed with TCE Metabolites via Drinking Water

In this study, the TCE metabolites, TCA and DCA, were administered in

drinking water at 5 g/L to male B6C3F1 mice for 61 weeks, with terminal sacrifice at week 65. The incidence of HCC was significantly ($p < 0.01$) increased in animals dosed with either compound. Time-to-tumor information was unavailable for this study.

The dosed mice in this study weighed approximately 30 g and were assumed to drink an average of 6 mL/day of the treated water, yielding a daily dose D of 1000 mg/kg of TCA or DCA. Adjusting for the partial (61 of 65 weeks in the experiment) and truncated (65 weeks of a 104-week lifetime) exposure regimen involved in these bioassays, the equivalent lifetime TWA-metabolite doses (see Table 5-1) for these studies were calculated using the relationship:

$$M = D \times 61/65 \times (65/104)^3 = 0.229D. \qquad (5\text{-}6)$$

CARCINOGENIC POTENCY EXTRAPOLATION BASED ON ANIMAL BIOASSAY-DATA

The carcinogenic potency assessment is based on a quantitative analysis of animal bioassay data sets under the assumption that TCE is carcinogenic to both animals and humans at the low environmental dose levels of regulatory concern. The rationale for using this assumption for TCE is discussed in detail elsewhere (U.S. EPA, 1980, 1986b; Anderson et al., 1983; CDHS, 1985). Arguments against using this assumption for TCE in dose-response extrapolation focus on the possibility that the observed carcinogenicity of compounds like TCE in bioassays conducted at high doses may be caused primarily by increased cellular and/or subcellular (e.g., peroxisome) proliferation (i.e., by tumor promotion or some epigenetic mechanism), rather than by initiation or some genotoxic mechanism, and that this induced cell behavior has a threshold-type, dose-response relationship implying a carcinogenic potency of zero at very low dose levels (Schumann et al., 1980; Stott et al., 1982; Buben and O'Flaherty, 1985; Elcombe, 1985; Elcombe et al., 1985; Green and Prout, 1985; Prout et al., 1985; Mirsalis et al., 1985; Goldsworthy and Popp, 1987).

Low-dose potency extrapolation from dichotomous tumor-response information in given animal-bioassay data sets was performed using a computer program (Crump and Watson, 1979) to numerically fit parameters to the multistage dose-response extrapolation model:

$$\text{Increased Risk} = R = 1 - e^{-\sum_{i=1}^{g} q_i d^i}, \qquad (5\text{-}7)$$

in which g is the number of exposed groups in the bioassay, d is the dose level in mg/kg-day at which the risk function is evaluated, and q_i, in units of $(mg/kg\text{-}d)^{-1}$, are the multistage parameters (q_1 = the low-dose "potency" parameter) that are estimated by using an iterative maximum-likelihood procedure (U.S. EPA, 1980, 1986b; Anderson et al., 1983; CDHS, 1985).

The input to this program for each bioassay data set consisted of the values for total lifetime TWA metabolized dose M (in mg/kg-d) and corresponding tumor-incidence data given in Table 5-1. For each data set, the Crump and Watson (1979) approach was used to calculate a one-tailed 95% upper confidence limit (UCL) for the linear parameter q_1 of the multistage model, denoted $q_1^*(M)$. Note that, according to the assumptions of the multistage model, the value of q_1 in Equation (5-7) must be greater than or equal to 0, whereas the value of $q_1^*(M)$ is restricted to be greater than 0. At any given very low dose, the multistage model predicts an increased UCL risk that is approximately equal to the product of $q_1^*(M)$ and dose. Calculated values of $q_1^*(M)$ corresponding to each of the bioassay data sets considered are given in Table 5-1. Both the GLOBAL79 and GLOBAL86 versions of the program gave similar results.

Time-to-tumor data were available for the NCI (1976) mouse-bioassay data, the NTP (1983) mouse-bioassay data, and the NTP (1983) rat-bioassay data. Thus, it was possible to use a more complex, time-dependent version of the multistage model that takes into account the observed differential survival of control and exposed bioassay groups. The computer implementation (WEIBULL82) of the time-dependent multistage model was developed by Howe and Crump (1983) and Crump and Howe (1984). This model is similar to the one defined by Equation (5-7), except that the exponentiated polynomial in that equation is multiplied by the new factor $(T\text{-}T_0)^k$, in which T_0 and k are new, additional parameters that are estimated using an iterative, maximum-likelihood procedure in which T refers to experimentally observed survival times for each animal. A 95% UCL potency, $q_1^*(M)$, calculated using the model is equal to a 95% evaluation of the term $q_1(T\text{-}T_0)^k$ in the time-dependent multistage model, where T is set equal to the bioassay durations of 90 weeks for the NCI bioassay and 104 weeks for the NTP bioassays.

In the "incidental-tumor" (IT) version of the time-dependent multistage model, the survival time for any animal with the tumor of interest represents a time greater than or equal to the time at which the tumor first appears in that animal. In the "lethal-tumor" (LT) version of this model, survival time for a

tumor-bearing animal represents the time of death caused by that tumor. For the purpose of potency estimates using the latter version, all tumor-bearing animals observed at terminal sacrifice are treated as if they were tumor free. In the absence of specific information on the lethality of each tumor observed in the NCI and NTP bioassays, both the IT and LT versions of the time-dependent multistage model are used here for the purpose of potency assessment. All potencies thus calculated are presented in Table 5-1. Such potencies are absent in Table 5-1 for the data sets involving the incidence of HCC in female mice of the NCI (1976) bioassay and RTAC in male rats of the NTP (1983) bioassay, because with these data sets the model failed to converge on optimized parameter estimates due to clustering of tumor observations at the time of terminal sacrifice.

CARCINOGENIC POTENCY OF HUMAN EQUIVALENT METABOLIZED DOSE

If it is assumed that the above calculated values of $q_1^*(M)$ are adequate to approximate expected tumor incidence among animals subjected to very low levels of metabolized dose M, then the application of these values in human cancer risk assessment raises the questions of whether or not these calculated potency values should be considered valid only for the exposure route, ingestion or inhalation, actually used in the corresponding bioassay, and whether or not a given value of M, should be considered equipotent for different species. These questions are addressed below.

Exposure-Route Extrapolation

Various physiological processes, such as the "first pass effect" involving liver-mediated metabolism, may result in differing values of metabolized dose for the same applied dose given by different exposure routes. In the case of TCE, carcinogenicity is hypothesized to be mediated by a reactive metabolite whose rate of production, according to the "oncogene mutation" theoretic basis of the multistage model, should be linearly related to increased tumor risk at low levels of increased risk, i.e., at levels of increased risk $R \ll 0.01$ such that $(1-e^{-R}) \simeq R$. Thus, according to this risk-prediction model, one would expect that a given metabolized dose, M, would be equipotent regardless of exposure route, as long as the distribution of this metabolized dose within susceptible target tissues was not affected by exposure route. The

distribution of TCE metabolites within potential target tissues in mice and rats is generally similar for both ingestion and inhalation exposure routes (Stott et al., 1982; Zenick et al., 1984). (Table 5-1 indicates potency values are similar for different routes of exposure.)

Interspecies Dose-Equivalence Extrapolation

Following the suggestion of Mantel and Schneiderman (1975), the U.S. EPA and the California Department of Health Services assume that mg/(surface area) is an equivalent measure of lifetime TWA dose among different species for carcinogens (U.S. EPA, 1980, 1986b; Anderson et al., 1983; CDHS, 1985). Specifically, for purposes of carcinogen-risk assessment, it is assumed that a daily, TWA lifetime dose expressed in units of $mg/kg^{2/3}$ is equivalent among different species because, to a close approximation, surface area is proportional to the 2/3rd power of body weight, as would be the case for perfect spheres of equal uniform density. Given this assumption and given a daily lifetime TWA metabolized dose for an animal of M_a mg/kg-d, then the equivalent human TWA dose would be $M_a f$, where the dose-equivalence factor f is given by:

$$f = \left(\frac{w_a}{h}\right)^{1/3} , \qquad (5\text{-}8)$$

in which w_h and w_a are the weights of a person (assumed to be 70 kg) and of the test animal, respectively.

Analyses of available bioassay data indicate that extrapolation of lifetime-animal-cancer-risk estimates based on either a mg/(surface area) or a mg/(kg dosage) scale may lead to reasonable projections of corresponding predicted human cancer risk (Crouch and Wilson, 1979; Hogan and Hoel, 1982; Crouch, 1983). However, the basis upon which interspecies potency correlations were made on either dosage scale in these particular studies has been called into question (Bernstein et al., 1985). The Mantel and Schneiderman (1975) suggestion, relied on by the U.S. EPA, explicitly offered only a "first approximation...[where the] basic assumption is that the locus of action of any drug is on some surface area," an approximation that was suggested for use because no "suitable data exist on the quantitative extrapolation of carcinogenic effects from animal to man."

In the absence of definitive empirical data, however, it is simply not

known which interspecies dose-extrapolation assumption better reflects reality in the context of extrapolating tumor-response data in animals to anticipated response in humans. Existing data do not rule out either approach. The surface area correction factor can be used as an uncertainty factor to adjust for the potential variability in human response to TCE exposure. In addition, there are many uncertainties involved in PBPK approaches. Based on current practice by both EPA and DHS, extrapolation of potency values from animals to humans is carried out using a dose-per-body-surface-area (SA) extrapolation method, but a dose-per-body-weight (BW) extrapolation is also shown for comparison.

Human Metabolism

A PBPK approach was used to estimate the extent of TCE metabolism in humans exposed to low environmental levels of TCE. Under these exposure conditions, in accordance with the estimates presented in Section 3, humans are assumed to metabolize 100% of all ingested TCE and 72% of all TCE respired through alveolar air. Thus, the UCL carcinogenic potency $q_1^*(D_i)$ of an applied lifetime TWA ingested dose D_i of TCE to humans is estimated to be equal to $q_1^*(M)$ based directly on the bioassay data for TCE-exposed animals considered in Table 5-1. The potency $q_1^*(D_r)$ of a respired dose D_r of TCE to humans is estimated to be equal to $0.72q_1^*(M)$, where M is in mg/kg total body weight per day or mg/m^2 total body surface area per day. The latter dose metric for metabolized dose was selected for use in this risk assessment for the following reasons. Although the animal tumor incidence data that serve here as a basis for potency extrapolation primarily involve liver tumors, 3 other tumor sites (lung, kidney, and lymphoreticular tissue) are also reflected in the bioassay data, so a single tissue-specific metric for metabolized dose is not possible for all relevant data sets on TCE-induced cancer in animals. While it is most desirable when using a PBPK approach to risk assessment to employ a dose metric that corresponds to a particular target tissue at risk (Andersen et al., 1987), it is not possible to use this strategy when a compound exhibits toxicity in multiple target tissues. For TCE in particular, this point is underscored by the fact that the major metabolites (TCA, TCEL, and chloral hydrate) persist and circulate systemically prior to excretion and/or further metabolism. While it would be possible to specify several different plausible alternative target tissues and potentially carcinogenic metabolites for which to extrapolate TCE's carcinogenic potential in humans based on animal bioassay data, and to generate a matrix of corresponding alternative potency values, the uncertainties currently inherent

in high- to low-dose potency extrapolation so greatly outweigh those involved in the identification of the most suitable target tissue(s) as to recommend the simpler approaches of applied dose and metabolized dose per kg of total body weight or per m^2 total body surface area. Of course, if toxicity is presumed to occur only at the site of metabolism, i.e., of reactive metabolite formation, then the latter approach to interspecies extrapolation would be expected to yield results identical to those of a tissue-specific approach for any metabolizing tissue comprising a fraction of body weight or surface area that is equal in both humans and the animal species from which a dose-response relationship is being extrapolated. For example, liver comprises about 4% of total body weight in mice, rats, and humans (Foster et al., 1983; NRC, 1986; Ward et al., 1988), so metabolized dose per kg or m^2 liver is approximately equivalent, as far as interspecies dose-response extrapolation is concerned, to that per kg or m^2 total body weight for this tissue in these 2 species.

