TECHNICAL SUPPORT DOCUMENT

PART B

PROPOSED IDENTIFICATION OF

PERCHLOROETHYLENE

AS A TOXIC AIR CONTAMINANT

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State of California

Air Resources Board

HEALTH EFFECTS OF
TETRACHLOROETHYLÈNE (PCE)

CALIFORNIA DEPARTMENT OF HEALTH SERVICES

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1. SUMMARY

Tetrachloroethylene, commonly referred to as perchloroethylene (PCE), is a volatile organic hydrocarbon with a chloroform-like odor used as a solvent in dry cleaning operations, a metal degreaser and as, a chemical intermediate in the synthesis of fluorocarbons. Perchloroethylene is a lipophilic compound which readily diffuses into the blood and subsequently into adipose tissue where it accumulates due to its relative stability and slow metabolism. The main metabolic pathway for PCE in humans appears to involve its oxidation by cytochrome P-450-dependent mixed-function oxidases (referred to as P-450) to 1,1,2,2-tetrachloroethylene oxide. Following an apparently spontaneous chloride migration, the metabolites trichloroacetyl chloride and trichloroacetic acid are formed. Trichloroacetic compounds account for 60 to 80% of the metabolites of PCE in rodents. This drops to 4% measured in humans with 20-60% of the dose being unaccounted for in the studies. Trichloroacetic acid accounts for most of the trichlorocompounds found in urine after exposure to PCE and trichloroethanol has been the next most frequently reported human metabolite. Following P-450 metabolism and several dechlorination steps, oxalic acid and carbon dioxide (CO_2) also may be produced in humans since they are common metabolic products in animals. Thus, while the rodent data is well defined, the human PCE metabolic data are very incomplete.

Perchloroethylene is eliminated from the body by two major processes: At high concentrations (>50 ppm), the majority of absorbed PCE is excreted unchanged in the exhaled air while the remainder may undergo metabolism and excretion as urinary or fecal metabolites. At lower concentrations, relatively less PCE is exhaled. In humans a mass balance of PCE absorption and elimination is not available. Models describing the kinetics of PCE have been developed. The primary uncertainties in the models are identification of the toxic metabolite and the amount of the metabolite produced by humans.

Perchloroethylene has moderate acute toxicity, with the liver being its principal target organ. Acute inhalation exposure of mice to 200 ppm for four hours produced moderate fatty infiltration in the liver. Chronic exposure of laboratory animals to 100 ppm PCE caused major liver damage. The products of PCE metabolism are thought to promote liver toxicity. The extent of PCE metabolism was directly proportional to observed liver cell damage in animal experiments. The method by which PCE causes liver toxicity is not known, but metabolites of PCE may bind covalently to cell components, such as mitochondria.

Perchloroethylene causes skin and eye irritation. Prolonged PCE exposure can produce erythema, burns, and blistering. It has been associated with tachycardia and sudden death from cardiac failure, and PCE may sensitize the heart to the effects of endogenous epinephrine. Massive acute exposure to PCE induces central nervous system (CNS) depression that can progress to loss of consciousness, anesthesia, and respiratory failure. Inhalation exposure of pregnant rodents to 300 ppm PCE produced maternal toxicity and fetotoxicity manifested as developmental delays and altered performance in behavioral tests in the offspring of exposed mice and rats. However, PCE is not considered to be a teratogen.

The No-Observed-Adverse-Effect-Level (NOAEL) for chronic inhalation in rats was reported to be 70 ppm PCE. However, rats are less susceptible than mice to hepatotoxicity, the most sensitive noncancer endpoint for toxicity. Mice chronically exposed to 100 ppm PCE exhibited liver degeneration and necrosis, whereas 200 ppm PCE given to rats under the same regimen did not result in hepatic lesions. A NOAEL for mice has not been established. A Lowest-Observed-Adverse-Effect Level (LOAEL) in Mongolian gerbils exposed to PCE for 3 months was reported to be 60 ppm, based on a finding of reduced brain DNA concentration. Humans have shown signs of liver toxicity after chronic exposure to 232-385 ppm PCE, indicating that humans may be as susceptible as mice to the hepatotoxic effects of PCE. The population-weighted mean ambient outdoor concentration of PCE in the South Coast air basin was 0.44 ppb, a level well below concentrations associated with adverse effects. Consequently, California Department of Health Services (CDHS) staff do not expect noncarcinogenic adverse health effects to occur from acute or chronic exposure to PCE at ambient air concentrations. However, maximum 24 hour average concentrations of PCE measured in urban areas were approximately 5 ppb, and the EPA Reference Dose of 1×10^{-2} mg/kg/day corresponds to exposures to ambient PCE concentrations of 35 ug/m^3 (5 ppb), long-term exposure to these higher concentrations could be associated with adverse health effects.

The current 8-hour occupational standard for PCE in the workplace is set at 25 ppm, to provide a margin of safety against eye and respiratory discomfort and subjective complaints. The National Institute for Occupational Safety and Health (NIOSH) does not suggest a safe exposure level but recommends reducing exposure to the lowest feasible limit to prevent potential carcinogenic effects.

Perchloroethylene increased the incidence of hepatocellular tumors in laboratory mice after oral and inhalation exposure and mononuclear cell leukemia in rats after inhalation. A lifetime inhalation bioassay was conducted by the National Toxicology Program (NTP) in which mice (of both sexes) were exposed to 100 or 200 ppm of PCE and rats (of both sexes) were exposed to 200 or 400 ppm PCE (NTP, 1986). Both concentrations of PCE resulted in a statistically significant increase in hepatocellular carcinoma and adenoma in treated mice of both sexes. The incidence of mononuclear cell leukemia was significantly increased in rats of both sexes. The study also revealed an elevated incidence of kidney tumors in rats though it was not statistically significant. The NTP concluded that, under the conditions of their study, there was clear evidence of carcinogenicity of PCE for male F344/N rats, some evidence of carcinogenicity of PCE for female F344/N rats, and clear evidence of carcinogenicity of PCE for both sexes of B6C3F1 mice. In an earlier study by the National Cancer Institute (1977) male and female mice developed hepatocellular carcinoma after oral gavage exposure to PCE 5 days/week over 78 weeks. Rats receiving PCE via gavage did not develop significant increases in tumors, but early mortality in treated animals limits the usefulness of this study in characterizing the oral carcinogenicity of PCE in rats.

Epidemiological studies have provided some indication that the use of dry-cleaning solvents, primarily PCE, poses an increased risk of cancer for exposed workers. However, investigators were unable to differentiate among exposures to various solvents, and possible confounding factors like smoking

were not evaluated. Therefore, the usefulness of these reports in the assessment of the human cancer risk from PCE exposure is limited.

The International Agency for Research on Cancer (IARC) reviewed the carcinogenicity data on PCE and placed it in category 2B, (possible human carcinogen) with sufficient evidence of carcinogenicity in animals and inadequate evidence in humans (IARC, 1987). While the U.S. Environmental Protection Agency (EPA) staff has recommended classification of PCE in category B2, i.e., a probable human carcinogen, the Halogenated Solvents Subcommittee of Science Advisory Board has disagreed. The B2 recommendation has not been confirmed by the EPA administrator, so the 1985 classification in category C is still in effect. The CDHS staff agree with IARC's and EPA's conclusions that PCE is a potential human carcinogen.

Perchloroethylene has generally produced negative results in genotoxicity assays using bacterial systems, although a few positive responses have been reported, and an increase in mitotic recombinations and gene conversions was also reported in Saccharomyces cerevisiae. At least four known or presumed metabolites of PCE have shown evidence of genotoxicity. Perchloroethylene oxide, the first intermediate formed by microsomal oxidation, exhibited a dose-dependent mutagenic response in Salmonella typhimurium TA 1535. Trichloroethanol, another metabolite of PCE, induced sister chromatid exchange in cultured human lymphocytes. S-(1,2,2-trichlorovinyl)-glutathione and S-(1,2,2-trichlorovinyl)-L-cysteine have been shown to be mutagenic to Salmonella typhimurium TA100 following activation with kidney microsomes. These responses indicate that PCE itself and/or some of its metabolites are potentially genotoxic. However, the production of trichloroacetic acid and subsequent peroxisome proliferation may also play and important role in the carcinogenicity of PCE. The EPA reviewed the literature on the mutagenicity of PCE and concluded that inadequate information exists to classify PCE as either a mutagen or a nonmutagen. The staff of the CDHS agrees that PCE's mutagenicity has not been clearly established, but the positive results in some of the genotoxicity assays with PCE indicate a possible interaction with DNA and the potential for PCE to be an active genotoxin. CDHS staff have found no evidence of a carcinogenic threshold level, and the staff recommends that PCE be considered as not having a threshold for carcinogenicity.

Results from the 1986 NTP inhalation study were used as the basis for estimating the carcinogenic risk of PCE to humans. In this bioassay, PCE was 99.9% pure, and animals were exposed 6 hours/day, 5 days/week for 103 weeks. The mice in the 100 and 200 ppm dose groups were exposed to a time-weighted average (TWA) of 16 and 32 ppm, respectively (e.g., 100 ppm x 6 hours/24 hours x 5 days/7 days). Similarly, rats in the 200 and 400 ppm dose groups were exposed to a TWA of 33 and 66 ppm, respectively.

The CDHS staff used the metabolized dose, adjusted to continuous lifetime exposure, to calculate the carcinogenic potency of PCE. There are several uncertainties that accompany the metabolized dose adjustment. The metabolized dose approach, as it has been applied to PCE, assumes that oxidative metabolism leads to the production of carcinogenic metabolites; but the ultimate carcinogen(s) has not been well characterized. A mutagenic glutathione conjugate has also been identified. Metabolism of PCE has not been well-quantified in humans, and 20-40% of the absorbed PCE has not been accounted for. The high storage capacity and the slow release of

PCE from adipose tissue prevent verification of the mass balance relationship proposed by pharmacokinetic models between PCE absorbed and PCE metabolized or excreted either as urinary metabolites or unchanged by the lungs. The residual PCE could be metabolized to trichloro compounds over time, or to nonchlorinated metabolites such as CO_2 and oxalic acid that could not be easily associated with PCE metabolism without use of radiolabeled PCE. Data on the amount of PCE metabolized at ambient concentrations (less than 1 ppb) are not available. Several studies indicate that PCE metabolism increases as the concentration decreases. However, these concerns regarding metabolism can be taken into account for the most part by assuming an upper bound for metabolism of 25%. The upper range of human metabolism at low concentrations has been estimated to be as high as 73%.

Pharmacokinetic models generally do not account for individual differences in metabolism and storage. A high variability of body burden of PCE was found for different people tested. The body burden depended on such factors as age, sex, exercise or workload, body mass, adipose tissue mass, pulmonary dysfunctional states, and individual differences in the intrinsic capacity to metabolize PCE. Induction of PCE metabolism by diet or lifestyle factors can also increase the variability of PCE metabolism in humans. Human variability in PCE metabolism and interspecies differences could be accounted for through the incorporation of the surface area correction. DHS staff have chosen to utilize the metabolized dose approach with the surface area correction. Finally, chronic studies in mice and occupational studies in humans indicate that mice and humans appear to have similar sensitivity to PCE's induction of noncarcinogenic liver effects. Since carcinogenic and noncarcinogenic effects are thought to occur via the same metabolic pathway, a large species difference in response would not be expected.