Potential Human Cancer Potency of TCE based on Metabolized Dose

Based on the bioassay data considered, 25 alternative values of q_1^*(M) for TCE are provided in Table 5-1 that are based surface-area (SA) methods of interspecies dose-equivalence extrapolation. (An equal number of values based on the body weight (BW) method of extrapolation are shown for comparison.) The potencies in Table 5-1 based on the SA method of interspecies dose-equivalence extrapolation, which apply only to humans, range from 0.0020 to 0.098 $(mg/kg\text{-}d)^{-1}$, a 49-fold range, and have an arithmetic mean of 0.023 $(mg/kg\text{-}d)^{-1}$. (The potencies in Table 5-1 based on the BW method, which apply to animals and humans alike, range from 0.00034 to 0.0074 $(mg/kg\text{-}d)^{-1}$, an approximately 22-fold range, and have an arithmetic mean value of 0.0019 $(mg/kg\text{-}d)^{-1}$.) The highest potency value obtained is based on the data of Henschler et al. (1980) on malignant leukemias in female Han:NMRI mice exposed to TCE by inhalation.

Caution should be used in the selection or weighting of potency values from Table 5-1 for predicting human cancer risk and their use must be conditioned on the assumptions in this potency assessment, because all the corresponding bioassay data are not equally valid or relevant. For example, the Henschler et al. (1980) and Bell et al. (1978) mouse studies and the NTP (1983) rat studies were all judged by the U.S. EPA to have defects that made their results inadequate to use in the quantification of TCE's carcinogenic

potency, despite the support they lent to EPA's weight-of-evidence evaluation that TCE is a probable human carcinogen (U.S. EPA, 1985a-c). No attempt was made by the U.S. EPA (1985a) to quantify the extent to which each of these studies are "inadequate."

Two pairs of potency values are given in Table 5-1 for the TCE metabolites, TCA and DCA, which were each associated with significantly ($p < 0.01$) increased HCC incidence in male B6C3F1 mice when they were administered in drinking water for 61 weeks. These potency estimates allow a qualitative test of the hypothesis that one or both of these metabolites may account for the estimated carcinogenic potency of the parent compound, TCE. For the purpose of this test, only BW-based potencies are considered. The UCL potencies for TCA and DCA (see Table 5-1) must be adjusted using the appropriate molecular-weight ratios to reflect the corresponding TCE-equivalent values of 0.0059 and 0.017 (mg TCE-equiv./kg-d)$^{-1}$, respectively. Next, these values must be further adjusted to reflect the fact that neither TCA nor DCA represent 100% of the TCE-equivalent doses of TCE metabolites received by the mice in the bioassays for TCE-carcinogenicity in mice considered in Table 5-1. The DCA is known to be only a minor metabolite of TCE administered by gavage in mice, accounting for less than 1% of an applied TCE dose (Hathaway, 1980; Dekant et al., 1984; Green and Prout, 1985), whereas TCA may constitute 10 to 20% of the metabolites produced after chronic oral doses of TCE in male B6C3F1 mice (Green and Prout, 1985). Thus, based on the potency analysis of the Herren-Freund bioassay data, the TCE-equivalent potencies of TCA would be 0.001 to 0.002 (mg TCE-equiv./kg-d)$^{-1}$ and that for DCA would be 0.0002 (mg TCE-equiv./kg-d)$^{-1}$. These values may be compared to the restricted set of TCE-potencies referred to earlier, which have a mean value (and range) of 0.002 (0.0003 to 0.005) (mg/kg-d)$^{-1}$. Based on this qualitative comparison, it is unlikely that DCA accounts for most of the estimated carcinogenic potency of TCE in mice because the TCE-equivalent potency for DCA is roughly an order of magnitude lower than the average of those calculated for TCE in mice. The TCE-equivalent potency obtained for TCA, however, is consistent with the hypothesis that TCA can account for most of the estimated carcinogenic potency of TCE in mice.

Comparison with Earlier Potency Estimates using Metabolized Dose

Potency values for the NCI (1976) and NTP (1983) bioassay data sets listed

in Table 5-1 may be compared to corresponding values calculated by the U.S. EPA (1985a) listed in Table 5-2, which appears after Table 5-1 at the end of this section. The approach taken by the U.S. EPA for extrapolation of cancer potency in animals from these data sets is similar to the present approach, as is reflected in the similar values of $q_1^*(M)$ for the corresponding data sets in Tables 5-1 and 5-2 The values differ by less than 60%. The differences are due primarily to the fact that, in contrast to the U.S. EPA (1985a), the relevant tumor-incidence data were adjusted to account for early mortality among the mice.

The approach to extrapolate $q_1^*(M)$ potency values to equivalent values as a function of human applied dose, i.e., to values of $q_1^*(D)$, differs from that used in U.S. EPA (1985a), since the relationship between human applied and metabolized dose derived in the U.S. EPA (1985a) report is quite different. A comparison of the values of $q_1^*(M)$ in Table 5-1 (by the SA method) and $q_1^*(D)$ in Table 5-2 reveals that the U.S. EPA assumed that, at very low levels of exposure, humans and mice metabolize approximately 85% of an ingested dose of TCE, where 85% is simply the ratio of the values of V_{max} and K_m that were used by U.S. EPA (1985a) to fit a Michaelis-Menten relation to the data of Prout et al. (1985) on TCE metabolism in male B6C3F1 mice (see earlier subsection on NCI (1976) Study of Mice Dosed with TCE by Gavage). On the other hand, the PBPK analysis in Section 3 was used here to estimate that approximately 100% of a very small ingested TCE dose is metabolized. In addition, although DHS uses the surface area (SA) extrapolation as does the EPA, both SA and body weight (BW) extrapolations are presented in Table 5-1 for comparative purposes.

To estimate its recommended carcinogenic potency for inhaled TCE, the U.S. EPA (1985a) relied on the data of Monster et al. (1976) on TCE metabolism in 4 subjects exposed to 70 ppm TCE for 4 hours, including 1 hour of 100-watt (strenuous) exercise. These subjects metabolized a median amount of 439 mg TCE, representing 90% of the retained TCE dose, which the U.S. EPA used to obtain its estimate of 6.9×10^{-3} mg/(μg/m^3)-day (or 0.48 mg/ppm-d) as the expected amount of TCE metabolized upon human exposure to a constant, small concentration of TCE in air expressed as μg/m^3 (or ppm). Assuming an average alveolar ventilation rate of 353.5 L/hour (Table 3-9), the U.S. EPA analysis implies that 82.5% of ventilated TCE is metabolized for low concentrations of TCE in air, whereas the PBPK analysis in Section 3 implies that the percent of TCE metabolized is 72%. This discrepancy is due to the

fact that the former approach extrapolated the Monster et al. (1976) data on metabolite yield resulting from a 4-hour TCE exposure with strenuous exercise for 25% of the exposure period to an expected metabolite yield for a typical 24-hour period. In Section 3 it was shown that data of Monster et al. (1976) on TCE uptake and metabolism in humans, as well as similar data from other studies, are consistent with the prediction that 72% of alveolarly ventilated TCE is ultimately metabolized under low-level ambient exposure conditions.

Consideration of Potencies Derived from Inhalation Exposures

To make the risk assessment process specific to air exposures, carcinogenicity by inhalation was selected. The 4 studies of carcinogenicity using an inhalation route of exposure (Bell et al., 1978; Henschler et al., 1980; Fukuda et al., 1983; Maltoni et al., 1986) yielded 5 values for potency using the surface area extrapolation procedure (Table 5-1). From the Bell et al. (1978) study, the incidence of hepatocellular adenomas and carcinomas combined was selected since adenomas may have the potential to become carcinomas. The resulting 4 potency values have a range of 0.0098 to 0.098 (mg M/kg-d)$^{-1}$ with a mean value of 0.041 (mg M/kg-d)$^{-1}$. Note that this range includes the most sensitive sex, site, and species - lymphoma in female mice - consistent with DHS guidelines (California Department of Health Services, 1985).

Potencies Derived from Applied Dose

Prior risk assessments by DHS staff have used applied dose to calculate carcinogenic potencies. Carcinogenic potencies based on applied dose were determined for the 4 inhalation studies. Estimated inhalation rates in mice were based on the EPA formula:

$$I = 0.0345[\text{mouse wt(kg)}/0.025\text{(kg)}]^{2/3} \text{m}^3/\text{day},$$

where I = inhalation rate (Anderson et al., 1983). Thus, as an example, the 41 g mice in the Maltoni et al. (1986) study would breathe $0.0345(41/25)^{2/3}$ = 0.048 m^3 in 24 hours and 0.014 m^3 during a 7-hour exposure to TCE. At the lowest concentration of 100 ppm, which is equal to 538 mg/m^3 TCE, the mice would breathe 7.53 mg of TCE.

The values of inhaled TCE were then adjusted to account for a 5-day-per-week exposure and, in the case of the Henschler et al. (1980) and the Maltoni et al. (1986) studies, for the less than lifetime exposures. Resulting values of the lifetime dose are shown in Table 5-3. Human equivalent doses were then calculated by dividing the animal applied dose by (human body weight/mouse body weight)$^{1/3}$, the surface area correction. Thus, as an example, for 41 g mice, the animal applied dose was divided by (70 kg/0.041 kg)$^{1/3}$ or 11.9. Application of the surface area correction accounts for sensitivity differences across species. The resulting human equivalent doses were used with the animal tumor incidences in the Global 86 computer program. The 4 studies yielded a range of 95% Upper Confidence Limit (UCL) potency slopes based on applied dose of 0.004 to 0.034 (mg/kg-d)$^{-1}$ with a geometric mean value of 0.010 (mg/kg-d)$^{-1}$. The range of potency values overlaps that determined using the metabolized dose, where the range was 0.0098 to 0.098 (mg/kg-day)$^{-1}$. EPA (1985a) calculated a potency (q_1^*) of 1.1×10^{-2} (mg/kg-day)$^{-1}$ based on the applied dose of TCE.

Comparision of Metabolized and Applied Dose Approaches

The range of potency values derived using the applied dose, 0.004 to 0.034 (mg/kg-day)$^{-1}$, overlaps the range obtained using the metabolized dose (range = 0.0098 to 0.098 (mg/kg-day)$^{-1}$. This is not surprising since greater than 50% of TCE is metabolized and several metabolites of TCE (see Section 4) are mutagenic and therefore potentially carcinogenic. In fact TCA has been directly shown to be carcinogenic (Herren-Freund et al., 1987). The range of potency values suggested for TCE includes both values. The suggested range is 0.004 to 0.098 (mg/kg-d)$^{-1}$, a 24-fold range from the lower end of potency values derived using applied dose to the upper end of the range derived using metabolized dose.

Estimate of Statewide Cancer Incidence Due to Exposure to Ambient TCE

Ambient air measurements of TCE at 20 stations throughout California in 1986 and 1987 indicated a population-weighted mean concentration of 0.22 ppb (1.18 $\mu g/m^3$), as discussed in part A. A person breathing 20 m^3 per day of air containing 1.18 $\mu g/m^3$ TCE would inhale 23.6 ug or 0.0236 mg per day, and, dividing by a body weight of 70 kg, 3.37×10^{-4} (mg/kg-d). Multiplying this daily exposure dose by the range of potency values of 0.004 to 0.034 (mg/kg-day)$^{-1}$ yields a range of individual risks of 1×10^{-6} to

$1x10^{-5}$. Multiplying the range of individual risk values by the estimated statewide population of $28x10^6$ Californians yields a range of 28 to 280 cancer cases (upper bound excess population burden) due to lifetime exposure to current ambient levels of TCE or 1 to 10 additional cases per million people exposed.

Using the PBPK model in Chapter 3, the amount of TCE metabolized in 24 hours by a person breathing 0.22 ppb TCE is estimated to be:

$$\begin{aligned} 24\ A_m &= 24(0.72\ Q_a C_{in}) = 17\ Q_a C_{in} \\ &= 17 \times 0.3535\ m^3/\text{hour} \times 0.22\ \text{ppb} \times 5.38\ \mu g/m^3/\text{ppb} \\ &= 7.1\ \mu g/\text{day} = 0.0071\ \text{mg/day} \end{aligned} \qquad (5\text{-}9)$$

Dividing by a body weight of 70 kg yields an estimate for metabolized dose of $1x10^{-4}$ mg/kg-day from breathing 0.22 ppb TCE. Multiplying the metabolized dose by the range of risks from inhalation exposures of 0.0098 to 0.098 $(mg/kg\text{-}d)^{-1}$ yields a range of individual risk from exposure to ambient TCE of $1x10^{-6}$ to $1x10^{-5}$. Multiplying the range of individual risk values by the estimated statewide population of $28x10^6$ yields a range of 28 to 280 cancer cases (upper bound excess population burden) due to lifetime exposure to current ambient levels of TCE.

Combining the results from metabolized and applied dose estimates yields a range of individual risk values of $1x10^{-6}$ to $1x10^{-5}$ and a range of 28 to 280 excess cancer cases due to lifetime exposure to current ambient levels of TCE.

Estimate of Unit Risk Value

The current statewide mean concentration of 1.18 $\mu g/m^3$ TCE is only slightly greater than 1 $\mu g/m^3$, the concentration upon which the unit risk value is based. A person breathing 20 m^3 per day of air containing 1 $\mu g/m^3$ would inhale 20 μg or 0.02 mg per day and $2.9x10^{-4}$ mg/kg-d. this, approximately $1.3x10^{-4}$ mg/kg-d would be absorbed. Multiplying this dose by the range of potency values of 0.004 to 0.034 $(mg/kg\text{-}d)^{-1}$ derived from applied dose yields a range of values for unit risk of $1x10^{-6}$ to $1x10^{-5}$ $(\mu g/m^3)^{-1}$. The daily metabolized dose for 1 $\mu g/m^3$ using equation (5-9) above is 0.006 mg per 70 kg or $8.6x10^{-5}$ mg/kg-day. Multiplying this metabolized dose by the range of potencies for metabolized dose from the inhalation studies yields a range of values for unit risk of

$8x10^{-7}$ to $8x10^{-6}$ $(\mu g/m^3)^{-1}$. Combining the values from metabolized and applied dose approaches indicates a range of $8x10^{-7}$ to $1x10^{-5}$ $(\mu g/m^3)^{-1}$.

As a best estimate of the unit risk value, in order to provide a single number for use in risk assessment, the geometric means of the four values derived from metabolized dose and of the four values from applied dose were calculated. Use of geometric means to average several potency estimates of TCE was earlier done by the EPA (U.S. EPA 1985a). From the metabolized dose approach a unit risk of $2x10^{-6}(ug/m^3)$-1 was calculated, while from applied dose consideration of unit risk of $3x10^{-6}(ug/m^3)$ was determined.

Table 5-1. Dose-Response Data and Corresponding Estimates of Carcinogenic Potency for TCE.

Study Species Strain	Sex, Weight (Dosed Animals)	Daily Experimental Applied Dose or Conc., D	LTWA Metabolized Dose[a], M (mg/kg-d)	Tumor Type[b]	Tumor Incidence[c]	95% UCL Potency[d] of Metabolized Dose = q_1^* $(M)^{-1}$ in (mg M/kg-d)$^{-1}$ BW[e]	SA[f]
NCI 1976	M	0 mg/kg	0		1/20		
Mice	34 g	1169 mg/kg	369.6	HCC	26/48	0.0025	0.032
B6C3F1		2339 mg/kg	739.4		31/40		
					TT-IT	0.0033	0.042
					TT-LT	0.00034	0.0044
	F	0 mg/kg	0		0/18		
	29 g	869 mg/kg	274.7	HCC	4/42	0.00073	0.0098
		1739 mg/kg	549.8		11/37		
NTP 1983	M	0 mg/kg	0		8/48		
Mice	37 g	1000 mg/kg	563	HCC	30/50	0.0019	0.023
B6C3F1					TT-IT	0.0029	0.036
					TT-LT	0.0013	0.016
		0 mg/kg	0	HCC or	11/48		
		1000 mg/kg	563	HCA	38/50	0.0029	0.036
					TT-IT	0.0048	0.059
					TT-LT	0.0018	0.022

Table 5-1. (Continued)

Study Species Strain	Sex, Weight (Dosed Animals)	Daily Experimental Applied Dose or Conc., D	LTWA Metabolized Dose[a], M (mg/kg-d)	Tumor Type[b]	Tumor Incidence[c]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M) in (mg M/kg-d)$^{-1}$ BW[e]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M) in (mg M/kg-d)$^{-1}$ SA[f]
	F 33 g	0 mg/kg 1000 mg/kg	0 563		2/41 13/41	0.00096	0.012
				HCC	TT-IT	0.0011	0.014
					TT-LT	0.00077	0.0099
	1000 mg/kg	0 mg/kg 563	0	HCC or HCA	4/41 19/41	0.0014	0.018
					TT-IT	0.0019	0.024
					TT-LT	0.0011	0.014
NTP 1983 Rats F344/N	M 340 g	0 mg/kg 500 mg/kg 1000 mg/kg	0 198 282	RTC	0/33 0/20 3/16	0.00074	0.0043
		0 mg/kg 500 mg/kg 1000 mg/kg	0 198 282	RTC or RTA	0/45 2/39 3/26	0.00065	0.0038
					TT-IT	0.00034	0.0020
					TT-LT	0.00068	0.0040

Table 5-1. (Continued)

Study Species Strain	Sex, Weight (Dosed Animals)	Daily Experimental Applied Dose or Conc., D	LTWA Metabolized Dose[a], M (mg/kg-d)	Tumor Type[b]	Tumor Incidence[c]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M) in (mg M/kg-d)$^{-1}$ BW[e]	SA[f]
Bell et al. 1978 Mice B6C3F1	M 35 g (?)	0 ppm-6 h	0		18/99		
		100 ppm-6 h	42.3		28/95		
		300 ppm-6 h	127	HCC	31/100	0.0020	0.026
		600 ppm-6 h	254		43/97		
		0 ppm-6 h	0		20/99		
		100 ppm-6 h	42.3	HCC or	35/95		
		300 ppm-6 h	127	HCA	38/100	0.0028	0.036
		600 ppm-6 h	254		53/97		
Henschler et al. 1980 Mice Han:NMRI	F 30 g (?)	0 ppm-6 h	0		9/29		
		100 ppm-6 h	33.2	ML	17/30	0.0074	0.098
		500 ppm-6 h	166		18/28		
Fukuda et al. 1983 Mice ICR	F 30 g (?)	0 ppm-7 h	0		1/49		
		50 ppm-7 h	25.8		3/50		
		150 ppm-7 h	77.4	LA	8/50	0.0014	0.019
		450 ppm-7 h	232		7/46		
Maltoni et al. 1986 Mice Swiss	M 41 g	0 ppm-7 h	0		4/90		
		100 ppm-7 h	35.3		2/90		
		300 ppm-7 h	106	MH	8/90	0.00082	0.0098
		600 ppm-7 h	212		13/90		

Table 5-1. (Continued)

Study Species Strain	Sex, Weight (Dosed Animals)	Daily Experimental Applied Dose or Conc., D	LTWA Metabolized Dose[a], M (mg/kg-d)	Tumor Type[b]	Tumor Incidence[c]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M)$^{-1}$ in (mg M/kg-d)$^{-1}$ BW[e]	SA[f]
Herren-Freund 1987 Mice B6C3F1	M 30 g	0 mg/kg TCA[g]	0 TCA		0/22		
		1000 mg/kg TCA	229 TCA	HCC	7/22	0.0029	0.0039
		0 mg/kg DCA[g]	0 DCA		0/22		
		1000 mg/kg DCA	229 DCA	HCC	21/26	0.011	0.14

[a] Lifetime, time-weighted-average metabolized dose, M, in mg/kg-day. See text for derivation as a function of D.

[b] HCC = hepatocellular carcinoma, HCA = hepatocellular adenoma, RTC = renal tubular-cell adenocarcinoma, RTA = renal tubular-cell adenoma, ML = malignant lymphoma, LA = lung adenocarcinoma, MH = malignant hepatoma.

[c] Tumor-incidence denominator excludes animals dying before the occurrence of the first corresponding tumor type observed in the NCI (1976) and NTP (1983) studies. TT-IT = time-to-tumor data using an "incidental-tumor" model, TT-LT = time-to-tumor data using a "lethal-tumor" model.

[d] "Potency" here means the low-dose dose-response slope expressed by an upper-bound linear multistage coefficient such that, at very low doses, risk = (potency x dose), according to a multistage (or, with time-to-tumor data as input, a time-dependent multistage) risk prediction model (U.S. EPA, 1980; Anderson et al., 1983; Crump and Howe, 1984). 95% UCL = one-tailed 95% upper confidence limit.

[e] BW = Body weight interspecies dose-extrapolation method; equivalent doses assumed to be in mg/kg, so $M_{human} = M_{animal}$.

[f] SA = Surface Area interspecies dose-extrapolation method; equivalent doses assumed to be in mg/kg$^{2/3}$, so $M_{human} = M_{animal}[(\text{animal weight})/70\ \text{kg}]^{1/3}$.

[g] TCA = trichloroacetic acid, DCA = dichloroacetic acid.

Table 5-2. U.S. EPA Estimates of Carcinogenic Potency for TCE.[a]

Study Species Strain	Sex	Daily Experimental Applied Dose or Conc., D	LTWA Metabolized Dose[b], M (mg/kg-d)	Tumor Type	Tumor Incidence[c]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M) in (mg M/kg-d)$^{-1}$ BW[e]	95% UCL Potency[d] of Metabolized Dose = q_1^* (M) in (mg M/kg-d)$^{-1}$ SA[f]	95% UCL Potency of Human Applied Dose = q_1^* (D) in (mg D/kg-d)$^{-1}$ SA[f]
NCI 1976 Mice B6C3F1	M	0 mg/kg 1169 mg/kg 2339 mg/kg	0 375.7 714.6	Hepato-cellular carcinoma	1/20 26/50 31/48	0.0016[g]	0.021	0.018
	F	0 mg/kg 869 mg/kg 1739 mg/kg	0 285.5 554.0	Hepato-cellular carcinoma	0/20 4/50 11/47	0.00050[h]	0.0069	0.0058
NTP 1983 Mice B6C3F1	M	0 mg/kg 1000 mg/kg	0 571	Hepato-cellular carcinoma	8/48 30/50	0.0018	0.023	0.019
	F	0 mg/kg 1000 mg/kg	0 575	Hepato-cellular carcinoma	2/48 13/49	0.00075	0.036	0.0080

See Footnotes next page

Footnotes for Table 5-2

[a] Information derived from U.S. EPA (1985a), Tables 8-26, 8-33, 8-34, and 8-35, except that the adjustment for partial lifetime in the NCI (1976) study, using the factor $(90/104)^3$, is included here in the derivation of lifetime time-weighted average metabolized dose (see footnote b).

[b] Lifetime time-weighted average metabolized dose, M, in mg/kg-d. See footnote a for derivation as a function D.

[c] Tumor-incidence denominator is unadjusted for animals dying before the occurrence of the first corresponding tumor type observed in the NCI (1976) and NTP (1983) studies.

[d] "Potency" here means the low-dose, dose-response slope expressed by an upper-bound linear multistage coefficient such that at very low doses, risk ≈ (potency x dose), according to a multistage risk prediction model (U.S. EPA, 1980; Andersen et al., 1983). 95% UCL = one-tailed 95% upper confidence limit.

[e] BW = Body weight interspecies dose-extrapolation method; equivalent doses assumed to be in mg/kg, so $M_{human} = M_{animal}$.

[f] SA = Surface Area interspecies dose-extrapolation method; equivalent doses assumed to be in $mg/kg^{2/3}$, so $M_{human} = M_{animal}[(\text{animal weight})/70\ kg]^{1/3}$.

[g] The "linearized multistage" model (see footnote d) failed to yield this value reported by the U.S. EPA using the indicated input values; instead, the value 0.0020 $(mg/kg\text{-}d)^{-1}$ is obtained.

[h] The "linearized multistage" model (see footnote d) failed to yield this value reported by the U.S. EPA using the indicated input values; instead, the value 0.00058 $(mg/kg\text{-}d)^{-1}$ is obtained.

Table 5-3 Estimates of Carcinogenic Potency for TCE Based on Applied Dose to Mice

Study	Group	Inhaled Air I(m^3)[a]	TCE Dose mg/d[b]	TCE Dose mg/kg.d[c]	Adjusted to lifetime[d]	HED[e]	Tumor Incidence	q_1^*(mg/kg.d)$^{-1}$
Bell	0 ppm	.011	0	0	0	0	20/99[f]	
et al.,	100 ppm	.011	5.92	169	121	9.6	35/95	0.012
1978	300 ppm	.011	17.75	507	362	28.7	38/100	
B6C3F1	600 ppm	.011	35.51	1015	725	57.5	53/97	
Henschler	0 ppm	.010	0	0	0	0	9/29	
et al.,	100 ppm	.010	5.38	179	96	7.2	17/30	0.034
1980	300 ppm	.010	26.90	897	481	36.3	18/28	
Han:NMRI								
Fukuda	0 ppm	.011	0	0	0	0	1/49	
et al.,	50 ppm	.011	2.96	99	71	5.4	3/50	0.007
1983	150 ppm	.011	8.88	296	211	15.9	8/50	
ICR	450 ppm	.011	26.63	888	634	47.8	7/46	
Maltoni	0 ppm	.014	0	0	0	0	4/90	
et al.,	100 ppm	.014	7.53	184	99	8.3	2/90	0.004
1986	300 ppm	.014	22.60	551	295	24.7	8/90	
Swiss	600 ppm	.014	45.19	1102	590	49.4	13/90	

[a] Calculated from $I = 0.0345\ [Wt\ (kg)/0.025\ (kg)]^{2/3}\ m^3$/day (Anderson et al. 1983) and adjusting to either a 6 or 7 hours exposure.