For the low-dose PCE risk assessment, the Crump multistage polynomial (Crump, 1981) was chosen. This model, rather than a time dependent form of the multistage model, was chosen because most tumors were discovered only at the time of sacrifice, and survival in this study was relatively good.

Using the data of both rats and mice from the NTP 1986 studies, the upper bound of extra cancer cases predicted from a lifetime exposure to 1 ppb (6.78 $\mu g/m^3$) of PCE was estimated to be between 2 to 72 x 10^{-6} (per million persons exposed). This corresponds to a range of unit risks of 0.3 to 10.6 x 10^{-6} for a lifetime continuous exposure to 1 $\mu g/m^3$ of PCE. This range represents the upper limit of plausible excess cancer cases and incorporates the estimates of staff at EPA and others. Considering the quality of the studies and the importance of incorporating a 25% estimate of metabolism in humans, the best value for the 95% UCL was 54 x 10^{-6} $(ppb)^{-1}$. This compares with the current EPA estimate of 6.5 x 10^{-6}/ppb. These estimates are associated with male mouse heptocellular adenoma and carcinoma. Utilizing this metabolized dose approach predicted lower carcinogenic risks for humans than for rodents. Higher risks can be obtained with other metabolized dose approaches and the risk could be increased 3-fold if the physiological upper limit of human metabolism, 73%, was used in the metabolic model.

The mean environmental concentration measured by the Air Resources Board staff is 0.43 ppb in the South Coast Air Basin; that is, more than four orders of magnitude below the time-weighted average values used in the animal bioassay. Based on the CDHS potency evaluation and the annual average of 0.43 ppb PCE in the South Coast Air Basin, an upper limit of 233 additional lifetime cancer cases are estimated in the 10 million residents of the South Coast Air Basin as a result of PCE exposure. The calculations represent the upper range of plausible excess cancer risk: the actual risk, which cannot be calculated, may be insignificant. Based on the finding of carcinogenicity and the results of the risk assessment, the staff of CDHS concludes that PCE is an air pollutant which may cause or contribute to an increase in mortality or in serious illness, or which may pose a present or potential hazard to human health.

EVALUATION PERSPECTIVE

I. Exposure Sources

A. Air Levels

1. Population-weighted mean ambient levels measured outdoors in the South Coast Air basin: 0.44 ppb.

2. Ambient levels estimated in "hot spots": Modeling of dispersion from 8 PCE emission sources near City of Industry and Burbank indicates over 2000 persons would be exposed to PCE (from these sources) at annual average concentrations exceeding 6 ppb.

3. Indoor Air: PCE concentrations measured in indoor air varydepending on the quantity released from water and consumer product sources, the time since release, and the size of the room. The mean indoor air concentration measured in the U.S. ranged from 0.34 to 1.01 ppb. Maximum concentrations in homes reached levels of 14.1 ppb.

B. Reported Levels in Water

1. National Data: Ambient waters: mean concentration was 1 ug/L in 1,102 surface water measurements in 45 states.

2. California drinking water: PCE was present in 199 wells out of 2,947 sampled. The median concentration of the 199 wells was 1.9 ug/L with the highest concentration being 166 ug/L.

C. Reported Levels In Food

1. Highest levels were reported in butter, margarine, and olive oil. Butter contained 13 ug/kg and olive oil and margarine contained 7 ug/kg.

II. Pharmacokinetics

A. Absorption

1. Approximately 50 to 62% of the respired PCE is absorbed by humans.

B. Metabolism

1. The amount of PCE metabolized by P-450 varies depending on the animal species tested and concentration applied. The metabolite most often measured in urine is tricholoracetic acid (TCA). Other metabolites found are trichloroethanol, oxalic acid, carbon dioxide (CO_2), chloride, dichloroacetic acid, ethylene glycol, and possibly, thioether. Up to 47% of the PCE inspired may be metabolized by P-450. Much of the PCE inspired by humans is unaccounted for in disposition studies.

C. Excretion

1. Much of the PCE inhaled is exhaled unchanged by lungs. Some PCE has been detected in urine as trichloro metabolites. PCE

has a considerably longer half-life in humans than in rodents.

D. Bioaccumulation
   1. PCE accumulates in tissues following chronic exposure.

E. Conclusions
   1. Metabolism of PCE is complex and only partially characterized in humans. Particularly, the products and rates of metabolism are poorly understood.

   2. Metabolism and storage are influenced by factors such as age, sex, exercise or workload, body mass and adipose tissue mass, and pulmonary dysfunction.

III. Quantitative Risk Assessment

A. Shape of the Dose-Response Curve
   1. Animal: The data for male mice liver tumors (adenomas and carcinomas combined) were consistent with a linear dose-response relationship. However, only three data points were available: a control and two exposures.

   2. Human: NA.

B. Range of extrapolation
   1. Ratio of animal experimental concentration to ambient concentrations: Approximately 10^5.

C. Range of Risks
   1. The human risks associated with a continuous, lifetime exposure to perchloroethylene have been estimated using the linearized multistage model from animal carcinogenicity bioassays. Human risks estimated from animal data range from 2 to 72 x 10^{-6}/ppb or 0.3 to 10.6 x 10^{-6} $(ug/m^3)^{-1}$.

   2. The range of risks, estimated by fitting the multistage polynomial Crump, 1981) and the metabolized dose to the 1986 NTP rat and mouse inhalation study ranges from 14.4 -to-9.1-fold.

   MLE to 95% UCL:
   - male mice --12.7-fold
   - female mice --14.4-fold
   - male rats --10-fold
   - female rats --9.1-fold

IV. National and International Evaluation

A. U.S. Environmental Protection Agency
   1. Genotoxicity Tests: Inadequate information to classify PCE as a mutagen or nonmutagen.

   2. Animal carcinogenicity tests: Prior to the NTP (1986) inhalation bioassay EPA determined there was limited evidence

of animal carcinogenicity (EPA, 1985a). EPA Human Health Assessment Group (HHAG) has since concluded that there is sufficient animal evidence for PCE carcinogenicity. However, until this is finalized, the "C" classification stills stands.

3. Human Evidence: Insufficient data to assess human carcinogenicity.

4. The range of EPA potencies is 2 to 6.5 x 10^{-6}/ppb.

5. The EPA placed PCE in the fourth quartile of 53 carcinogens from which EPA has calculated potencies.

6. Conclusions: EPA HHAG determined that PCE is a probable human carcinogen and has categorized PCE in class B2. However, the Halogenated Solvents Subcommittee of the Science Advisory Board disagreed with the B2 classification. Thus, the final determination is to be made by the EPA administrator.

B. International Agency for Research on Cancer (IARC)

1. Animal carcinogenicity assays: Sufficient evidence of animal carcinogenicity by inhalation.

2. Human Evidence: Inadequate evidence of human carcinogenicity.

3. Conclusion: PCE is a possible human carcinogen, classified by IARC as a category 2B carcinogen (IARC, 1987).

C. Conclusions

1. Inadequate information exists to clearly classify PCE as genotoxic or nongenotoxic.

2. Data from epidemiological studies are of very limited use for the risk assessment of PCE due to the presence of other causative factors.

3. PCE should be considered a potential human carcinogen.

4. Risk estimates should be based on "metabolized dose," but the best value assumes a 25% human metabolic rate to account for the uncertainity in human metabolism of PCE.

5. The upper 95% CI of human risks estimated from animal data range from 2 to 72 x 10^{-6}/ppb (0.3 to 10.6 x 10^{-6} $(ug/m^3)^{-1}$).

6. The best value for the 95% UCL is 54 x 10^{-6}/ppb.

2. PHARMACOKINETICS AND METABOLISM

Perchloroethylene is readily absorbed through the lungs and gastrointestinal tract and, to a lesser extent, may be absorbed through the skin. Once in the body, PCE disperses into all tissues. Steady-state tissue concentrations are a function of the absorbed dose, partitioning factors, and pharmacokinetic properties, such as rate of metabolic conversion and elimination. The percentage of PCE absorbed by humans via inhalation has been reported to be between 50 and 70% (Bolanowska and Golacka, 1972; Imbriani et al., 1988; Ohtsuki et al., 1983).

The primary metabolic pathway of PCE is thought to involve oxidation to an epoxide as the first step, although this epoxide intermediate has never been isolated *in vivo*. The epoxide undergoes rearrangement to form trichloroacetyl chloride and, ultimately, trichloroacetic acid (TCA), which has been identified in urine (Yllner, 1961; Daniel, 1963; Moslen et al., 1977; Costa and Ivanetich, 1980). Studies in which radiolabeled PCE was administered to animals have occasionally recovered oxalic acid as a significant urinary metabolite (Yllner, 1961; Dimitrieva, 1967; Pegg et al., 1979). Carbon dioxide is also commonly produced (Pegg et al., 1979; Schumann et al., 1980). Other metabolites detected include trichloroethanol, chloride, dichloroacetic acid, ethylene glycol, S-(1,2,2-trichlorovinyl)glutathione, N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine, N-trichloroacetyl-aminoethanol, and N-oxalyl-aminoethanol (Daniel, 1963; Dekant et al., 1985; Dimitrieva 1967, Green et al., 1990, Ikeda and Ohtsuji, 1972; Ikeda et al., 1972; Koppel et al., 1985; Monster et al., 1983; Yllner, 1961).

This section presents an overview of published studies on the absorption, distribution, metabolism, and elimination of PCE. Studies that have defined the rate and extent of each of these processes in humans and in rodents are emphasized. Proposed metabolic pathways are discussed in some depth because metabolism is responsible for the transformation of PCE to one or more reactive species. Several reviews of the pharmacokinetics of PCE have also been published (Bolt, 1985; Travis, 1988, Ward et. al. 1988).

ABSORPTION

This section reviews the relevant data on PCE uptake through ingestion, dermal absorption, and inhalation.

Ingestion

Absorption of PCE from the gastrointestinal tract has been measured indirectly as percent of dose recovered. The percentage of dose recovered after administration of PCE is similar in mice and rats, varying between 80 to 100%. Peak blood concentrations measured in rats one hour after a 500 mg/kg dose shows PCE is absorbed rapidly (Pegg et al., 1979).

Little information exists regarding amount or rate of oral absorption of PCE in humans. Koppel and associates (1985) reported that the blood concentration of PCE following an oral dose of 400 mg was described by a two-compartment model with half-lives of 160 minutes and 33 hours (unpublished data cited in Koppel et al., 1985). The same authors (Koppel et al., 1985) measured the concentration of PCE in blood at 21.5 μg/ml within one hour of ingestion of 12 to 16 grams. Although far from definitive, these reports suggest fairly rapid and complete oral absorption.

Dermal absorption

Jakobsen and co-workers (1982) measured the absorption of PCE through guinea pig skin. Animals were in contact with liquid PCE for 6 hours. During the exposure, blood PCE concentrations rose rapidly, and peaked within 30 minutes. Tsuruta (1975) estimated the rate of absorption of PCE through mouse skin to be 24 $nmol/min\text{-}cm^2$ of skin. In a follow-up study Tsuruta (1989) reported on skin absorption of PCE in male BALB/cAnNCrj-nu/nu nude mice. Animals were placed in a chamber containing PCE at 200, 1000, or 3000 ppm for 2, 4, or 6 hours, and a respirator was used to prevent inhalation of PCE. Tissue extracts were subjected to GC analysis. The skin absorption rate for PCE was calculated using a single compartment model. Absorption rates correlated linearly with exposure concentration and duration. A skin absorption coefficient of 1.00 cm/hr was calculated.