[b] Calculated from 1 ppm TCE = 5.38 mg/m^3.

[c] Adjusted using the estimated body weight of mice in the study.

[d] Values from previous column are multiplied by 5/7 since animal exposures were only 5 days/week. In the cases of the Henschler and Maltoni studies, there was an additional multiplication by 78/104 since the exposures in these studies were less than lifetime.

[e] The HED (human equivalent dose) was estimated by dividing the estimated animal absorbed dose by $[70\ (kg)/mouse\ weight\ (kg)]^{1/3}$

[f] Incidence in the Bell et al. study are for hepatacellular adenomas and carcinomas combined.

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APPENDIX A

TABLE OF ABBREVIATIONS FOR

APPENDIX A

A-1254	Aroclor 1254
ALA-D	δ-aminolevulinic acid dehydratase
ALA-S	δ-aminolevulinic acid synthetase
av	average
B(a)P	benzo[a]pyrene
BSP	sulfobromophthalein
CNS	central nervous system
CS_2	carbon disulfide
DCE	dichloroethylene
DDC	sodium diethyldithiocarbamate
ED_{50}	dose required to elicit a specific response in 50% of animals
ET_{50}	time required to elicit a specific response in 50% of animals
G-6-P	glucose-6-phosphate
GSH	glutathione
IP	intraperitoneal
IV	intravenous
LAP	Leukocyte alkaline phosphatase
LC_{50}	concentration that is lethal to 50% of animals
LD_{50}	dose that is lethal to 50% of animals
LD_{90}	dose that is lethal to 90% of animals
Lilly 18947	2,4-dichloro-6-phenylphenoxyethyldiethylamine hydrobromide
LT_{50}	time to reach 50% mortality
3-MC	3-methylcholanthrene
PB	phenobarbital
PO	oral
ppmv	parts per million by volume
PSP	phenolsulfonphthalein
SC	subcutaneous
SGOT	serum glutamic-oxalacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SICD	serum isocitric dehydrogenase
SKF 525A	2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride
S-OCT	serum ornithine carbamoyltransferase

Table A-1. Acute inhalation toxicity of TCE.

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rat	100 ppmv	8 h	5 g/kg ethanol (gavage), 16 to 18 h prior to TCE	No observed effect	Cornish and Adefuin, 1966
Rat	200 ppmv	3 h		No effect on response time or food-motivated conditioned response	Grandjean, 1960
Rat	400 ppmv	6 h		Slight decrease in performance of swim test	Grandjean, 1963
Rat	500 ppmv	4 h		No observed effect	Cornish and Adefuin, 1966
Rat	800 ppmv	3 h		No effect on response time or food-motivated conditioned response	Grandjean, 1960
Rat	800 ppmv	6 h		Significant deterioration of swimming performance, but no effect if test was conducted 60 min after exposure ceased	Grandjean, 1963
Rat	1000 ppmv	4 h	10 g/kg ethanol (gavage), 16 to 18 h prior to TCE	No observed effect	Cornish and Adefuin, 1966
Rat	1200 ppmv	0.6, 1, 2, 5, 6, 7, or 8 h		Mortality: 0/20, 2/5, 2/5, 9/10, 4/5, 5/5, 9/10	Adams and Spencer, 1951
Rat	1600 ppmv	6 h		Statistically significant ($p < 0.01$) reduction in motor activity	Grandjean, 1963
Rat	2000 ppmv	4 h	5 g/kg ethanol (gavage), 16 to 18 h prior to TCE	No marked changes in serum enzyme levels (SGOT, SGPT, and SICD)	Cornish and Adefuin, 1966

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rat	2000 ppmv	4 h	5 g/kg ethanol (gavage), 16 to 18 h prior to TCE	No observed effect	Cornish and Adefuin, 1966
Rat	3000 ppmv	8 or 14 h		Mortality: 0/30, 2/10	Adams and Spencer, 1951
Rat	4800 ppmv	1.4, 4, 6, or 8 h		Mortality: 0/20, 3/10, 4/9, 6/30	ibid.
Rat	5000 ppmv	4 h	5 g/kg ethanol (gavage), 16 to 18 h prior to TCE	Fatty degeneration of liver	Cornish and Adefuin, 1966
Rat	5000 ppmv	4 h	5 g/kg ethanol (gavage) 16 to 18 h prior to TCE	With ethanol: statistically significant increase in SGPT ($p < 0.001$), SGOT ($p < 0.05$), and SICD ($p < 0.001$) activity; fatty infiltration of liver	ibid.
Rat	6400 ppmv	1, 1.4, 2, or 4 h		Mortality: 0/20, 0/10, 1/10, 4/10	Adams and Spencer, 1951
Rat	6900 ppmv	2 h		Statistically significant decrease in G-6-P and increase in SGOT and SGPT ($p < 0.05$)	Carlson, 1974
Rat	6900 ppmv	2 h	40 mg/kg 3-MC (IP), daily for 2 d prior to TCE	Statistically significant decrease in G-6-P and increase in SGPT and SGOT ($p < 0.05$)	ibid.
Rat	7800 ppmv	2 h		Slight increase in SICD	ibid.

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rat	7800 ppmv	2 h	40 mg/kg 3-MC (IP), daily for 2 d prior to TCE	Statistically significant ($p < 0.05$) increase in SICD	Carlson, 1974
Rat	9000 ppmv	15 min		Slight difficulty in locomotion; CNS depression, loss of righting reflex	Utesch et al., 1981
Rat	9600 ppmv	0.8, 1, or 2 h		Mortality; 0/20, 3/20, 8/15	Adams and Spencer, 1951
Rat	10000 ppmv	1.5 h	5 g/kg ethanol (gavage), 16 to 18 h prior to TCE	Marked elevation of SGOT activity	Cornish and Adefuin, 1966
Rat	10000 ppmv	2 h	400 mmol/kg PB (PO), daily for 7 d prior to TCE	Statistically significant decrease in cytochrome P-450 content ($p < 0.001$), increase in cytochrome b_5 ($p < 0.05$), and decrease in GSH content ($p < 0.005$) in liver fractions at 1 and 2 h; statistically significant ($p < 0.005$) increase in GSH content at 8 h	Moslen et al., 1977b
Rat	10400 ppmv	2 h		Statistically significant ($p < 0.05$) increase in SGPT and SGOT	Carlson, 1974
Rat	10400 ppmv	2 h	50 mg/kg PB (IP), daily for 4 d prior to TCE	Statistically significant ($p < 0.05$) increase in SGOT and SGPT; decrease in G-6-P	ibid.

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rat	12000 ppmv	10 min		Loss of righting reflex	Utesch et al., 1981
Rat	14000 ppmv	5 min		Loss of righting reflex; irritation of eyes and respiratory tract	ibid.
Rat	16000 ppmv	2 h	40 mg/kg 3-MC (IP), daily for 2 d prior to TCE	4/5 deaths	Carlson, 1974
Rat	20000 ppmv	0.3, 0.4, 0.6, 0.8, 1, 3, 4, or 5 h		Mortality: 0/20, 2/20, 4/20, 1/5, 1/10, 3/10, 7/10, 20/20	Adams and Spencer, 1951
Rat	25000 ppmv	20, 30, or 40 min	50 mg/kg SKF 525A (IP), 30 min prior to TCE	0.5, 1, 2, or 4 µg/kg epinephrine induced cardiac arrhythmia	White and Carlson, 1979
Rat	25000 ppmv	1 h	75 mg/kg PB (IP), daily for 4 d	>4.0 µg/kg epinephrine did not induce cardiac arrhythmia	ibid.
Rat	25000 ppmv	1 h	25 mg Aroclor 1254/kg (IP), daily for 6 d	>4.0 µg/kg epinephrine did not induce cardiac arrhythmia	ibid.
Rat	25000 ppmv	1 h	30 mg/kg Lilly 18947 (IP), 30 min prior to TCE	>4 µg/kg epinephrine did not induce cardiac arrhythmia	ibid.
Mouse	1600 ppmv	4 h		No observed effect	Kylin et al., 1963

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Mouse	3200 ppmv	4 h		Moderate fatty infiltration of liver; no significant difference in S-OCT activity	Kylin et al., 1963
Mouse	5500 ppmv	46 to 585 min		ED_{50}, induction of anesthesia = 46 min; ET_{50}, significant increase in SGPT ($p < 0.05$) = 400 min; LT_{50} = 585 min	Gehring, 1968
Mouse	6400 ppmv	4 h		No observed effect	Kylin et al., 1962
Mouse	8450 ppmv			LC_{50}	Friberg et al., 1953
Rabbit	3000 ppmv	7.5 to 60 min	40 mg/kg (IP), daily for 4 d prior to TCE	No cardiac arrhythmia without epinephrine; at 30 to 60 min of TCE exposure, >1.0 µg/kg epinephrine induced cardiac arrhythmia	White and Carlson, 1979
Rabbit	3000 ppmv	7.5 to 60 min	25 mg/kg Aroclor 1254 (IP), daily for 6 d prior to TCE	No cardiac arrhythmia without epinephrine; at 30 to 60 min of TCE exposure, ≥2.0 µg/kg epinephrine induced cardiac arrhythmia	ibid.
Rabbit	3000 ppmv	15 to 60 min	50 mg/kg SKF 525A (IP), 30 min prior to TCE after 15 min of TCE	Cardiac arrhythmia without epinephrine; after 60 min of TCE exposure, 0.5 to 4.0 µg/kg epinephrine induced cardiac arrhythmia	ibid.
Rabbit	3000 ppmv	15 to 60 min	30 mg/kg Lilly 18947 (IP), 30 min prior to TCE	Cardiac arrhythmia without epinephrine; after 60 min of TCE exposure, epinephrine (>0.5 µg/kg) induced arrhythmia after 7.5 min	ibid.

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rabbit	6000 ppmv	7.5 to 60 min		No cardiac arrhythmia without epinephrine; 1.0 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 15 to 60 min TCE exposure	White and Carlson, 1981
Rabbit	6000 ppmv	7.5 to 60 min	1 g/kg ethanol (PO), 30 min prior to TCE	No cardiac arrhythmia without epinephrine; 0.5 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 30 to 60 min TCE exposure	ibid.
Rabbit	6000 ppmv	7.5 to 60 min	1 g/kg ethanol (IV), 30 min prior to TCE	Cardiac arrhythmia occurred after 30 to 45 min TCE exposure; 0.5 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 15 to 60 min TCE exposure	ibid.
Rabbit	6000 ppmv	7.5 to 30 min	1.35 mmol/kg disulfiram (PO), 6 and 24 h prior to TCE	No cardiac arrhythmia when challenged with up to 3.0 µg/kg epinephrine	Fossa et al., 1982
Rabbit	8100 ppmv	7.5 to 60 min	40 mg/kg B(a)P (IP), 48 and 72 h prior to TCE	No cardiac arrhythmia without epinephrine; 1.0 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 30 to 60 min TCE exposure	Carlson and White, 1983
Rabbit	8100 ppmv	7.5 to 60 min		No cardiac arrhythmia without epinephrine; 1.0 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 30 to 60 min TCE exposure	ibid.
Rabbit	8100 ppmv	7.5 to 60 min	40 mg 3-MC (IP), 48 and 72 h prior to TCE	No cardiac arrhythmia without epinephrine; 2.0 to 3.0 µg/kg epinephrine induced cardiac arrhythmia after 7.5 min TCE exposure	ibid.

Table A-1. (Continued)

Species	Concentration	Duration	Pretreatment	Effect	Reference
Rabbit	9000 ppmv	7.5 to 30 min	1.35 mmol/kg CS_2 (IP), 6 and 24 h prior to TCE	No cardiac arrhythmia without epinephrine; 0.5 to 3.0 µg/kg epinephrine induced arrhythmia after 7.5 min TCE exposure	Fossa et al., 1982
Rabbit	9000 ppmv	7.5 to 60 min	1.35 mmol/kg disulfiram (IP), 6 and 24 h prior to TCE	Cardiac arrhythmia after ≥30 min TCE; increased incidence of arrhythmia after 0.5 to 3.0 µg/kg epinephrine	ibid.
Rabbit	9000 ppmv	1 h	1.35 mmol/kg DDC (IP), 6 and 24 h prior to TCE	No cardiac arrhythmia without epinephrine; 0.5 to 3.0 µg/kg epinephrine induced arrhythmia after 7.5 min TCE exposure	ibid.
Dog	200 to 2000 ppmv	1 h		Decrease in leukocyte count	Hobara et al., 1984
Dog	5000 ppmv	10 min		No observed effect	Reinhardt et al., 1973
Dog	10,000 ppmv	10 min		Ventricular fibrillation after 8 mg/kg epinephrine; increased sensitivity to epinephrine	Reinhardt et al., 1973
Cat	20 ppmv	1 or 1.5 h		Liver and kidney lesions; hemorrhage of spleen; lymph node hypertrophy	Mosinger and Fiorentini, 1955

Table A-2. Acute oral toxicity of TCE.

Species	Dose	Pretreatment	Effect	Reference
Rat	0.65 mmol/kg		No effect on hepatic and serum lipoproteins	Danni et al., 1981
Rat	1.29 mmol/kg	5 or 8 mg/kg PB (IP), 0, 6, and 24 h prior to TCE	Without PB: no effect on liver weight; with PB: statistically significant increase in hepatic triglycerides (females, $p < 0.005$; males, $p < 0.025$) and liver weight (males, $p < 0.005$)	ibid.
Rat	2.58 mmol/kg		No effect on hepatic and serum lipoproteins	ibid.
Rat	5.16 mmol/kg		No lipoperoxidation after 30 min; LD_{90} = 5.16 mmol/kg body weight	ibid.
Mouse	2443 mg/kg		LD_{50} (females)	Tucker et al., 1982
Mouse	2402 mg/kg		LD_{50} (males)	ibid.

Table A-3. Acute intraperitoneal toxicity of TCE.

Species	Dose	Pretreatment	Effect	Reference
Rat	0.3, 0.5, 1.0, or 2.0 mL/kg	50 mg/kg PB (IP), 2 exposures/d, 1 or 2 d prior to TCE	Treatment had no significant effect on SGOT activity; PB did not potentiate effects	Cornish et al., 1973
Rat	1.25, 1.0, or 2.0 mL/kg	30 mg/kg cobaltous chloride (SC), 2 exposures/d prior to TCE	69% decrease in hepatic cytochrome P450	Allemand et al., 1978
Rat	1.25, 1.0, or 2.0 mL/kg	1360 mg/kg piperonyl butoxide (IP), 30 min prior to TCE exposure	No observed effect	ibid.
Rat	0.25, 1.0, or 2.0 mL/kg		61% decrease in hepatic GSH 4 h post-treatment; 108% increase in hepatic GSH 16 h post-treatment	ibid.
Rat	0.25, 1.0 or 2.0 mL/kg	70 mg/kg PB (IP), daily for 5 d prior to TCE	132% increase in hepatic cytrochrome P450; significant increase ($p < 0.05$) in SGPT activity	ibid.
Rat	350 to 2940 mg/kg		>1500 mg/kg induced hepatotoxicity	Elcombe et al., 1981
Mouse	650 mg/kg		LD_{50}	ibid.
Mouse	0.6 mL/kg		No observed effect	Plaa and Larson, 1965
Mouse	1.0 mL/kg	5 g/kg ethanol (gavage), 12 h or 3 d prior to TCE	Pretreatment had no effect on BSP retention or PSP excretion	Klaassen and Plaa, 1966

Table A-3. (Continued)

Species	Dose	Pretreatment	Effect	Reference
Mouse	1.0, 1.5 mL/kg	2.5 mL/kg isopropyl alcohol (gavage), 18 h prior to TCE	Statistically significant ($p < 0.05$) increase in SGPT activity	Traiger and Plaa, 1974
Mouse	1.5 mL/kg	2.5 mL/kg acetone (gavage), 18 h prior to TCE	Statistically significant ($p < 0.05$) increase in SGPT activity	ibid.
Mouse	1.6 mL/kg		ED_{50}, increase in SGPT activity	Klaassen and Plaa, 1966
Mouse	2.0 mL/kg		ED_{50}, retention of BSP	ibid.
Mouse	2.0 mL/kg		"Moderate increase in SGPT activity and liver calcium content"	Masuda and Nakayama, 1982
Mouse	2.0 mL/kg	DDC (10, 30, 100 mg/kg) or CS_2 (3, 10, 30 mg/kg) (PO), 30 min before TCE	"Suppression" of TCE-induced hepatotoxicity	ibid.
Mouse	2.2 mL/kg		LD_{50}	Klaassen and Plaa, 1966
Mouse	2.5 mL/kg		80% mortaility (8/10)	Plaa and Larson, 1965
Mouse	368, 735, 1470 mg/kg		Dose dependent increase in hepatic nonprotein sulfhydryl content	Elcombe et al., 1981

Table A-3. (Continued)

Species	Dose	Pretreatment	Effect	Reference
Mouse	2000 mg/kg		Damage to bronchiolar epithelium; marked decrease in pulmonary cytochrome P-450 and aryl hydrocarbon hydroxylase activity	Forkert et al., 1985
Mouse	2500 mg/kg		10% mortality (1/10); pathological changes in alveolar cells	ibid.
Mouse	3000 mg/kg		60% mortality (6/10); increased accumulation of pulmonary calcium; increase inanesthesia recovery time	ibid.
Guinea pig	6.7 mmol/kg		Depression of righting reflex; slowed respiration, heart rate, and atrioventricular transmission of excitation	Mikiskova and Mikiska, 1966
Dog	0.57 mL/kg		ED_{50}, increase in SGPT activity	Klaassen and Plaa, 1967
Dog	0.57 mL/kg	5 g/kg ethanol (gavage), 24 h prior to TCE	Significant ($p < 0.05$) increase in SGPT activity	ibid.
Dog	1.9 mL/kg		LD_{50}	ibid.

Table A-4. Acute intravenous toxicity of TCE.

Species	Concentration	Effect	Reference
Dog	50 mg/kg	No observed effect	Hobara et al., 1984

Table A-5. Acute subcutaneous toxicity of TCE.

Species	Concentration	Effect	Reference
Mouse	5.7, 8.6, 10.0, 12.0, 14.0 mmol/kg	ED_{50} for increase in sleeping time = 12 mmol/kg	Plaa et al., 1958
Mouse	120.0 mmol/kg	LD_{50}	ibid.

Table A-6. Subchronic inhalation toxicity of TCE.

Species	Concentration	Duration	Effect	Reference
Rat	30 ppmv	Continuously for 90 d	No observed effect	Prendergast et al., 1967
Rat	50 ppmv	5 h/d for 28 d	Statistically significant ($p < 0.05$) increase in hepatic cytochrome P-450; slight increase in aminopyrinedemethylase activity	Norpoth et al., 1974
Rat	50 ppmv	Continuously for 48 h or 240 h, after pretreatment with 75 mg/kg PB (IP), daily for 4 d	Increase in ALA-D activity in blood; inhibition of ALA-D activity in liver	Koizumi et al., 1984
Rat	55 ppmv	8 h/d, 5 d/wk for 14 wk	Statistically significant ($p < 0.01$) increase in liver weight	Kimmerle and Eben, 1973a
Rat	100 ppmv	Continuously, 5 d/wk for 1 month	Statistically significant ($p < 0.05$) reduction of total social behavioral activity	Silverman and Williams, 1975
Rat	100 ppmv	Continuously, 5 d/wk for 3 months	No significant effect on social behavioral activity; statistically significant decrease ($p < 0.05$) in exploration-thirst test time	ibid.
Rat	125 ppmv	4 h/d, 5 d/wk for 25 d	Statistically significant reduction in avoidance efficiency ($p < 0.01$)	Goldberg et al., 1964a
Rat	125 ppmv	4 h/d, 5 d/wk for 5 wk	Statistically significant behavioral changes were noted from day 3 through day 26 ($p < 0.01$)	ibid.
Rat	150 ppmv	Continuously for 30 d	Statistically significant ($p < 0.001$) increase in liver weight	Kjellstrand et al., 1982a

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Rat	150 ppmv	Continuously for 30 d	Decrease in weight gain of females, significant increase in liver weight ($p < 0.05$)	Kjellstrand et al., 1981
Rat	200 ppmv	7 h/d, 151 exposures in 205 d	No significant effect	Adams and Spencer, 1951
Rat	200 ppmv	6 h/d for 5 d	Decrease in brain RNA content (statistical significance not provided)	Savolainen et al., 1977
Rat	200 ppmv	4 h/d, 5 d/wk for 10 d	No effect on avoidance and escape responses	Goldberg et al., 1964b
Rat	200 ppmv	5 d/wk for 1 month	No significant change in social behavioral activity, statistically significant ($p < 0.05$) increase in exploration-thirst test time	Silverman and Williams, 1975
Rat	200 ppmv	Continuously for 1 month	No observed effect	Honma et al., 1980
Rat	400 ppmv	7 h/d, 173 exposures in 243 d	Statisdically significant ($p < 0.01$) increase in liver and kidney weight	Adams and Spencer, 1951
Rat	400 ppmv	Continuously for 1 month	No observed effect	Honma et al., 1980
Rat	400 ppmv	Continuously for 48 h or 240 h, after pretreatment with 75 mg/kg PB (IP) daily for 4 d	Statistically significant decrease in ALA-D activity in liver ($p < 0.05$) and blood ($p < 0.01$)	Koizumi et al., 1984

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Rat	400 ppmv	Continuously for 48 h or 240 h	At 48 h, statistically significant ($p < 0.01$) decrease in liver, bone, and bone marrow ALA-D activity; at 240 h, statistically significant ($p < 0.05$) increase in ALA-S activity in liver	Koizumi et al., 1984
Rat	400 ppmv	8 h/d, 5 d/wk for 44 wk	Decrease in performance in swimming test, enhanced exploratory behavior in maze; no change in learning ability (Hebb test and conditioned avoidance response)	Battig and Grandjean, 1963
Rat	470 ppmv	5 h/d for 10 d	Statistically significant ($p < 0.02$) increase in aminopyrinedemethylase activity	Norporth et al., 1974
Rat	500 ppmv	Continuously, 5 d/wk for 1 month	Reduction of total social behavioral activity ($p < 0.05$); no significant difference in exploration-thirst test time	Silverman and Williams, 1975
Rat	560 ppmv	4 h/d, 5 d/wk for 10 d	No effect on avoidance and escape responses; decrease in learning ability	Goldberg et al., 1964b
Rat	650 ppmv	8 h/d, 5 d/wk for 6 wk	Lung congestion	Prendergast et al., 1967
Rat	800 ppmv	Continuously for 1 month	Statistically significant ($p < 0.05$) reduction in acetylcholine content in the striatum; slight decrease in brain norephinephrine content	Honma et al., 1980

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Rat	800 ppmv	Continuously for 48 h or 240 h	Weight loss; pronounced inhibition of ALA-D; increase in SGPT activity	Koizumi et al., 1984
Rat	1000 ppmv	5 d/wk for 1 month	Reduction of total social behavioral activity ($p < 0.01$); significant difference ($p < 0.05$) in exploration-thirst test time	Silverman and Williams, 1975
Rat	1568 ppmv	4 h/d, 5 d/wk for 10 d	Slight ataxia; decrease in learning ability	Goldberg et al., 1964b
Rat	2300 ppmv	5 h/d for 10 d	Slight decrease in aminopyrine-demethylase activity	Norporth et al., 1974
Rat	2600 ppmv	30 min/d, 6 d/wk for 80 d	Statistically significant ($p < 0.05$) decrease of ambulation in open field test	Ikeda et al., 1980b
Rat	3000 ppmv	7 h/d, 20 exposures in 36 d	Statistically significant ($p < 0.001$) increase in liver and kidney weight	Adams and Spencer, 1951
Rat	4380 ppmv	4 h/d, 5 d/wk for 10 d	Gross ataxia; inhibition of learning ability	Goldberg et al., 1964b
Rat	7.9 µmol/L	6 h/d for 5 d	Decrease in brain RNA content; increase in hepatic cytochrome P-450	Vainio et al., 1978
Mouse	37 ppmv	Continuously for 30 d	Statistically significant increase in liver weight (females, $p < 0.05$; males, $p < 0.001$)	Kjellstrand et al., 1983b