McDougal and associates studied whole-body dermal penetration of perchloroethylene in rats (McDougal et al., 1990). Rats with closely clipped fur were exposed for 4 hours to 12500 ppm PCE while breathing uncontaminated air through a mask. Blood concentrations were determined by sampling through jugular cannulas. A physiologically based pharmacokinetic model was used to predict permeability constants. A dermal flux of 0.054 mg/cm2/h was determined and a permeability constant of 0.67 cm/hr was estimated. From this data it was estimated that 3.5% of PCE absorption of rats subjected to both inhalation and dermal exposure would be as a result of dermal absorption.

Percutaneous penetration of PCE vapor in humans exposed to ambient air concentrations of 600 ppm is approximately one percent of pulmonary absorption (Riihimaki and Pfaffli, 1978). Studies in which volunteers immersed their thumbs in liquid PCE measured a peak concentration of 0.3 ppm in expired air within 40 minutes; concentrations decreased thereafter. PCE has a relatively slow rate of elimination in breath. The relatively low concentration of PCE in exhaled air suggests that dermal absorption is limited (Hake and Stewart, 1977; Stewart and Dodd, 1964).

Pulmonary Uptake

PCE is effectively absorbed through the lungs during inhalation. Yllner (1961) reported the average pulmonary absorption of mice exposed to approximately 2000 ppm (reported as 1.3 mg PCE/g of mouse in a 2.7 liter chamber) to be 70%. In these animals, absorption varied from 42 to 87%.

In a study by Pegg and co-workers (1979), the peak blood concentration of PCE in rats during a 6-hour exposure to 600 ppm was approximately 10 μg/mL.

During inhalation exposure, PCE diffuses across the lungs and dissolves into the bloodstream. The rate of transfer depends on the blood/gas partition coefficient for PCE. Three estimates of the human blood/air partition coefficient are available: 10.3 (Gargas et al., 1986), 13.1 (Sato and Nakajima, 1979), and 16 (Monster et al., 1979). These values reflect the fact that PCE is lipophilic and readily diffuses into the blood. The uptake of PCE by the lungs is also determined by the alveolar ventilation rate, exposure concentration, exposure duration, and metabolism.

Several experimental human studies have quantified PCE absorption in terms of "percent uptake" (one minus the ratio of alveolar to ambient air concentration and multiplied by 100%). However, unless a steady state absorption rate is approximated at very low exposure concentration (near 1 ppb), the net quantities of PCE retained following environmental exposure may not be accurately quantified. The percentage of PCE absorbed in humans through the lungs has been estimated at 51 to 70% (Bolanowska and Golacka, 1972; Imbriani et al., 1988). After a 6-hour exposure of five volunteers to 54 ppm PCE (reported as 0.39 mg/L), retention of PCE reportedly stabilized after 1 1/2 hours and ranged from 55 to 70% (Bolanowska and Golacka, 1972). Reports of a steady state condition by Bolanowska and Golacka (1972) differ from the data of Fernandez and associates (1976) who studied humans exposed to 100 ppm PCE. The latter investigators found that alveolar air concentrations continued to steadily increase after 8 hours although the air level had reached 55 ppm. Ohtsuki and co-workers (1983) estimated that the percentage of PCE absorbed by humans was 50%, although the specific derivation of the value was not described. Imbriani and colleagues exposed human volunteers to 3.6 to 316 mg/m^3 (0.5 to 46 ppm) PCE for 2 to 4 hours at rest (10 cases) and while undertaking light exercise (5 cases) (Imbriani et al., 1988). The median measured PCE concentrations were 66 mg/m^3 (9.6 ppm). Mean relative uptake declined from 58 to 60% after 20 minutes exposure to 51% (after 100 minutes at exercise) to 55% (after 2450 minutes at rest).

Monster and colleagues (1979) observed an inverse relationship between uptake and exposure duration in humans over the course of a 4-hour inhalation exposure to 72 or 144 ppm PCE. The net uptake at the end of 4 hours was approximately 60% of that during the first hour. This observation indicates that net uptake decreases as blood and tissue concentrations of PCE equilibrate with PCE in the air space of the lungs. Net uptake is affected by differences in ventilation rate. When volunteers were exposed to 142 ppm while under a work load (i.e., an increased ventilation rate), the uptake of PCE increased to over two times what it was at rest (Monster et al., 1979).

Fernandez and co-workers (1976) exposed humans to 100 ppm PCE for 8 hours and measured the concentration in alveolar air. Alveolar concentration rose rapidly in the first half hour and then continued to increase throughout the experiment although at a slower rate, reflecting a sustained decline in percent uptake observed.

At steady state, the amount of PCE taken up or retained will be equal to the amount of PCE metabolized. As pointed out by Guberan and Fernandez (1974), the alveolar concentration of PCE rises more rapidly during the first phase of uptake. PCE concentrations in rapidly perfused tissues will reach approximate steady state concentrations long before they are achieved in poorly perfused or highly lipophilic tissues. For example, during an 8-hour exposure to 100 ppm, tissues such as the liver will reach half of their maximum concentration in 34 minutes, while adipose tissue will reach half of its maximum concentration in 3 1/4 hours. Following exposure, PCE may continue to perfuse into adipose tissue and excretion from this tissue will be slow (Guberan and Fernandez, 1974). Since the uptake or retention rate varies as a function of tissue, time and a number of other variables, the results in these studies relating uptake to metabolism may overestimate metabolism since the quantity of PCE stored in adipose tissue has not been accounted for.

Table 2-1 summarizes the absorption and recovery of experimentally administered PCE in animals. Table 2-2 lists parameters of absorption, metabolism, and disposition of PCE in humans.

DISTRIBUTION AND BIOACCUMULATION

PCE diffuses into the bloodstream and distributes to tissues, primarily to organs and fat. Seventy-two hours after oral or inhalation exposure to tetrachloro[^{14}C]ethylene, measurable radioactivity was found in the liver, kidneys, fat, lungs, heart, and adrenal glands of rats. The major part of the radioactivity was concentrated in the liver, kidneys, and fat (Pegg et al., 1979). A similar distribution was observed by Frantz and Watanabe after PCE was administered to rats in a saturated drinking-water solution (containing approximately 150 ppm PCE) (Frantz and Watanabe, 1983). Radiolabelled PCE was found to cross the placenta in pregnant mice exposed for 10 to 60 minutes (Ghantous et al., 1986). In addition to amniotic fluid, radioactivity was found in maternal fat, brain, blood, liver, kidney and lungs.

The binding of metabolites of PCE to hepatic macromolecules has been measured by Pegg and associates (1979), Schumann and co-workers (1980), Mitoma and colleagues (1985), and Mazzullo and associates (1987). Savolainen and associates (1977) gave rats 200 ppm PCE for 6 hour/day for 4 days, and observed substantial levels of PCE in perirenal fat within 17 hours after the end of the exposure (Savolainen et al., 1977).

Information on the distribution of PCE in humans comes largely from reports of accidental exposures (Stewart et al., 1961a; Stewart, 1969; Hake and Stewart, 1977; Koppel et al., 1985). Over exposure can result in central nervous system (CNS) depression, cardiac arrhythmias, alteration of kidney function, and liver injury. These observations provide indirect evidence that PCE distributes to the nervous system, liver, and kidneys. Additionally, tissue concentrations in the liver and brain have been measured at levels ten to 50 times greater than those in blood following fatal intoxication (Lukaszewski, 1979).

TABLE 2-1. ABSORPTION AND RECOVERY OF TETRACHLOROETHYLENE (PCE) IN ANIMALS

Parameter	Value	Species (weight)	Route	Concentration or dose	Exposure duration	Reference
Percent absorbed (average)	70.0 (range 42 to 87)	Mouse	Inhalation	1.3 mg/g	2 h	Yllner, 1961
Percent of dose recovered[a,b] (48 h post exposure)	79.65	Mouse	Oral	900 mg/kg	5 d/wk; 4 wk	Mitoma et al., 1985
(72h post exposure)	99.65	Mouse	Oral	800 mg/kg	Single exposure	Dekant et al., 1985
Percent of dose recovered (72 h post exposure)	94.6	Mouse (18-31 g)	Oral	500 mg/kg	Single exposure	Schumann et al., 1980
Percent of dose recovered (48 h post exposure)	84.4	Rat	Oral	1000 mg/kg	5 d/wk; 4 wk	Mitoma et al., 1985
Percent of dose recovered[a,b] (72 h post exposure)	97.5	Rat	Oral	800 mg/kg	Single exposure	Dekant et al., 1985
Percent of dose recovered (72 h post exposure)	103.0	Rat (250 g)	Oral	1 mg/kg	Single exposure	Pegg et al., 1979
Percent of dose recovered (72 h post exposure)	91.2	Rat (250 g)	Oral	500 mg/kg	Single exposure	Pegg et al., 1979
Percent of dose recovered (72 h post exposure)	100.0	Rat (275 to 285 g)	Oral, in drinking water	8.1 mg/kg (approx.)	12 h	Frantz and Watanabe, 1983

TABLE 2-1. (Continued)

Parameter	Value	Species (weight)	Route	Concentration or dose	Exposure duration	Reference
Percent of dose recovered (72 h post exposure)	99.9	Rat (250 g)	Inhalation	10 ppm	6 h	Pegg et al., 1979
Percent of dose recovered (72 h post exposure)	100.0	Rat (250 g)	Inhalation	600 ppm	6 h	Pegg et al., 1979
Percent of dose recovered (72 h post exposure)	88.0	Mouse	Inhalation	10 ppm	6 h	Schumann et al., 1980
Percutaneous absorption rate	24 n/mol/min/cm^2	Mouse	Dermal			Tsuruta, 1975
	1.00 cm/h	Mouse	Dermal	200-3000 ppm	2-6h	Tsuruta, 1989
	0.668 cm/h (0.0541 mg/cm/h)	Rat	Dermal	12500 ppm	0.5-4h	McDougal et al., 1990
Time to peak blood concentration	1 h (approx.)	Rat (250 g)	Oral	500 mg/kg	Single exposure	Pegg et al., 1979
Time to peak blood concentration	0.5 h	Guinea pig	Dermal		6 h	Jakobsen et al., 1982
Total pulmonary uptake	0.40 mg/animal (16.5 mg/kg)	Mouse	Inhalation	10 ppm	6 h	Pegg et al., 1979
Total pulmonary uptake	1.48 mg/animal (5.9 mg/kg)	Rat	Inhalation	10 ppm	6 h	Pegg et al., 1979
	28.1 mg/animal (79.9 mg/kg)	Rat	Inhalation	500 ppm	2 h	Dallas et al., 1989b
	3.9 mg/animal (11.2 mg/kg)	Rat	Inhalation	50 ppm	2 h	Dallas et al., 1989b

TABLE 2-1. (Continued)

Parameter	Value	Species (weight)	Route	Concentration or dose	Exposure duration	Reference
Total pulmonary uptake	77.5 mg/animal (310 mg/kg)	Rat	Inhalation	600 ppm	6 h	Pegg et al., 1979

[a] Percent of dose recovered refers to the portion of administered dose of PCE accounted for by measurements of breath, metabolites, excreta, and tissue.