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Mouse	75 ppmv	Continuously for 30 d	Statistically significant ($p < 0.001$) increase in plasma butyrylcholinesterase activity (males), liver weight (females), and kidney weight	ibid.
Mouse	150 ppmv	Continuously for 2, 5,9,16 or 30 d	No effect on spleen weight or body weight gain; significant increase in liver weight ($p < 0.05$)	Kjellstrand et al., 1981
Mouse	150 ppmv	Continuously for 30 d	Increase in liver weight; minor changes in kidney and spleen weight; increase in plasma butyrylcholinesterase activity in males of seven strains	Kjellstrand et al., 1983a
Mouse	150 ppmv	Continuously for 120 d	Statistically significant ($p < 0.05$) increase in plasma butyrylcholinesterase activity (males), liver and kidney weight, and decrease in spleen weight (males)	Kjellstrand et al., 1983b
Mouse	150 ppmv	Continuously for 30 d	Statistically significant increase in plasma butyrylcholinesterase activity, liver and kidney weight ($p < 0.05$)	ibid.
Mouse	150 ppmv	Continuously for 30 d	Statistically significant ($p < 0.001$) increase in liver weight, increase in acid phosphatase activity in brain stem	Kjellstrand et al., 1982a
Mouse	150 ppmv	Continuously for 30 d	Significant increase in kidney weight ($p < 0.05$)	Kjellstrand et al., 1983b

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Mouse	170 ppmv	Continuously for 30 d	No effect on activity of brain acetylcholinesterase, glutamine synthetase, acid phosphatase, or glutamate dehydrogenase; increase in blood butyrylcholinesterase activity (males); statistically significant ($p < 0.01$) decrease in body weight (males) and liver weight ($p < 0.001$)	Kanje et al., 1981
Mouse	225 ppmv	16 h/d, 7 d/wk for 30 d	Statistically significant ($p < 0.001$) increase in plasma butyrylcholinesterase activity and liver and kidney weight	Kjellstrand et al., 1983b
Mouse	300 ppmv	Continuously for 30 d	Statistically significant ($p < 0.001$) increase in plasma butyrylcholinesterase activity, liver and kidney weight, and decrease in spleen weight	ibid.
Mouse	450 ppmv	8 h/d, 7 d/wk for 30 d	Statistically significant increase in plasma butyrylcholinesterase activity ($p < 0.05$, males), and in liver and kidney weight ($p < 0.001$, males)	ibid.
Mouse	900 ppmv	4 h/d, 7 d/wk for 30 d	Statistically significant increase in plasma butyrylcholinesterase activity ($p < 0.01$, males), liver weight ($p < 0.001$), and kidney weight ($p < 0.001$, females; $p < 0.01$, males)	ibid.

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Mouse	900 ppmv	4 h/d, 7 d/wk for 120 d	Statistically significant increase in plasma butyrylcholinesterase activity ($p < 0.001$, males), liver weight ($p < 0.001$), and kidney weight ($p < 0.001$, males)	ibid.
Mouse	900 ppmv	4 h/d, 7 d/wk for 120 d	No significant effect observed	ibid.
Mouse	1600 ppmv	4 h/d, 6 d/wk for 1, 2, 4, or 8 wk	Statistically significand ($p < 0.01$) increase in liver fat	Kylin et al., 1965
Mouse	1800 ppmv	2 h/d, 7 d/wk for 30 d	Statistically significant increase in plasma butyrylcholinesterase activity ($p < 0.001$, males), liver weight ($p < 0.001$), and kidney weight ($p < 0.01$, males)	Kjellstrand et al., 1983b
Mouse	3600 ppmv	1 h/d, 7 d/wk for 30 d	Statistically significant increase in plasma butyrylcholinesterase activity ($p < 0.01$, males), liver weight ($p < 0.001$), and kidney weight ($p < 0.01$, females; $p < 0.001$, males)	ibid.
Mouse	10,000 ppmv	1 or 4 h/d for 5 d	Statistically significant ($p < 0.01$) increase in liver NADPH cytochrome c reductase (4 h/d); statistically significant ($p < 0.01$) decrease in lung NADPH cytochrome c reductase	Lewis et al., 1984
Mouse	1 v/v%	1 h for 12 d	40% mortality (4/10)	Hunter, 1949
Mouse	1.5 v/v%	1 h for 8 d	100% mortality (10/10)	ibid.

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Gerbil	60 ppmv	Continuously for 3 months	Significant increase ($p < 0.05$) in S100 protein in hippocampus, cerebellar vermis, and brainstem	Haglid et al., 1981
Gerbil	150 ppmv	Continuously for 30 d	Increased weight gain in females; significant increase in liver and kidney weight ($p < 0.001$)	Kjellstrand et al., 1981
Gerbil	150 ppmv	Continuously for 30 d	Statistically significant ($p < 0.001$) increase in liver weight (males); increase in acid phosphatase activity in brain stem	Kjellstrand et al., 1982b
Gerbil	230 ppmv	Continuously for 28 d (0 to 28 d of age)	36% mortality (14/39)	ibid.
Gerbil	230 ppmv	Continuously for 21 d (7 to 28 d of age)	8% mortality (3/39)	ibid.
Gerbil	230 ppmv	Continuously for 14 d (14 to 28 d of age)	13% mortality (5/40)	ibid.
Gerbil	230 ppmv	Continuously for 21 d (21 to 28 d of age)	2% mortality (1/43)	ibid.
Gerbil	320 ppmv	Continuously for 3 months	Significant increase ($p < 0.05$) in DNA content of cerebellar vermis and sensory motor cortex	Haglid et al., 1981
Guinea pig	30 ppmv	Continuously for 90 d	No observed effect	Prendergast et al., 1967
Guinea pig	100 ppmv	7 h/d, 132 exposures in 185 d	Slight increase in liver weight	Adams and Spencer, 1951

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Guinea pig	200 ppmv	7 h/d, 163 exposures in 227 d	Statistically significant ($p < 0.001$) decrease in body weight	ibid.
Guinea pig	400 ppmv	7 h/d, 167 exposures in 235 d	Statistically significant ($p < 0.01$) decrease in liver weight	ibid.
Guinea pig	650 ppmv	8 h/d, 5 d/wk for 6 wk	Lung congestion	Prendergast et al., 1967
Guinea pig	1200 ppmv	7 h/d, 5 d/wk (418 to 1159-h total exposure)	29% mortality (4/14)	Barrett et al., 1938
Rabbit	30 ppmv	Continuously for 90 d	No observed effect	Prendergast et al., 1967
Rabbit	200 ppmv	7 h, 178 exposures in 248 d	No significant effect	Adams and Spencer, 1951
Rabbit	400 ppmv	7 h, 161 exposures in 225 d	Slight increase in liver weight	ibid.
Rabbit	650 ppmv	8 h/d, 5 d/wk for 6 wk	Weight loss	Prendergast et al., 1967
Rabbit	3000 ppmv	7 h/d, 27 exposures in 36 d	No significant effect	Adams and Spencer, 1951
Dog	30 ppmv	Continuously for 90 d	No observed effect	Prendergast et al., 1967
Dog	650 ppmv	8 h/d, 5 d/wk for 6 wk	Weight loss	ibid.

Table A-6. (Continued)

Species	Concentration	Duration	Effect	Reference
Dog	2000 ppmv	6 h/d, 5 d/wk for 6 months	Congestion of lungs and liver	Taylor, 1936
Monkey	200, 400 ppmv	7 h/d, 48 exposures in 212 d and 161 exposures in 225 d, respectively	No significant effect	Adams and Spencer, 1951
Monkey	189 mg/m^3	Continuously for 90 d	No observed effect	Prendergast et al., 1967
Monkey	3825 mg/m^3	8 h/d, 5 d/wk for 6 wk	No observed effect	ibid.

Table A-7. Subchronic oral toxicity of TCE.

Species	Dose	Duration	Effect	Reference
Rat	1000 mg/kg	Daily for 10 d	No alteration in activity of SGOT or SGPT; statistically significant ($p < 0.05$) decrease in hepatic DNA content	Elcombe et al., 1981
Rat	1100 mg/kg	Daily for 3 d	No observed effect	Stott et al., 1982
Rat	1100 mg/kg	5 d/wk for 3 wk	Statistically significant ($p < 0.01$) increase in lifer weight; slight increase in DNA synthesis	ibid.
Mouse	24 mg/kg	Daily for 14 d	Increase in liver weight not significant	Tucker et al., 1982
Mouse	24 mg/kg	Daily for 14 d	Decrease in cell-mediated immune response	Sanders et al., 1982
Mouse	100, 200, 400 mg/kg	5 d/wk for 6 wk	Statistically significant ($p < 0.01$) increase in liver weight	Buben and O'Flaherty, 1985
Mouse	240 mg/kg	Daily for 14 d	Decrease in cell-mediated immune response	Sanders et al., 1982
Mouse	240 mg/kg	Daily for 14 d	Statistically significant increase in liver weight ($p < 0.05$)	Tucker et al., 1982
Mouse	250 mg/kg	5 d/wk for 3 wk	No significant effect	Stott et al., 1982

Table A-7. (Continued).

Species	Dose	Duration	Effect	Reference
Mouse	500 mg/kg	5 d/wk for 3 wk	Statistically significant ($p < 0.01$) increase in liver weight; statistically significant decrease in DNA synthesis ($p < 0.05$) and DNA content ($p < 0.01$)	ibid.
Mouse	500 mg/kg	Daily for 10 d	No alteration in activity of SGOT or SGPT; statistically significant ($p < 0.05$) decrease in hepatic DNA content	Elcombe et al., 1981
Mouse	800 mg/kg	5 d/wk for 6 wk	Statistically significant ($p < 0.001$) increase in liver weight, and decrease in G-6-P activity ($p < 0.01$)	Buben and O'Flaherty, 1985
Mouse	1200 mg/kg	5 d/wk for 3 wk	Statistically significant increase in liver weight ($p < 0.01$) and decrease in DNA content ($p < 0.01$)	Stott et al., 1982
Mouse	1600 mg/kg	5 d/wk for 6 wk	Statistically significant ($p < 0.001$) increase in liver weight and decrease in G-6-P activity	Buben and O'Flaherty, 1985
Mouse	2400 mg/kg	5 d/wk for 6 wk	Statistically significant ($p < 0.001$) increase in liver weight and decrease in G-6-P activity; statistically significant ($p < 0.01$) increase in liver triglycerides and SGPT activity	ibid.
Mouse	3200 mg/kg	5 d/wk for 6 wk	Statistically significant ($p < 0.001$) increase in liver weight and decrease in G-6-P activity; statistically significant ($p < 0.05$) increase in SGPT activity	ibid.

Table A-7. (Continued).

Species	Dose	Duration	Effect	Reference
Mouse	2400 mg/kg	5 d/wk for 3 wk	Statistically significant increase in liver weight ($p < 0.01$) and decrease in DNA content ($p < 0.01$)	Stott *et al.*, 1982
Mouse	2400 mg/kg	Daily for 3 d	Statistically significant increase in liver weight ($p < 0.01$) and decrease in DNA content ($p < 0.01$)	*ibid.*

Table A-8. Subchronic toxicity of TCE administered in drinking water.

Species	Concentration	Duration	Effect	Reference
Mouse	0.1 mg/mL	ad libitum for 4 or 6 months	Significant increase ($p < 0.05$) in brain weight (females, 4 months)	Tucker et al., 1982
Mouse	0.1 mg/mL	ad libitum for 4 or 6 months	Statistically significant ($p < 0.05$) inhibition of cell-mediated immunity and bone marrow stem cell colonization (females, 4 months)	Sanders et al., 1982
Mouse	1.0 mg/mL	ad libitum for 4 or 6 months	Significant increase ($p < 0.05$) in brain weight (females, 4 months)	Tucker et al., 1982
Mouse	1.0 mg/mL	ad libitum for 4 or 6 months	Statistically significant ($p < 0.05$) inhibition of cell-mediated immunity and bone marrow stem cell colonization (females, 4 months)	Sanders et al., 1982
Mouse	2.5 mg/mL	ad libitum for 4 or 6 months	Significant increase ($p < 0.05$) in brain weight (females, 4 months)	Tucker et al., 1982
Mouse	2.5 mg/mL	ad libitum for 4 or 6 months	Statistically significant ($p < 0.05$) inhibition of humoral and cell-mediated immunity (females, 4 months); inhibition of bone marrow stem cell colonization	Sanders et al., 1982
Mouse	5.0 mg/mL	ad libitum for 4 or 6 months	Significant decrease ($p < 0.05$) in body weight (females), significant increase ($p < 0.05$) in brain weight (females) and kidney weight (both sexes), elevated protein and ketone levels; decreased erythrocyte and leukocyte counts	Tucker et al., 1982
Mouse	5 mg/mL	ad libitum for 4 or 6 months	Statistically significant ($p < 0.05$) inhibition of humoral and cell-mediated immunity; significant ($p < 0.05$) inhibition of bone marrow stem cell colonization	Sanders et al., 1982

Table A-9. Subchronic intraperitoneal toxicity of TCE.

Species	Dose	Duration	Effect	Reference
Mouse	330 mg/kg	Every other day for 5 d	Statistically significant ($p < 0.05$) increase in hepatic microsomal NADPH cytochrome c reductase activity	Lewis *et al.*, 1984
Rabbit	2 mL (2.92 g)	2 d/wk for 41 to 247 d	Widespread neurological changes	Bartonicek and Brun, 1970
Rabbit	3 mL (4.38 g)	3 d/wk for 29 d	Widespread neurological changes	*ibid.*

Table A-10. Subchronic subcutaneous toxicity of TCE.

Species	Dose	Duration	Effect	Reference
Rabbit	2.5 g (3 mL/d)	3 to 24 injections	Large areas of necrosis at site of injection	Barrett et al., 1938

Table A-11. Human health effects from oral exposure to TCE.

Quantity	Duration	Effect	Reference
Unknown	Single exposure	Vomiting, abdominal pain, marked oliguria; death resulting from hepatorenal failure	Kleinfeld and Tabershaw, 1954
1 Tablespoon	Single exposure	Vomiting, weakness; recovered in 5 d	Stephens, 1945
0.5 oz	Single exposure	Coma, weakness; recovered in 10 d	Stephens, 1945
Unknown	Single exposure	Acute paranoid psychosis with Lilliputian hallucinations; coma	Todd, 1954
350 mL	Single exposure	Pronounced arrhythmia; unconscious for 4 d	Dhuner et al., 1957
500 mL	Single exposure	Arrhythmia, unconscious for 8 d; death	Dhuner et al., 1957

Table A-12. Human health effects from experimental inhalation exposure to TCE.

Concentration	Duration	Effect	Reference
27, 81, 201 ppmv	4-h single exposure	Irritation of eyes and throat; headache at >81 ppmv; dizziness, anorexia, and skin irritation at 201 ppmv	Nomiyama and Nomiyama, 1977
100, 200, 300, 500 ppmv	1.5-h single exposure	Dose-dependent decrease in performance on psychophysiological tests	Stopps and McLaughlin, 1967
100, 200 ppmv	1 h/d for 5 d	Sleepiness, eye irritation	Stewart et al., 1970
100, 300 ppmv	2-h single exposure	No significant effects observed	Vernon and Ferguson, 1969
110 ppmv	4 h for two exposures (separated by 1.5 h)	Statistically significant decrease ($p < 0.05$ to 0.001) in performance on tests of immediate memory and complex reaction time; no disturbances of motor function, coordination-equilibrium, or behavior patterns were observed	Salvini et al., 1971
150, 300 ppmv	2.5-h single exposure	No observed effect	Ettema et al., 1975
200 ppmv	2.5-h single exposure	Impairment of mental capacity (individual also ingested 0.35 g/kg ethanol)	Windemuller and Ettema, 1978
1000 ppmv	2-h single exposure	Statistically significant ($p < 0.05$) decrease in performance on Howard-Dolman, steadiness, and pegboard tests; no effect on flicker fusion, form perception, or code substitution tests	Vernon and Ferguson, 1969
1000 ppmv	2-h single exposure	Reduction in optokinetic fusion limit	Kylin et al., 1967

Table A-13. Human health effects from inhalation of mixtures containing TCE.

Concentration	Duration	Effect	Reference
Unknown (cleaning fluid)	3 consecutive d	Drowsiness, headache, anorexia, vomiting, sore throat, fever, jaundice, dark urine	Baerg and Kimberg, 1970
Unknown (cleaning fluid)	2 to 5 exposures/wk for 6 wk	Nausea, vomiting, and pain in right upper quadrant; oliguria, chills, and fever	ibid.
Unknown (cleaning fluid)	Intermittent for 1 y	Drowsiness, abnormal hepatic function, malaise, abdominal pain, vomiting, nausea	ibid.
Unknown	Unknown	Abnormal liver function, jaundice, nausea, gastroenteritis	Litt and Cohen, 1969
Unknown	Unknown	Four deaths from sniffing typewriter correction fluid	King et al., 1985

Table A-14. Human health effects from occupational exposure to TCE (inhalation and dermal).

Concentration	Occupation	Effect	Reference
1 to 13 ppmv	Paint-stripper operator	Lightheadedness, headache	Hervin et al., 1974
27 to 297 ppmv	Chemist	Hepatitis, jaundice, coma; death due to massive liver necrosis	Joron et al., 1955
43 ppmv (58 to 508 mg/m^3)	Degreaser	Lightheadedness, headache	Hervin et al., 1974
166 to 3700 ppmv	Floor tiler	Fever, emesis, tachycardia, pulmonary congestion, hyperkalemia, dyspnea, and acute renal failure	Gutch, et al., 1965
1714 to 3310 ppmv	Tank painter	Loss of consciousness	Longley and Jones, 1963
215 ppmv (approximately)	Degreaser	Abnormal hepatic function	Suciu and Olinici, 1983
Unknown (mixture of 40% TCE, 60% DCE)	Degreaser	Dizziness, numbness and pain in fingers and feet, double vision, hoarseness, loss of weight, pain and tenderness in leg muscles, inability to walk properly, inability to straighten fingers, fever; recovered in one year	McBirney, 1954
Not provided	Mechanic; cleaned metal parts	Erythematous dermatitis, edema of eyelids and parotid areas, conjunctivitis, tearing, hoarseness, muscle weakness, decrease in libido, headache	Bauer and Rabens, 1974
Not provided	Cleaned bomb casings	Generalized scarlatiniform dermatitis, edema of eyelids, dizziness, sleepiness	ibid.

Table A-14. (Continued).

Concentration	Occupation	Effect	Reference
Not provided	Cleaner of metal parts in a metal-plating plant	Generalized papulovesicular eruptions, exfoliative dermatitis, sore throat, conjunctivitis, anorexia, headache, lethargy, irritability	Bauer and Rabens, 1974
Not provided	Laundry and dry cleaning workers	Statistically significant ($p < 0.05$) increase in malignant neoplasms (lung, trachea, cervix, and skin); statistically significant ($p < 0.005$) increase in all circulatory diseases	Blair et al., 1979
200 to 8000 ppmv (estimate)	Degreaser	Nausea, death; autopsy revealed no gross anatomical abnormalities	Kleinfeld and Tabershaw, 1954
Not provided	Tank degreaser	Dizziness, emesis; death	Kleinfeld and Tabershaw, 1954
Not provided	Maintenance engineer	Impotence, gynecomastia, peripheral neuropathy, pigmentation lymphadenopathy, scleroderma, Raynaud's syndrome	Saihan et al., 1978
Not provided	Degreaser for 5 y	Jaundice; periods of inebriation, confusion, nausea, emesis, anorexia, fatigue	Thiele et al., 1982
Not provided	Not provided	No statistically significant difference in mortality pattern	Tola et al., 1980
Not provided	Laundry and dry cleaning workers	No overall increase in total cancer; elevated risk of homicide, lung cancer, and kidney cancer; decreased risk of ischemic heart disease and breast cancer	Duh and Asal, 1984
Not provided	Not provided	No significant difference in nerve conduction velocity	Triebig et al., 1982

Table A-14. (Continued)

Concentration	Occupation	Effect	Reference
Not provided	Not provided	Nausea, emesis, respiratory irritation, loss of consciousness; death	Salvini et al., 1971
Not provided	Laundry and dry cleaning workers	Elevated risk of kidney and genital cancers; decreased risk of bladder, cancer, skin cancer, and lymphosarcoma	Katz and Jowett, 1981
Not provided	Tank degreaser	Addiction; permanent paresis of the olfactory nerves, intermittent gastric disturbance; death	James, 1963
Not provided	Cutlery factory worker	Intense itching, subcorneal pustular eruption, erythema	Conde-Salazar et al., 1983
Not provided	Degreaser	Severe headache, loss of appetite, abdominal pain, nausea, emesis, extreme weakness; recovered after 10 d	Nomura, 1962
Not provided	Metal degreaser	Vertigo, depression, analgesia of the trigeminal nerve; loss of taste	Mitchell and Parsons-Smith, 1969
Not provided	Industrial workers	288 fatalities due to occupational over exposure were reported to HM Factory Inspectorate between 1961 and 1980	McCarthy and Jones, 1983
Not provided	Tank cleaners	Headache, nausea, emesis, dizziness	Buxton and Hayward, 1967
Not provided	Paint stripping machine worker	Emesis, abdominal cramps, erratic heart beat, episodes of flushing and weakness	Milby, 1968
Not provided	Electronics factory worker	Erythema multiform-like lesions	Phoon et al., 1984

Table A-14. (Continued)

Concentration	Occupation	Effect	Reference
Not provided	Degreaser	Erythema, headache, loss of appetite	McBirney, 1954
Not provided	Cleaner of optical lenses	Total loss of tactile sense, loss of muscular coordination	ibid.
Not provided	Not specified	Impairment of auditory and vestibular nerves, sleepiness, headache	Szule-Kuberska and Tronczyrska, 1976
Not provided	Degreaser	Death due to acute pulmonary edema	Teare, 1948
Not provided	Degreaser of metal parts	Erythema, loss of consciousness, convulsions	Maloof, 1949
Not provided	Fur cleaner	Asthenia, fever, abdominal pain, vomiting, lower back pain, oliguria, icterus, moderate liver enlargement, hepatorenal insufficiency	Suciu and Olinici, 1983
Not provided	Fur cleaner	Nausea, vomiting, abdominal pain	ibid.
Not provided	Degreaser	Nausea, vomiting, abdominal pain, dysphoria	Seage and Burns, 1971
Not provided	Degreaser	Loss of consciousness, severe cyanosis, bronchopneumonia, fever	Pies, 1939
Not provided	Electrical equipment cleaner	Addiction	O'Connor, 1954
Not provided	Not specified	Myocardial infarction	Morreale, 1976

Table A-14. (Continued)

Concentration	Occupation	Effect	Reference
Not provided	Not specified	Increase in LAP score	Friborska, 1969
Not provided	Not provided	Gastroduodenitis, electroencephalographic signs of cerebral cortex irritation, zonal perception hearing loss	Tomasini and Sartorelli, 1971
Not provided	Not specified	Increase in threshold for trigeminal nerve evoked potential	Barret et al., 1982
Not provided	Metal plater	Irritation of eyes, nose, and throat	Nomiyama and Nomiyama, 1977

APPENDIX B

Table B-1. Carcinogenicity bioassays for TCE administered by gavage to mice.

Study	Strain	Sex	Dose	Tumor type	Incidence	Statistical significance[a]
NCI, 1976	B6C3F1	M	0 mg/kg (matched)	Hepatocellular carcinoma	1/20	
			0 mg/kg (colony)		5/77	
			1169 mg/kg[b]		26/50	$p = 0.004$
			2339 mg/kg		31/48	$p < 0.001$
		F	0 mg/kg (matched)	Hepatocellular carcinoma	0/20	
			0 mg/kg (colony)		1/80	
			869 mg/kg[b]		4/50	$p = 0.090$
			1739 mg/kg		11/47	$p = 0.008$
NTP, 1983	B6C3F1	M	0 mg/kg	Hepatocellular carcinoma	8/48	
			1000 mg/kg[c]		30/50	$p < 0.001$
		M	0 mg/kg	Hepatocellular carcinoma or adenoma	11/48	
			1000 mg/kg[c]		38/50	$p < 0.001$
		F	0 mg/kg	Hepatocellular carcinoma	2/48	
			1000 mg/kg[c]		13/49	$p < 0.05$
		F	0 mg/kg	Hepatocellular adenoma	2/48	
			1000 mg/kg[c]		8/49	$p < 0.05$
		F	0 mg/kg	Hepatocellular carcinoma or adenoma	4/48	
			1000 mg/kg[c]		19/49	$p < 0.001$
Van Duuren et al., 1979	ICR/Ha-Swiss	M	0 mg/kg			
			2.8 mg/kg[d]	No statistically significant observed effect		
		F	0 mg/kg			
			2.8 mg/kg[d]	No statistically significant observed effect		

Table B-2. Carcinogenicity bioassays for TCE administered by gavage to rats.