[b] The percent recovered is considered to be indicative of percent absorption; however, the two parameters are not identical (D'Souza et al., 1985; U.S. EPA, 1985a).

TABLE 2-2. ABSORPTION, METABOLISM, AND DISPOSITION OF PCE IN HUMANS

Parameter	Value	Route	Concentration or dose	Duration	Reference
Percent absorbed through lungs	50	Inhalation			Ohtsuki et al., 1983
Percent absorbed through lungs	62 to 64	Inhalation[a]	72 or 144 ppm	4 h	Monster et al., 1979
Average pulmonary uptake	1350 mg	Inhalation	150 ppm	8 h	Fernandez et al., 1976
Average pulmonary uptake	455 mg	Inhalation	72 ppm	4 h	Monster et al., 1979
Average pulmonary uptake	945 mg	Inhalation	144 ppm	4 h	Monster et al., 1979
Average pulmonary uptake	0.77 mg/min	Inhalation		20 min	Imbriani et al., 1988
Average pulmonary uptake	0.63	Inhalation		1 h	Imbriani et al., 1988
Average pulmonary uptake	0.57	Inhalation		2 h	Imbriani et al., 1988
Average pulmonary uptake	0.54	Inhalation		3 h	Imbriani et al., 1988
Average pulmonary uptake	0.52	Inhalation		4 h	Imbriani et al., 1988
Percent retention	55 to 70	Inhalation	54 ppm (390 mg/L)	4-6 h	Bolanowska and Golacka, 1972
Percent retention	55 to 60	Inhalation	36.1-240.8mg/m^3	20 min-4h	Imbriani et al., 1988
Time to peak concentration					
- exhaled air	15 to 40 min	Dermal		10 min	Stewart and Dodd, 1964
- lung tissue	8 h	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
- whole body	8 h	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
- organs	8 h	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
- fat	12 h	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
- blood	0.5 h	Inhalation	72 to 142 ppm	4 h	Monster et al., 1979

TABLE 2-2. (Continued)

Parameter	Value	Route	Concentration or dose	Duration	Reference
Percent metabolized	1 to 3	Inhalation	50 ppm	8 h	Ikeda et al., 1972 Ohtsuki et al., 1983
Percent metabolized	47	Inhalation	57 ppm (390 mg/L)	4-6 h	Bolanowska and Golacka, 1972
Percent eliminated unchanged via lungs	38	Inhalation	50 ppm	8 h	Ikeda et al., 1972 Ohtsuki et al., 1983
Percent eliminated unchanged via lungs	25	Inhalation	57 ppm (390 mg/L)	4 to 6 h	Bolanowska and Golacka, 1972
Body burden	1000 mg	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
Adipose burden	500 mg (approximate)	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
Predicted biological $t_{1/2}$[b]	71.5 h	Inhalation	100 ppm	8 h	Guberan and Fernandez, 1974
Respiratory $t_{1/2}$	65 h	Inhalation	100 ppm	7 h (single exposure)	Ikeda and Imamura, 1973 (based on Stewart et al., 1970)
Urinary metabolite $t_{1/2}$	144 h	Inhalation	10 to 100 ppm	8 h/d;5 d	Ikeda and Imamura, 1973

TABLE 2-2. (Continued)

Parameter	Value	Route	Concentration or dose	Duration	Reference
Time to steady-state equilibrium, adipose tissue	125 h	Inhalation	100 ppm	8 h/d	Monster et al., 1979
Saturation of metabolism	NA[c]	Inhalation	100 ppm	8 h	Ohtsuki et al., 1983
Elimination $t_{1/2}$	160 min 33 h	Oral	400 mg	Single exposure	Koppel et al., 1985

[a] Values calculated from average estimated uptake/min.
[b] $t_{1/2}$ denotes half-life.
[c] NA: not applicable.

Perchloroethylene is a relatively stable molecule and is metabolized slowly. Perchloroethylene is soluble in lipids and this factor, along with its slow rate of metabolism lead to its accumulation in tissue following repeated exposure (Filser and Bolt, 1979; Loew et al., 1983).

On the basis of a model of PCE uptake, distribution, and elimination, Guberan and Fernandez (1974) predicted that the solvent would disperse primarily to three body compartments: adipose tissue, muscle, and tissues rich in blood vessels. It was estimated that the body burden of a 70-kg man exposed to 100 ppm PCE for 8 hours would be 1000 mg. More than one half of the PCE would accumulate in fat; substantial amounts would also be concentrated in muscles.

The rate of uptake and the time required for equilibration of tissue concentrations (for a given concentration of PCE in inspired air) depend on the solubility of the substance, the volume of each tissue, and on the rate of blood flow through the tissue. Because the blood supply to adipose tissue is less than to other tissues and because PCE is more soluble in fat than in blood, it takes a correspondingly greater time for equilibrium concentrations to be reached. Assuming a single 8-hour exposure to PCE, the time required for fat to reach about 50% of its capacity is 25 hours (Monster et al., 1979; Guberan and Fernandez, 1974).

Once whole-body, steady-state concentrations are reached (compared to concentrations in air), the amount of PCE in each tissue depends on the tissue/blood partition coefficient. Since PCE is lipophilic, the adipose/blood partition coefficient is the highest of any tissue type (at 37°C the adipose/blood partition coefficient is about 107) (Guberan and Fernandez, 1974). Guberan and Fernandez state that daily occupational exposure to PCE at 100 ppm, 8 hours/day, would lead to accumulation of PCE in fat.

Savolainen and co-workers noted accumulation of PCE in the blood, liver, fat, kidneys, and brain of rats following 5 days of inhalation exposure at 200 ppm (Savolainen et al., 1977). The PCE levels in perirenal fat, brain, and lungs rose continuously during the experiment. Concentrations in blood and liver also increased, but the rate of accumulation slowed by the third day.

Evidence of PCE accumulation is also available from a study that measured the concentration of PCE in exhaled air. When human volunteers were exposed to 100 ppm, 7 hours/day for 5 days, the concentration of PCE in expired air increased with each exposure. This suggests that PCE had accumulated in the tissues on each consecutive day of exposure and had not been eliminated between exposures (Hake and Stewart, 1977).

METABOLISM AND ELIMINATION

Among the most important enzyme systems for metabolism of toxic substances are the mixed-function oxygenases (MFO). These enzymes are concentrated in the liver, kidneys, lungs, and skin, and are present in other tissues as

well. Mixed-function oxygenases catalyze the addition of oxygen to compounds, which facilitates their excretion from the body. Oxidation of a compound can function as a mechanism of detoxification or can transform it to a reactive (toxic) substance. At least 16 microsomal P-450 genes are expressed in humans, with 4 of them being extrahepatic (Guengerich, 1989).

Evidence that MFO's are directly involved in the metabolism of PCE comes from the work of several investigators. Moslen and colleagues (1977) demonstrated that a number of substances, including phenobarbital (PBT) and Aroclor 1254, induced hepatic MFO. Pretreatment of rats with either of these compounds, followed by administration of PCE, increased the metabolism of PCE five to seven times over controls. Costa and Ivanetich (1980) showed that substances that inhibit cytochrome P-450, a component of MFO's, also inhibit metabolism of PCE in rats. Induction of cytochrome P-450 by PBT or pregnenolone-16a-carbonitrile increased the metabolism of PCE. The solvent was also shown to bind to the active site of P-450 in rat hepatic microsomes.

The first step in the general metabolism of PCE is thought to be transformation to an epoxide by the MFO, although this epoxide has never been isolated *in vivo* (Bonse et al., 1975; Greim et al., 1975). The epoxide of PCE apparently rearranges spontaneously with migration of a chlorine to form trichloroacetyl chloride and, ultimately, trichloroacetic acid (Moslen et al., 1977; Leibman and Ortiz, 1977; Reichert, 1983). Trichloroethanol has also been reported as a metabolite; however, a pathway has not been proposed that explains its formation (Ikeda and Ohtsuki, 1972; Ikeda et al., 1972; Monster et al., 1983; Koppel et al., 1985).

Although not consistently reported, the production of oxalic acid and CO_2 as metabolites of PCE has been documented in rodents (Yllner, 1961; Pegg et al., 1979; Schumann et al., 1980; Dekant et al., 1985). Their formation argues for a second and possibly minor pathway of oxidative metabolism for rats and mice.

Perchloroethylene is also be metabolized via a GSH-transferase to form a glutathione conjugate, S-(1,2,2-trichlorovinyl)glutathione (Dekant et al., 1986; Green et al., 1990), which is further metabolized to S-(1,2,2-trichlorovinyl)-L-cysteine (Dekant et al, 1987). The conjugate can be formed by rat microsomes and cytosolic GSH-S-transferases. This metabolite can be further metabolized to dichloroacetic acid and pyruvate.

It has been proposed that oxalic acid and CO_2 are formed as end-products of a metabolic pathway that also includes epoxide formation as the first step (Pegg et al., 1979). In this scheme, chloroethylene glycol is formed from the epoxide by the action of epoxide hydrolase. This reaction is followed by hydrolysis to oxalic acid and/or decarboxylation to CO_2 and possibly formic acid.

Chloride, dichloroacetic acid, and ethylene glycol have also been reported as urinary metabolites of PCE in rodents (Daniel, 1963; Yllner, 1961; Dimitrieva, 1967). It appears that these substances are minor metabolites, since each has been reported by only one investigator. Little is known

about their formation. Figures 2-1 and 2-2 show the structure of metabolites as well as the proposed metabolic pathways.

There are some similarities in the urinary metabolites produced by humans and rodents following exposure to PCE. Trichloroacetic acid has been identified in all species. Tables 2-3 and 2-4 list the metabolites identified in humans, mice, and rats, as well as the conditions of exposure.

Yllner was the first to analyze urinary metabolites of PCE in mice. Although 18% of the radiolabeled compound was not accounted for, 52% of the portion metabolized was recovered as trichloroacetic acid (TCA), 11% as oxalic acid, and a trace amount as dichloroacetic acid (Yllner, 1961). Daniel fed labeled PCE to rats and found TCA (0.6%) and inorganic chloride as the only metabolites (Daniel, 1963). Total urinary metabolite production in rats was measured by Moslen and co-workers (1977). They did not identify the actual metabolites, with the exception of TCA (which was the major metabolite produced). Pegg and associates and Dimitrieva identified oxalic acid as the primary product of PCE metabolism in rats (Pegg et al., 1979; Dimitrieva, 1967). Carbon dioxide has been recovered as a metabolite of PCE in mice (Schumann et al., 1980) and in rats (Pegg et al., 1979).

Trichloracetic acid, trichloroethanol, and an unidentified organic chloride have been measured in urine from humans exposed to PCE. Based on the Yllner (1961) study in mice, Ogata and colleagues measured only trichloro compounds in the urine of human volunteers (Ogata et al., 1971). In this study the major metabolite was an unidentified organic chloride. In some instances, identification of trichloroethanol has been by the Fujiwara reaction (Ikeda et al., 1972; Ikeda and Ohtsuji, 1972). The results of this test are qualitative and the accuracy is questionable. However, Monster and co-workers (1983) and Koppel and associates (1985) determined the presence of trichloroethanol by gas chromatography. Still other studies have failed to detect trichloroethanol; therefore, it is far from clear under what circumstances this substance is formed (Monster et al., 1979; Fernandez et al., 1976). Measurements of human metabolite production are confounded by the fact that CO_2, oxalic acid, and chlorine are normal products of metabolism. Their presence as products of PCE metabolism could be quantified only by the administration of radiolabeled PCE--a procedure that has not been undertaken in humans. Furthermore, studies in humans have primarily focused on quantifying the amount of trichloro compounds detectable in urine and not on accounting for all the metabolic products of PCE.

Lafuente and Mallol have reported the presence of a thioether derivative of PCE in the urine of women occupationally exposed to PCE (Lafuente and Mallol, 1986). A gradual increase in thioether production was observed over the course of a week. This increase appears to have been associated with continued exposure to PCE (with concomitant accumulation) over the work week. However, measured amounts of thioether in exposed women were reported not to be statistically different from levels found in nonexposed individuals.

The recent identification of thioether derivatives in humans exposed to PCE,

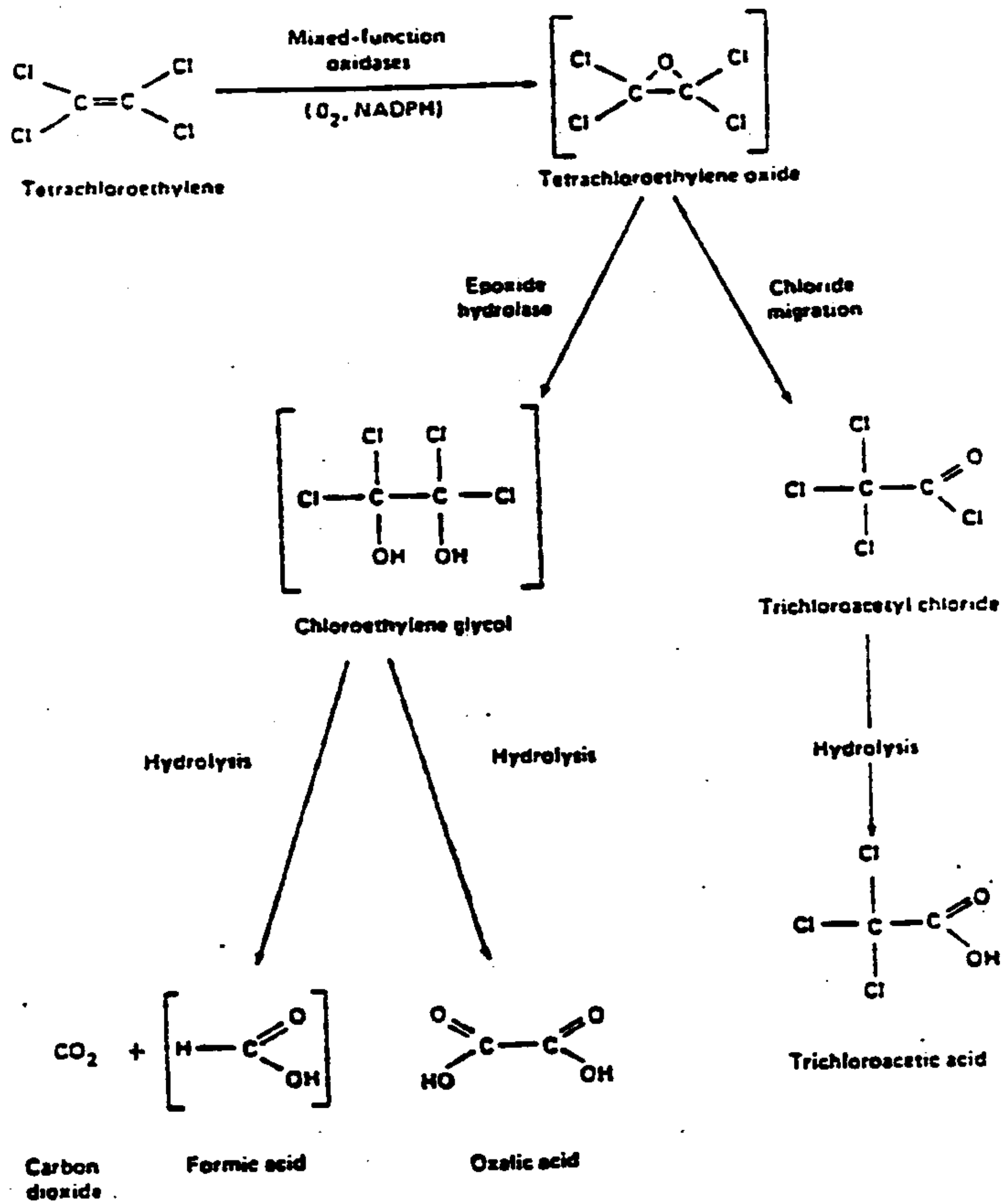

Figure 2-1. Metabolic pathways of PCE (brackets denote compounds that have not been isolated *in vivo*) (Daniel, 1963; Pegg, 1979; Costa and Ivanetich, 1980). Although trichloroethanol, dichloroacetic acid, ethylene glycol, and a thioether have been identified as urinary metabolites, the route(s) by which each formed has not been characterized.

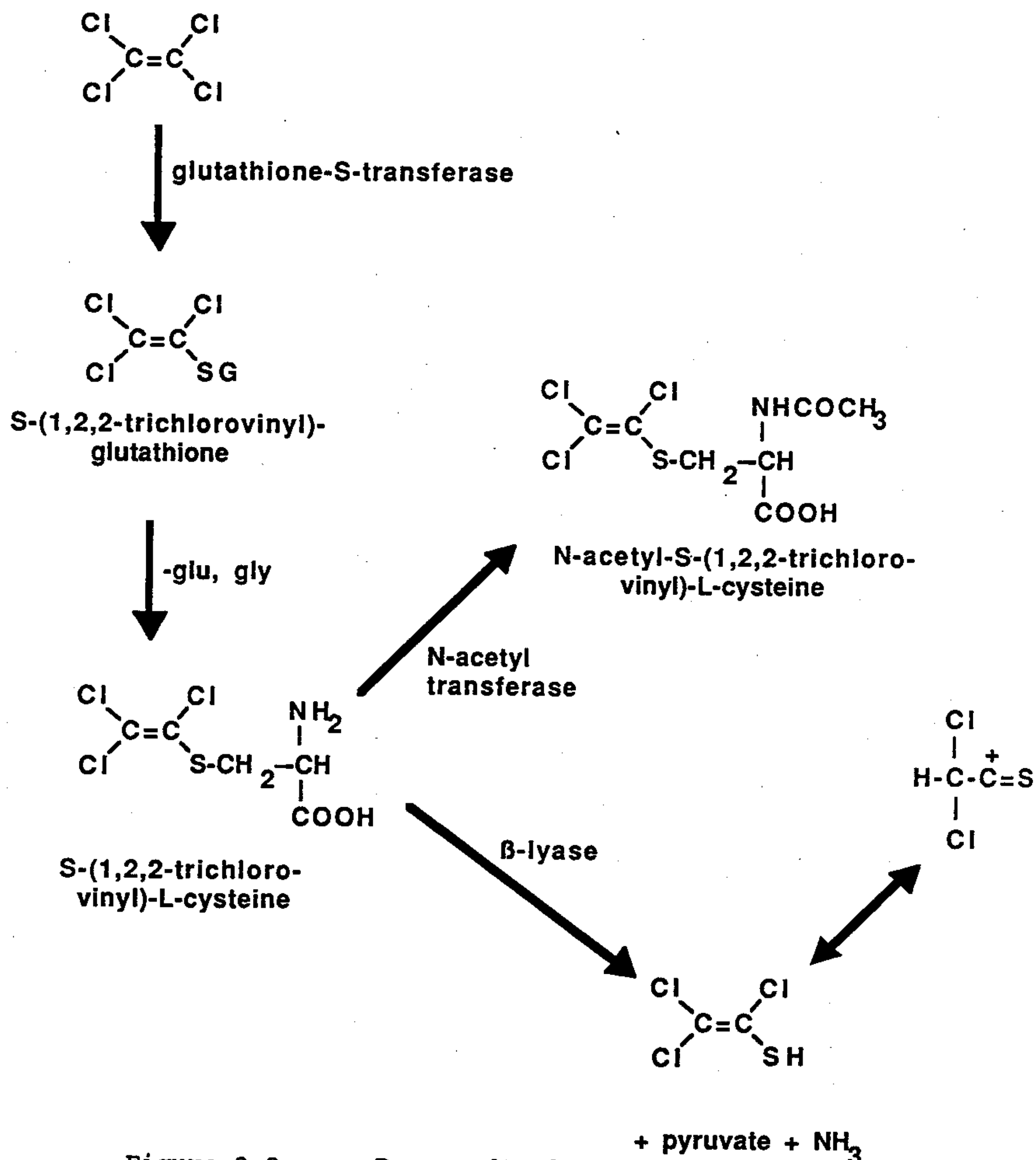

Figure 2-2 Proposed glutathione conjugation metabolic pathway for tetrachloroethylene (adapted from Green et al., 1990)

TABLE 2-3. PCE METABOLITE PRODUCTION IN RODENTS

Metabolite	Species (no. of animals)	Concentration or dose	Route of exposure	Duration of exposure	Reference
Trichloroacetic acid	Mouse (5)	1.3 mg/g	Inhalation	2 h	Yllner, 1961
	Rat (6)	1000 mg/kg	Oral	Single exposure	Daniel, 1963
	Rat (NA)	5 mg/L	Inhalation	5 h/d for 3 d	Dimitrieva, 1967
	Rat (8)	200 ppm	Inhalation	8 h	Ikeda and Ohtsuji, 1972
	Rat (7)	2.78 mmol/kg	Intraperitoneal	Single exposure	Ikeda and Ohtsuji, 1972
	Mouse (1)	200 ppm	Inhalation	8 h	Ikeda and Ohtsuji, 1972
	Mouse (2)	2.78 mmol/kg	Intraperitoneal	Single exposure	Ikeda and Ohtsuji, 1972
	Rat (4)	0.75 mL/kg	Gavage	Single exposure	Moslen et al., 1977
	Mouse (4 to 24 per dose level)	20 to 2000 mg/kg	Gavage	5 d/wk for 6 wk	Buben and O'Flaherty, 1985
	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
Trichloroethanol	Rat (8)	200 ppm	Inhalation	8 h	Ikeda and Ohtsuji, 1972
	Mouse (1)	200 ppm	Inhalation	8 h	Ikeda and Ohtsuji, 1972
	Rat (7)	2.78 mmol/kg	Intraperitoneal	Single exposure	Ikeda and Ohtsuji, 1972
	Mouse (2)	2.78 mmol/kg	Intraperitoneal	Single exposure	Ikeda and Ohtsuji, 1972
	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
Dichloroacetic acid	Mouse (5)	1.3 mg/g	Inhalation	2 h	Yllner, 1961
	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985

TABLE 2-3. (Continued)