Study	Strain	Sex	Dose	Tumor type	Incidence	Statistical significance[a]
NCI, 1976	Osborne-Mendel	M	0 mg/kg			
			549 mg/kg[b]	No statistically significant observed effect		
		F	0 mg/kg			
			1097 mg/kg[b]	No statistically significant observed effect		
NTP, 1983	F344/N	M	0 mg/kg	Renal tubular cell	0/48[c]	
			500 mg/kg[d]	adenocarcinoma	0/49	
			1000 mg/kg		3/49	p = 0.028[e]
		M	0 mg/kg	Renal tubular cell	0/48	
			500 mg/kg[d]	adenoma/adenocarcinoma	2/49	
			1000 mg/kg		3/49	p = 0.028[e]
		F	0 mg/kg			
			500 mg/kg[d]	No statistically significant observed effect		
			1000 mg/kg	No statistically significant observed effect		
NTP, 1987	ACI 9935	M/F	0 mg/kg			
			500 mg/kg[f]	No statistically significant observed effect		
			1000 mg/kg	No statistically significant observed effect		
	August 28807	M/F	0 mg/kg			
			500 mg/kg[f]	No statistically significant observed effect		
			1000 mg/kg	No statistically significant observed effect		
	Marshall 520	M	0 mg/kg (untreated)	Testicular	16/46	
			0 mg/kg (vehicle)	interstitial cell	17/46	
			500 mg/kg[f]	tumor (almost	21/48	
			1000 mg/kg	exclusively benign)	32/48	p = 0.002
	Marshall 520	F	0 mg/kg			
			500 mg/kg[f]	No statistically significant observed effect		
			1000 mg/kg	No statistically significant observed effect		

Table B-2. (Continued).

Study	Strain	Sex	Dose	Tumor type	Incidence	Statistical significance[a]
	Osborne-Mendel	M	0 mg/kg (untreated)	Renal tubular cell	0/50	
			0 mg/kg (vehicle)	adenoma	0/50	
			500 mg/kg[f]		6/50	p = 0.007[g]
			1000 mg/kg		1/50	
		M	0 mg/kg	Renal tubular cell	0/50	
			500 mg/kg[f]	adenoma/adenocarcinoma	6/50	p = 0.007[g]
			1000 mg/kg		2/50	
		F	0 mg/kg			
			500 mg/kg[f]	No statistically significant observed effect		
			1000 mg/kg	No statistically significant observed effect		
Maltoni et al., 1986 BT 301	Sprague-Dawley	M	0 mg/kg			
			50 mg/kg[h]	No statistically significant observed effect		
			250 mg/kg	No statistically significant observed effect		
		F	0 mg/kg			
			50 mg/kg[h]	No statistically significant observed effect		
			250 mg/kg	No statistically significant observed effect		

[a] Fischer Exact Test unless otherwise noted.
[b] Five doses/wk of industrial grade TCE (Epichlorohydrin (0.09%); 1,2-epoxybutane (0.19%)) for 78 wk; observed for 32 wk; terminated on wk 110.
[c] Terminal incidence (all tumors were observed upon terminal sacrifice at 103 wks).
[d] Five doses/wk of purified TCE for 103 wk; terminated between 103 to 107 wk.
[e] Using "Life Table" or "Incidental Tumor" tests referenced in NTP (1983).
[f] Five doses/wk of epoxide-free TCE for 103 wk; terminated between 110 to 111 wk.
[g] Using "Life Table" or "Incidental Tumor" tests referenced in NTP (1987).
[h] Four to five doses/wk of purified TCE for 52 wk (13-wk old at start).

Table B-3. Carcinogenicity bioassays for TCE based on inhalation in mice.

Study	Strain	Sex	Concentration	Tumor type	Incidence	Statistical significance[a]
Bell et al., 1978[b]	B6C3F1	M	0 ppmv	Hepatocellular carcinoma	18/99	
			100 ppmv[c]		28/95	p = 0.046
			300 ppmv		31/100	p = 0.026
			600 ppmv		43/97	p < 0.001
		M	0 ppmv	Hepatocelluar adenoma	2/99	
			100 ppmv[c]		7/95	
			300 ppmv		7/100	
			600 ppmv		10/97	p = 0.015
		M	0 ppmv	Hepatocellular carcinoma	20/99	
			100 ppmv[c]	or adenoma	35/95	p = 0.008
			300 ppmv		38/100	p = 0.004
			600 ppmv		53/97	p < 0.001
		F	0 ppmv	Hepatocellular carcinoma or adenoma	8/99	
			100 ppmv[c]		9/100	
			300 ppmv		10/94	
			600 ppmv		17/99	p = 0.04
Henschler et al., 1980	Han:NMRI	M	0 ppmv			
			100 ppmv[d]	No statistically significant observed effect		
			500 ppmv	No statistically significant observed effect		
		F	0 ppmv	Malignant lymphoma	9/29	
			100 ppmv[d]		17/30	p = 0.042
			500 ppmv		18/28	p = 0.012

Table B-3. (Continued).

Study	Strain	Sex	Concentration	Tumor type	Incidence	Statistical significance[a]
Fukuda et al., 1983	ICR	F	0 ppmv	Lung adenocarcinoma	1/49	
			50 ppmv[e]		3/50	
			150 ppmv		8/50	$p < 0.05$
			450 ppmv		7/46	$p < 0.05$
Maltoni et al., 1986 BT 303	Swiss	M	0 ppm			
			100 ppm[f]	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		
		F	0 ppm			
			100 ppm[f]	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		
Maltoni et al., 1986 BT 305	Swiss	M	0 ppm	Pulmonary Tumors	10/90	
			100 ppm[g]	(almost exclusively	11/90	
			300 ppm	benign)	23/90	$p < 0.05$
			600 ppm		27/90	$p < 0.01$
		M	0 ppm	Hepatomas	4/90	
			100 ppm[g]	(malignant)	2/90	
			300 ppm		8/90	
			600 ppm		13/90	$p < 0.05$
		F	0 ppm			
			100 ppm[g]	No statistically significant observed effect		
			300 ppm	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		

Table B-3. (Continued).

Study	Strain	Sex	Concentration	Tumor type	Incidence	Statistical significance[a]
Maltoni et al., 1986 BT 306/306 bis	B6C3F1	F	0 ppm	Pulmonary tumors	4/90	
			100 ppm[h]	(almost exclusively	6/90	
			300 ppm	benign)	7/90	
			600 ppm		15/90	$p < 0.05$
		M	0 ppm			
			100 ppm[h]	No statistically significant observed effect		
			300 ppm	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		
		M and F, combined	0 ppm	Hepatomas	4/180	
			100 ppm[h]	(malignant)	5/180	
			300 ppm		7/180	
			600 ppm		15/180	$p < 0.01$

[a] Fischer Exact Test against control incidence, unless otherwise noted.
[b] From U.S. EPA, 1985a, pp. 8-42.
[c] Six h/d, 5 d/wk, technical grade TCE (Epichlorohydrin (0.09%)) for 104 wk.
[d] Six h/d, 5 d/wk, purified TCE for 78 wk; observed for 52 wk; terminated on wk 130 (median survival time of controls = approx. 104 wk).
[e] Seven h/d, 5 d/wk, reagent grade TCE (Carbon tetrachloride (0.128%); benzene (0.019%); epichlorohydrin (0.019%); 1,1,2-trichloroethane (0.010%)) for 104 wk; terminated on wk 107.
[f] Seven h/d, 5 d/wk, epoxide-free TCE for 8 wk (11 wk old at start).
[g] Seven h/d, 5 d/wk, epoxide-free TCE for 78 wk (11 wk old at start) observed to wk 145 (most animals dead by wk 104).
[h] Seven h/d, 5 d/wk, epoxide-free TCE for 78 wk (12 wk old at start), observed to wk 145 (most males and females dead by wk 90 and 130, respectively).

Table B-4. Carcinogenicity bioassays for TCE based on inhalation in rats and hamsters.

Study	Strain	Sex	Concentration	Tumor type	Incidence	Statistical significance[a]
Bell et al., 1978	Charles River	M/F	0 ppmv			
			100 ppmv[b]	No statistically significant observed effect		
			300 ppmv	No statistically significant observed effect		
			600 ppmv	No statistically significant observed effect		
Henschler et al., 1980	Han:WIST	M/F	0 ppmv			
			100 ppmv[c]	No statistically significant observed effect		
			500 ppmv	No statistically significant observed effect		
Henschler et al., 1980	Syrian hamster	M/F	0 ppmv			
			100 ppmv[d]	No statistically significant observed effect		
			500 ppmv	No statistically significant observed effect		
Fukuda et al., 1983	Sprague-Dawley	F	0 ppmv			
			50 ppmv[e]	No statistically significant observed effect		
			150 ppmv	No statistically significant observed effect		
			450 ppmv	No statistically significant observed effect		
Maltoni et al., 1986 BT 302	Sprague-Dawley	M	0 ppm			
			100 ppm[f]	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		
		F	0 ppm			
			100 ppm[f]	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		

Table B-4. (Continued).

Study	Strain	Sex	Concentration	Tumor type	Incidence	Statistical significance[a]
Maltoni et al., 1986 BT 304/304 bis	Sprague-Dawley	M	0 ppmv	Testes	6/135	
			100 ppm[g]	Leydig-cell	16/130	$p < 0.05$
			300 ppm	tumor (almost	30/130	$p < 0.01$
			600 ppm	exclusively benign)	31/130	$p < 0.01$
		F	0 ppmv			
			100 ppm[g]	No statistically significant observed effect		
			300 ppm	No statistically significant observed effect		
			600 ppm	No statistically significant observed effect		

[a] Fischer Exact Test unless otherwise noted.
[b] Six h/d, 5 d/wk, technical grade TCE (Epichlorohydrin (0.09%)) for 104 wk.
[c] Six h/d, 5 d/wk, purified TCE for 78 wk; observed for 78 wk; terminated on wk 156.
[d] Six h/d, 5 d/wk, purified TCE for 78 wk; observed for 52 wk; terminated on wk 130.
[e] Seven h/d, 5 d/wk, reagent grade TCE (Carbon tetrachloride (0.128%); benzene (0.019%); epichlorohydrin (0.019%); 1,1,2-trichloroethane (0.010%)) for 104 wk; terminated on wk 107.
[f] Seven h/d, 5 d/wk, epoxide-free TCE for 8 wk.
[g] Seven h/d, 5 d/wk, epoxide-free TCE for 104 wk.

Table B-5. Carcinogenicity bioassays for TCE administered dermally, subcutaneously, and in drinking water.

Study	Strain	Sex	Dose or concentration	Tumor type	Incidence	Statistical significance[a]
Van Duuren et al., 1979	Ha ICR	M/F	0 mg 1.0 mg[b]	No statistically significant observed effect		
Van Duuren et al., 1979	Ha ICR	M/F	0 mg 0.5 mg[c]	No statistically significant observed effect		
Herren-Freund et al., 1986	B6C3F1	M/F	0 mg/L 3 mg/L[d,e] 40 mg/L[f]	No statistically significant observed effect No statistically significant observed effect		

[a] Fischer Exact Test unless otherwise noted.
[b] Three dermal applications/wk of purified TCE for 83 wk.
[c] One subcutaneous injection/wk of purified TCE for 89 wk.
[d] Exposed via drinking water for 61 wk; terminated on wk 65.
[e] Equivalent to 0.59 mg/kg-d, assuming a daily water intake of 6.3 mL/d for 32-g mice.
[f] Equivalent to 7.7 mg/kg-d, assuming a daily water intake of 6.7 mL/d for 35-g mice.

Table B-6. Carcinogenicity bioassays for the TCE metabolites, TCA and DCA, administered to mice in drinking water.

Study	Strain	Sex	Dose or concentration	Tumor type	Incidence	Statistical significance[b]
Herren-Freund et al., 1986	B6C3F1	M	0 mg DCA/L	Hepatocellular carcinoma	0/22	
			5000 mg DCA/L[b,c]		21/26	$p < 0.01$
		M	0 mg DCA/L	Hepatocellular adenoma	2/22	
			5000 mg DCA/L[b,c]		25/26	$p < 0.01$
Herren-Freund et al., 1986	B6C3F1	M	0 mg TCA/L	Hepatocellular carcinoma	0/22	
			5000 mg TCA/L[b,c]		7/22	$p < 0.01$
		M	0 mg TCA/L	Hepatocellular adenoma	2/22	
			5000 mg TCA/L[b,c]		8/22	$p < 0.01$

[a] Fischer Exact Test unless otherwise noted.
[b] TCA = trichloracetic acid, DCA = dichloroacetic acid. Exposed via drinking water for 61 wk; terminated on wk 65.
[c] Equivalent to 1000 mg/kg-d, assuming a daily water intake of 6.0 mL/d for 30-g mice.