Metabolite	Species (no. of animals)	Concentration or dose	Route of exposure	Duration of exposure	Reference
Oxalic acid	Mouse (5)	1.3 mg/g	Inhalation	2 h	Yllner, 1961
	Rat (NA)	5 mg/L	Inhalation	5 h/d for 3 d	Dimitrieva, 1967
	Rat (3)	10 or 600 ppm	Inhalation	6 h	Pegg et al., 1979
	Rat (3)	1 or 500 mg/kg	Gavage	Single exposure	Pegg et al., 1979
	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
N-trichloroacetyl-aminoethanol	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
N-oxalyl-aminoethanol	Mouse (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
	Rat (NA)	800 mg/kg	Oral	Single exposure	Dekant et al., 1985
S-(1,2,2-trichloro-vinyl)glutathione	Rat (3)	1500 mg/kg	Gavage	42 d	Green et al., 1990
N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine	Rat (3)	1500 mg/kg	Gavage	42 d	Green et al., 1990
	Rat (10)	400 ppm	Inhalation	6 hr/d, 28 d	Green et al., 1990
	Mouse (10)	400 ppm	Inhalation	6 hr/d, 28 d	Green et al., 1990

TABLE 2-3. (Continued)

Metabolite	Species (no. of animals)	Concentration or dose	Route of exposure	Duration of exposure	Reference
Carbon dioxide	Rat (3)	10 or 600 ppm	Inhalation	6 h	Pegg et al., 1979
	Rat (3)	1 or 500 mg/kg	Gavage	Single exposure	Pegg et al., 1979
	Mouse (3)	10 or 600 ppm	Inhalation	6 h	Schumann et al., 1980
	Mouse (3)	1 or 500 mg/kg	Gavage	Single exposure	Schumann et al., 1980
Ethylene glycol	Rat (NA)	5 mg/L	Inhalation	5 h/d for 3 d	Dimitrieva, 1967
Chloride	Rat (6)	1000 mg/kg	Oral	Single exposure	Daniel, 1963

TABLE 2-4. PCE METABOLITE PRODUCTION IN HUMANS

Metabolite	No. of individuals	Concentration or dose	Route of exposure	Duration of exposure	Reference
Trichloroacetic acid	85	10 to 400 ppm	Inhalation	8 h/d, 6 d/wk	Ikeda et al., 1972
	5	390 mg/L	Inhalation	6 h (single exposure)	Bolanowska and Golacka, 1972
	4	20 to 70 ppm	Inhalation	Daily (intermittent)	Ikeda and Ohtsuji, 1972
	66	200 to 400 ppm	Inhalation	Daily (intermittent)	Ikeda and Ohtsuji, 1972
	24	100 to 200 ppm	Inhalation	1 to 8 h	Fernandez et al., 1976
	6	70 or 140 ppm	Inhalation	4 h (single exposure)	Monster and Houtkooper, 1979
	5	7 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
	9	47 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
	9	53 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
	1	8 to 10 mL	Oral	Single exposure	Koppel et al., 1985
Trichloroethanol	85	10 to 400 ppm	Inhalation	8 h/d, 6 d/wk	Ikeda et al., 1972
	4	20 to 70 ppm	Inhalation	Daily (intermittent)	Ikeda and Ohtsuji, 1972
	66	200 to 400 ppm	Inhalation	Daily (intermittent)	Ikeda and Ohtsuji, 1972

TABLE 2-4. (Continued)

Metabolite	No.of individuals	Concentration or dose	Route of exposure	Duration of exposure	Reference
	24	100 to 200 ppm	Inhalation	Daily (intermittent)	Ikeda and Ohtsuji, 1972
	6	70 or 140 ppm	Inhalation	4 h (single exposure)	Monster and Houtkooper, 1979
	5	7 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
	9	47 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
	9	53 ppm[a]	Inhalation	37 to 54 h/wk	Monster et al., 1983
Thioether derivative	6	15 to 50 ppm	Inhalation	8 h/d, 5 d/wk	Lafuente and Mallol, 1986

[a]Values represent the median of the time-weighted-average exposures for individuals.

coupled with problems associated with accurate and complete identification of metabolites, and the fact that the bulk of an absorbed human dose has not been accounted for indicates that not all human metabolites of PCE have been identified. Based on present information, humans and rodents appear to metabolize PCE in qualitatively similar ways.

Green and associates have studied the role of the glutathione conjugation pathway in the metabolism of PCE, as shown in Figure 2-2 (Green et al., 1990). Male Fischer 344 rats (160-190 g) were administered 1500 mg/kg b.w. ^{14}C-PCE by gavage each day for 42 days. Bile collected for the 24 hr period following the final dose of PCE contained a radioactive constituent identified as S-(1,2,2-trichlorovinyl)glutathione through a combination of thin-layer and gas chromatography and mass spectroscopy. Pooled urine from groups of three gavaged rats collected on days 1, 17, and 42 were all found to contain N-acetyl-S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC-NAc). TCVC-NAc concentrations for the three days samples were 23.0, 41.1, and 32.7 μg/ml. Pooled urine samples from groups of five male or female Fischer 344 rats or male or female B6C3F1 mice also all had measurable concentrations of the metabolite TCVC-NAc on the three days tested (days 1, 7, and 14). Concentrations of this metabolite were higher in the urine of rats (0.55 to 2.04 μg/ml) compared with urine from similarly exposed mice (0 to 0.20 μg/ml).

In vitro studies of the metabolism of TCVC-NAc were conducted (Green et al., 1990). Metabolism by kidney cytosol fractions from mice, rats and humans were compared. Rat cytosol fraction was estimated to have the highest V_{max} and lowest K_m. V_{max} to K_m ratios for human and mice kidney cytosol fractions were similar (0.21-0.24 and 0.20-0.37, respectively). The V_{max}/K_m ratios for rat fraction were much higher (5.88 for males and 2.88 for females). Addition of 0.1 mM aminooxyacetic acid, an inhibitor of beta-lyase, reduced metabolism by 50%.

Similar *in vitro* studies with hepatic fractions showed that PCE metabolism via glutathione conjugation also occurred in rat and mouse liver (Green et al., 1990). Relative rates of hepatic glutathione conjugation estimated from *in vitro* metabolism studies were reported as: rat cytosol (18 pmol/min/mg protein) > rat microsomal fraction (6.4 pmol/min/mg) > mouse cytosol (3.4 pmol/min/mg) > human cytosol or microsomes. Activity in human liver fractions was reported to be below the limit of detection.

Relative formation of the metabolites trichloroacetic acid (TCA) and TCVC-NAc in male Fischer 344 rats (three animals per group) exposed to 10, 100, or 1000 ppm PCE for 6 hr was also studied (Green et al., 1990). Urine collected over the following 18 hr period showed that TCA was a more significant metabolite over the range of exposures tested. From the data shown (presented graphically only), TCA was at least 10-fold higher than TCVC-NAc following exposure to 100 ppm PCE (approximately 45 vs. 2 μg/ml), and 4-fold higher following exposure to 1000 ppm PCE (approximately 80 vs. 20 μg/ml). The relative rate of TCVC-NAc formation relative to PCE exposure increased at 1000 ppm, whereas the relative rate of TCA formation was greatest at lower concentrations.

Perchloroethylene is eliminated from the body by two major processes: metabolism followed by excretion of urinary or fecal metabolites and pulmonary elimination of unchanged PCE. Although some PCE may be eliminated through the skin, preliminary measurements indicate that this is a minor route in humans (Bolanowska and Golacka, 1972). Except for the Schumann and associates (1980) study, most of the PCE systemically absorbed under experimental conditions (e.g., in mice, rats, and humans) was eliminated unchanged in expired air, so that metabolic degradation and subsequent elimination appear to account for less than 50% of absorbed PCE. Experimental data reviewed below indicate that PCE metabolism is dose-dependent and saturable, and that the amount of PCE metabolized appears to be species-dependent as well.

When the production of urinary metabolites was measured in mice exposed for two hours to approximately 2000 ppm (reported as 1.3 mg/g of PCE in a 2.7 liter chamber), Yllner found that only 2% of an inhaled dose was excreted by this route (Yllner, 1961). Seventy percent of the parent compound was recovered in expired air. Pegg and colleagues (1979) compared the metabolism of PCE in Sprague-Dawley rats at different doses and routes of exposure. The PCE was administered by gavage (1 or 500 mg/kg) or by inhalation (10 or 600 ppm). The primary route of elimination of PCE occurred through the lungs as the unmetabolized parent compound. Urinary excretion of metabolites accounted for the majority of the remaining PCE. The percentage of PCE metabolized was dose dependent: elimination of unmetabolized PCE in expired air following oral exposure increased from 72% after 1 mg/kg to 90% after a dose of 500 mg/kg. There was a corresponding decrease in metabolites from 28 to 10%. An analogous pattern was seen after inhalation exposure. At 10 ppm, 68% of the dose was eliminated unchanged through the lungs and 32% was recovered as metabolites. Treatment at 600 ppm caused an increase in pulmonary elimination to 88% of the dose with a concomitant decrease in metabolites to 12%. Thus, it is likely that exposure to concentrations below 10 ppm would result in a decrease in the pulmonary elimination of PCE and in an increase in relative metabolism of PCE. Pulmonary elimination of PCE was linear and had a half-life of approximately 7 hours. The half-life was independent of dose or route of administration. The half-life of PCE in blood was 6 and 7 hours, after oral and inhalation exposure, respectively.

The pharmacokinetics of PCE in Sprague-Dawley rats and B6C3F1 mice were studied by Schumann and co-workers (1980). Rats were given a single oral dose of 500 mg/kg PCE or were exposed to 10 ppm ^{14}C-PCE by inhalation. The authors reported that at 10 ppm the major route of elimination was excretion of unmetabolized PCE in expired air, although supporting data were not provided. Some PCE was metabolized, and metabolites were recovered in the urine. After an oral dose of 500 mg/kg of labeled PCE, radioactivity was measured in the expired air, urine, feces, and carcass. The relative importance of each route in the elimination of PCE was not discussed.

The elimination of PCE by B6C3F1 mice differed, depending on the dose and possibly on the route of administration from that found in Sprague-Dawley rats. Metabolism, with urinary excretion of PCE, was the primary route of elimination after inhalation exposure at 10 ppm; 62.5% of the dose was

recovered as urinary metabolites and only 12% was excreted through the lungs. Eighty-three percent of a single oral dose (500 mg/kg) was eliminated through the lungs, while 10.3% appeared as urinary metabolites (Schumann et al., 1980).

An additional study (reported as an abstract only) of the fate of PCE in rats and mice following oral administration has been reported (Dekant et al., 1985). Female rats and mice (strains not specified) were given a single oral dose of 800 mg/kg ^{14}C-PCE. The main route of elimination was reported to be exhalation of unmetabolized PCE within 72 hours: 94% for rats and 91% for mice. A lesser amount of urinary (1% for rats, 8% for mice) and fecal (2% for rats, 0.5% for mice) metabolites were excreted over the same period. Using a combination of HPLC separation, chemical derivitization, and GC/MS analysis from pooled 72-hour urine samples, 7 conjugated and unconjugated metabolites were found. These were (as fraction of radioactivity): trichloroacetic acid (58%), trichloroethanol (8%), N-trichloroacetyl-aminoethanol (6%), N-oxalyl-aminoethanol (6%), dichloroacetic acid (4%), and oxalic acid (3%). It was not clear whether analyses were done from pooled samples from one or both experimental species.

The fate of PCE in Sprague-Dawley rats fed PCE in their drinking water was reported by Frantz and Watanabe (1983). Animals were given PCE in a saturated solution over a 12-hour period, resulting in an average dose of 8.1 mg/kg. Treatment was followed by a 72-hour observation period prior to sacrifice. Most of the PCE (87.9%) was eliminated unmetabolized via the lungs. Although urinary excretion was the second largest route of elimination, the amount metabolized was relatively small (7.2%). The half-life of pulmonary elimination was 7.1 hours. This is nearly identical to the value determined by Pegg and colleagues (1979) after oral doses of PCE were given to rats.

Mitoma and co-workers studied the metabolic disposition of PCE in Osborne-Mendel rats and B6C3F1 mice (Mitoma et al., 1985). However, the relatively short follow-up period of 48 hours used in this study tends to underestimate the quantity of metabolites excreted. Substantial differences between species in the amount of PCE eliminated unchanged in expired air were noted. There were also differences in the percentage of dose metabolized. Rats eliminated 79% through their lungs and metabolized 5% of a 1000 mg/kg dose. Pulmonary elimination by mice (of a 900 mg/kg dose) was 57.5%, while 22% was metabolized. The actual amount of PCE metabolized (measured as mmol/kg) also differed by species. Mice metabolized approximately four times as much of the total dose of PCE as rats. However, in both species as the dose was quadrupled, the amount metabolized only increased about 2.5-fold. These data suggest that metabolism approaches saturation at high doses in both species. Conversely, at low concentrations the proportion of PCE metabolized increases.

Buben and O'Flaherty also demonstrated that oxidative metabolism of PCE in Swiss mice decreases with increasing dose and appears to be a saturable process (Buben and O'Flaherty, 1985). Animals received 0, 20, 100, 200, 500, 1000, 1500, or 2000 mg/kg-day of PCE by gavage and were followed for 72 hours post exposure. Trichloroacetic acid was measured as an index of

PCE metabolite production; the estimated maximum rate of urinary metabolite excretion was 136 mg/kg-day. As the dose increased, the percentage of the dose metabolized decreased. At the lowest doses, approximately 25% of the PCE was metabolized. This amount decreased to 5% at the highest doses. Tables 2-5 and 2-6 summarize data on the metabolism of PCE in rats and mice, respectively. Table 2-7 lists some pharmacologic constants of PCE in rodents.

The data reviewed above indicate that metabolism of PCE in both rats and mice displays saturable kinetics. The percentage of dose metabolized decreases as the dose is increased until the amount metabolized is no longer a function of the dose (a zero-order reaction). The extent of metabolism of PCE appears species-dependent. Rats consistently metabolize a relatively smaller amount of PCE than mice regardless of the route of administration.

Measurements of PCE metabolism in humans have many uncertainties associated with them. Total trichlorinated metabolites in urine have usually been determined colorimetrically and therefore are difficult to evaluate quantitatively. By this method, production of chlorinated metabolites is equated with the amount metabolized. This approach does not account for the possibility that some metabolites may be produced that are not chlorinated, such as CO_2, the thioether, and oxalic acid.

Studies of PCE metabolism in humans have consistently considered TCA to be a principal metabolite. However, a major purpose of the studies has been to ascertain if PCE exposure in the workplace can be quantified by measurements of trichloro compounds in urine. Thus, the studies do not reflect an attempt to measure all possible metabolites of PCE.

Trichloroacetic acid production has also been measured to estimate the extent of metabolism of PCE and to characterize the kinetics of urinary elimination. Volunteers exposed to 87 ppm of PCE for 3 hours excreted about 0.40 mg/h of TCA (Ogata et al., 1971). Monster and colleagues analyzed the TCA content of blood and urine from volunteers exposed to 72 or 144 ppm of PCE for 4 hours (Monster et al., 1979). The mean production of TCA (over a 70-hour period) was 6.0 and 11.0 mg, respectively. Blood levels of TCA increased over the course of the experiment and continued to rise until about 20 hours after the end of exposure. The TCA was eliminated from blood by first-order processes; the half-life of elimination was 65 to 90 hours. Urinary elimination of TCA followed the disappearance of TCA from blood. Fernandez and associates measured excretion of TCA from individuals exposed to 150 ppm for 8 hours (Fernandez et al., 1976). Over a 72-hour collection period, the average amount of TCA produced was 25 mg. This is equivalent to about 0.34 mg of TCA per hour. Ikeda (1977) and Ikeda and Imamura (1973) qualitatively measured the half-life of urinary trichloro compounds and calculated the mean half-life to be 144 hours (range of 123 to 190 hours). The lengthy half-life may result from the continued formation of metabolites from PCE mobilized from tissues where it has accumulated.

Measurements of TCA production suggest that human metabolism of PCE via this pathway represents only a small portion of the absorbed dose. For example, the values published by Monster and associates (6.0 and 11.0 mg TCA after

TABLE 2-5. METABOLISM AND DISPOSITION OF TETRACHLOROETHYLENE IN RATS

Route[b] (n)	Concentration or dose[c]	Exposure duration	Post exposure period	Percent metabolized[a]		Percent eliminated unchanged via lungs	Reference
				Urinary metabolites	Total metabolites[d]		
Oral (3)	1 mg/kg	Single exposure	72 h	16.5	28.5	71.5	Pegg et al., 1979
Inhalation (3)	10 ppm (12.2 mg/kg)	6 h	72 h	18.7	32	68	Pegg et al., 1979
Oral (in drinking water) (4)	8 mg/kg	12 h	72 h	7.2	12	88	Frantz and Watanabe, 1983
Inhalation (3)	600 ppm (732 mg/kg)	6 h	72 h	6	12	88	Pegg et al., 1979
Oral (3)	500 mg/kg	Single exposure	72 h	4.6	10	90	Pegg et al., 1979
Oral (4)	1000 mg/kg	5 d/wk; 4 wk	42 h	2.4	5	79	Mitoma et al., 1985

[a] These values represent the percent of recovered dose that was metabolized.
[b] n = number of treated animals.
[c] For inhalation exposures, the dose in mg/kg was calculated assuming a breathing rate of 0.18 m3/d, and an average weight of 250 g per animal.
[d] These values represent metabolite recovery from all routes of excretion.

TABLE 2-6. METABOLISM AND DISPOSITION OF TETRACHLOROETHYLENE IN MICE

Route (n)[a]	Concentration or dose[b]	Exposure duration	Post exposure period	Percent metabolized: Urinary metabolites[c]	Percent metabolized: Total metabolites[d]	Percent eliminated unchanged via lungs	Reference
Oral (12-15)	20 mg/kg	5 d/wk; 6 wk	0 h	25	NA[e]	NA	Buben and O'Flaherty, 1985
Inhalation (3)	10 ppm (29 mg/kg)	6 hr	72 h	62.5	88.0	12	Schumann et al., 1980
Inhalation (N/A)	1.3 mg/g	2 h	96 h	20	NA	70	Yllner, 1961
Oral (12-15)	200 mg/kg	5 d/wk; 6 wk	0 h	15.5	NA	NA	Buben and O'Flaherty, 1985
Oral (12-15)	500 mg/kg	5 d/wk; 6 wk	0 h	12.6	NA	NA	Buben and O'Flaherty, 1985
Oral (3)	500 mg/kg	Single exposure	72 h	10.3	17.4	82.6	Schumann et al., 1980
Oral (4)	900 mg/kg	5 d/wk; 4 wk	48 h	14.4	22.2	57.5	Mitoma et al., 1985
Oral (12-15)	1000 mg/kg	5 d/wk; 6 wk	0 h	8.1	NA	NA	Buben and O'Flaherty, 1985

TABLE 2-6. (Continued)

Route (n)[a]	Concentration or dose[b]	Exposure duration	Post exposure period	Percent metabolized: Urinary metabolites[c]	Total metabolites[d]	Percent eliminated unchanged via lungs	Reference
Oral (4-6)	2000 mg/kg	5 d/wk; 6 wk	0 h	5.1	NA	NA	Buben and O'Flaherty, 1985

[a]n = number of treated animals.
[b]For inhalation exposure(s), the dose in mg/kg was calculated assuming a breathing rate of 0.0345 m^3/d, and an average weight of 20 g per animal.
[c]These values represent recovery of urinary metabolites only.
[d]These values represent recovery from all routes of exposure, with the exception of expired tetrachloroethylene.
[e]NA: not available.

TABLE 2-7. PHARMACOLOGIC CONSTANTS OF TETRACHLOROETHYLENE IN RODENTS

Parameter	Value	Species (weight)	Route	Concentration or dose	Exposure duration	Reference
Blood elimination $t_{1/2}$[a]	6 h	Rat (250 g)	Oral	500 mg/kg	Single exposure	Pegg et al., 1979
Blood elimination $t_{1/2}$[a]	7 h	Rat (250 g)	Inhalation	600 ppm	6 h	Pegg et al., 1979
Pulmonary elimination $t_{1/2}$[a]	7 h (approx.)	Rat (250 g)	Oral and inhalation	1 or 500 mg/kg and 10 or 600 ppm	Single exposure 6 h	Pegg et al., 1979
Blood k_{eq} (elimination constant)	0.12 h-1	Rat (250 g)	Oral	500 mg/kg	Single exposure	Pegg et al., 1979
Blood k_{eq} (elimination constant)	0.10 h-1	Rat (250 g)	Inhalation	600 ppm	6 h	Pegg et al., 1979
Pulmonary k_{eq} (elimination constant)	0.10 h-1	Rat (250 g)	Oral and inhalation	1 or 500 mg/kg and 10 or 600 ppm	Single exposure	Pegg et al., 1979
V_{max} (maximum amount of urinary metabolites formed and excreted in 24 h)	136 mg/kg·d	Mouse	Oral	20 to 2000 mg/kg	5 d/wk; 6 wk	Buben and O'Flaherty, 1985
V_{max} (maximal velocity metabolic elimination)	/7 mmol/h·kg	Rat (200 to 250 g)	Inhalation	1000 ppm (initial concentration)	12 h	Filser and Bolt, 1979
K_{eq} (equilibrium constant between gas phase and animal tissue)	105	Rat 200 to 250 g)	Inhalation	1000 ppm	12 h	Filser and Bolt, 1979

TABLE 2-7. (Continued)

Parameter	Value	Species (weight)	Route	Concentration or dose	Exposure duration	Reference
Body burden	5.9 mg/kg	Rat (250 g)	Inhalation	10 ppm	6 h	Pegg et al., 1979
Body burden	310 mg/kg	Rat (250 g)	Inhalation	600 ppm	6 h	Pegg et al., 1979
Body burden	16.5 mg/kg	Mouse	Inhalation	10 ppm	6 h	Schumann et al., 1980

[a] $t_{1/2}$ is the half-time of elimination.

a 4-hour exposure to 72 or 144 ppm PCE) respectively, represent only about 1 to 2% of the estimated absorbed dose (Monster et al., 1979). Ogata and co-workers reported that TCA excretion was equivalent to 1.8% of the retained PCE with total excretion of organic chloride accounting for only 2.8% of the retained dose (Ogata et al., 1971). These data agree with those of Fernandez and colleagues, who estimated that 1350 mg of PCE would be absorbed after exposure to 150 ppm PCE for 8 hours (Fernandez et al., (1976). Ikeda and co-workers and Ohtsuki and associates have estimated that only about 2% of an 8-hour exposure to 50 ppm PCE would be metabolized and that 38% would be eliminated through the lungs unchanged by the end of the exposure period; the remaining 60% of the inhaled dose was hypothesized to be stored in the body and available for subsequent metabolism and/or pulmonary elimination (Ikeda et al., 1972; Ohtsuki et al., 1983). Ohtsuki and colleagues found that human urinary metabolite production did not appear to be linearly related to exposure concentration. A graph of total trichloro compounds (from urine) plotted against PCE concentrations in air showed that metabolite production appeared to be dose-dependent, leveling off at approximately 400 ppm PCE (8-hour exposure). This suggested metabolic saturation; however, no statistical test of departure from linearity was performed in this study and a questionable nonlinear model was assumed.

Bolanowska and Golacka proposed that at steady state approximately 62% of respired PCE remains in the body, (based on their measurements of inspired and exhaled PCE) (Bolanowska and Golacka, 1972). Of the retained dose an estimated 25% is later exhaled. Thus, 75% of the retained dose, or 47% of the respired dose, is available for metabolism. At steady state, absorbed dose equals metabolized dose. However, since the steady state was probably not attained, less than 47% of the respired dose was metabolized, with the balance representing the quantity of PCE accumulating in adipose tissue. These calculations are much higher than those obtained by other studies of PCE uptake and metabolism in humans (Ogata et al., 1971; Ikeda et al., 1972; Fernandez et al., 1976; Ikeda, 1977; Monster et al., 1979; Ohtsuki et al., 1983). However, the difference in large part may be due to Bolanowska and Golacka's consideration of total metabolism and not focusing solely on trichloro compounds as other investigators have done. Ogata and co-workers only analyzed for the excretion of trichloroacetic acid and an unidentified organic chlorine compound (Ogata et al., 1971). Ikeda and associates only measured the production of trichloro compounds and creatinine (Ikeda et al., 1972). Fernandez and co-workers measured only the excretion of TCA (Fernandez et al., 1976). Monster and fellow researchers analyzed urine for TCA and trichloroethanol (Monster et al., 1976). Ohtsuki and colleagues measured only total trichloro compounds in the urine (Ohtsuki et al., 1983). In fact, Bolanowska and Golacka reported that trichloro compound excretion in urine accounted for a few percent of the PCE retained, which is similar to results reported in the other investigations. Consequently, it appears that up to approximately 47% of the respired PCE may be metabolized based on the 1972 Bolanowska and Golacka study, which examined inhaled, retained, and exhaled PCE in humans.

A long period of time is necessary for pulmonary elimination of unmetabolized PCE. Stewart and co-workers have analyzed the pulmonary

excretion of PCE following experimental human exposures and found that it is biphasic (Stewart et al., 1970; Hake and Stewart, 1977). Initially, elimination is rapid but the second phase is prolonged, with a half-life of approximately 65 hours. Monster and co-workers determined that human pulmonary elimination of PCE has three different phases, with half-lives of 12 to 16 hours, 30 to 40 hours, and 55 to 50 hours, respectively (Monster et al., 1979). Bolanowska and Golacka identified four phases for pulmonary excretion of PCE, with half-lives of 0.025 hours, 0.6 hours, 4.8 hours, and 34 hours, respectively (Bolanowska and Golacka, 1978). Fernandez and associates note that humans exposed to 100 ppm for 8 hours required about 2 weeks to eliminate PCE (Fernandez et al., 1976).

3. TOXIC EFFECTS IN ANIMALS

Estimates of human health risks resulting from exposure to a toxic substance are frequently based on an assessment of animal dose-response data because specific human data are often inadequate for this purpose. In this section, animal PCE toxicity studies are reviewed, including data from bioassays conducted to evaluate the carcinogenicity of PCE. Bioassay results are also used as the basis of the quantitative assessment of carcinogenic potency in Section 5. The toxicity of PCE has also been reviewed by IARC (1979), Reichert (1983), WHO (1984), and the U.S. EPA (1980, 1982, 1984b, 1985a, and 1985b).

The discussion of PCE toxicity begins with analyses of toxic effects to major body organs and systems. For these effects, Appendix A presents a review and summary of dose-response information for different routes and periods of exposure. The information in Appendix A would be relevant to the development of safety limits for PCE exposure in terms of preventing acute, subchronic, and noncarcinogenic chronic toxicological end points in the absence of adequate human-toxicity data. Studies dealing with the teratogenicity of PCE are examined. The section reviews the mutagenic potential of PCE and its metabolites and summarizes the results of animal carcinogenicity bioassays.

TOXIC EFFECTS ON ORGANS AND SYSTEMS

Hepatic Toxicity

Cornish and fellow researchers administered 0.3 to 2.0 mL/kg (0.33 to 4.95 mg/kg) of PCE intraperitoneally (IP) to rats. Liver damage was measured by an increase in serum glutamic oxalacetic transaminase levels (SGOT) and was observed at all doses (Cornish et al., 1973). Ogata and associates observed a decrease in the adenosine triphosphate (ATP) content of liver as well as an increase in the content of lipids and triglycerides after mice were exposed to 800 ppm for 3 hours (Ogata et al., 1968). Elevation of serum glutamic pyruvate transaminase (SGPT) levels in mice was elicited by exposure to 3700 ppm for 9 to 12 hours, as well as by IP administration of 3900 mg/kg (Gehring, 1968). Klaassen and Plaa also measured increased levels of SGPT in mice that received single intraperitoneal doses of 2.9 mL/kg (Klaassen and Plaa et al., 1966). These animals had enlarged hepatocytes and slight liver necrosis. A single intraperitoneal dose of 1.23 mL/kg elevated SGPT levels in dogs.

Schumann and associates dosed mice and rats orally with 100, 200, 500, or 1000 mg/kg of PCE daily for 11 days (Schumann et al., 1980). In mice, all dose levels produced hepatocellular swelling, a significant increase in absolute liver weight ($p<0.05$), and a significant decrease in hepatic DNA content ($p<0.05$). All of these changes are indicative of hypertrophy (the enlargement of an organ due to an increase in size of its constituent cells). Mice that received 100 mg/kg of PCE displayed an increase in hepatic DNA synthesis. In

contrast to the pathologies that developed in mice, only the highest PCE dose, 1000 mg/kg, induced hepatic toxicity in rats. These animals had a statistically significant increase in relative liver weight ($p<0.05$).

Lundberg and associates exposed female Sprague-Dawley rats for four hours to PCE at concentrations between 1/32 and 1/2 of the four-hour LC_{50} (50.4 g/m^3 or 7320 ppm), or a range of approximately 1.5 to 25 g/m3 (230 to 3660 ppm) (Lundberg et al., 1986). No significant increases in serum sorbitol dehydrogenase or histological alterations were found in liver sections from rats exposed to 50% of the LC_{50} (25 g/m^3 or 3660 ppm). Similarly, no adverse effects on serum SDH or liver histology were found in rats administered PCE IP at 1/8 to 1/2 of the single-dose LD_{50} (5060 mg/kg b.w.).

Odum and associates exposed male and female Fischer 344 rats and B6C3F1 mice of both sexes to 400 ppm PCE by inhalation, 6 hr/day for 14, 21, or 28 days or to 200 ppm for 28 days (Odum et al., 1988). Increased numbers of peroxisomes and increased peroxisomal cyanide-insensitive palmitoyl CoA oxidation were reported in mouse liver, but not in rat liver or in kidneys of rats or mice. Peak blood trichloracetic acid concentrations in mice exposed for a single 6-hr period to 400 ppm PCE were 13 times higher than observed in similarly exposed rats. It was estimated that mice formed more than 6 times more TCA than rats. A related study was conducted by Elcombe (1985). This work examined the species differences in peroxisome proliferation and trichloroacetic acid formation following trichloroethylene exposure.

Carpenter studied the subchronic and chronic inhalation toxicity of PCE in rats (Carpenter et al., 1937). Although no effects were observed in animals treated with 70 ppm PCE (8 hours/day, 5 days/week for 7 months), rats that received 150 exposures of 230 ppm had less glycogen storage than unexposed animals. Exposure to 470 ppm PCE (150 days) caused liver congestion and swelling. Rowe and co-workers exposed guinea pigs to 100 ppm 7 h/d over a period of 17 to 185 days (Rowe et al., 1952). No effects were apparent in animals that received 13 exposures in 17 days. However, when the number of exposures was increased to 132 over 185 days, females had a significant increase in liver weight ($p = 0.01$) and animals of both sexes exhibited lipid accumulation in the liver.

Kylin and co-workers examined the hepatotoxic effects of PCE administered subchronically to mice (Kylin et al., 1965). Groups of twenty albino mice (strain not specified) were exposed to 200 ppm PCE vapors for 4 hours a day, six days per week, for one, two, four, or eight weeks. No overt toxic effects were observed. Liver histopathology was graded on a scale of 0 to 4, with 4 representing massive fatty infiltration involving at least half of the lobules. At least 95% of exposed animals were found to have grade 2 peripheral fatty degeneration upon one week or more of exposure. An exposure duration-dependent increase in incidence of grade 4 fatty degeneration from 25 to 80% was noted for exposure periods of 1 to 4 weeks. Cirrhosis or necrosis were not observed.

Buben and O'Flaherty treated mice by gavage with PCE dosages ranging from 20 to 2000 mg/kg PCE, 5 days/week for 6 weeks (Buben and O'Flaherty et al., 1985). Liver weights and liver triglycerides were significantly greater than

those of controls at doses of 100 mg/kg of PCE and above ($p<0.001$). A dose-dependent increase in liver degeneration and karyorrhexis was also observed at PCE doses of 100 mg/kg and greater. Activity of glucose-6-phosphatase (G6P) was inhibited and a significant increase in SGPT occurred at 500, 1000, 1500, and 2000 mg/kg PCE ($p<0.001$). Hepatic DNA content was measured in mice treated with 200 or 1000 mg/kg PCE; animals that received 1000 mg/kg had significantly lower levels of DNA ($p<0.01$).

A bioassay sponsored by the National Toxicology Program (NTP) chronically administered tetrachloroethylene by gavage to mice at a time-weighted average (TWA) dose of 386 to 1972 mg/kg PCE and to rats at a TWA dose of 471 to 949 mg/kg PCE (NCI, 1977). There was no reported increase in any noncarcinogenic hepatic lesions in either mice or female rats. In male rats, the incidence of fatty metamorphosis (?) (2 of 39 control animals; 4 of 49 low dose animals, and 7 of 49 high dose animals) and bile duct hyperplasia (0 of 40 control animals; 1 of 50 low dose animals, and 6 of 50 high dose animals) were both elevated. The statistical significance of these data was not evaluated.

In an NTP-sponsored study of the effects of inhaled PCE on mice and rats, animals were exposed to PCE by inhalation 6 hours/day, 5 days/week for 103 weeks (NTP, 1986). Mice were exposed to 100 or 200 ppm PCE and rats to 200 or 400 ppm. Male and female mice of both exposure groups developed liver degeneration and necrosis. Development of these pathologies appeared to be dose related. The incidence of liver degeneration in male mice was as follows: controls, 2/49 animals; low dose, 8/49; and high dose, 14/50. The observed incidence of liver degeneration in female mice in the control group was 1/49 animals; in the low dose group, 2/50; and in the high dose group, 13/50. The number of male mice exhibiting necrosis in the treatment groups increased with increasing exposure concentrations (i.e., controls, 1/49 animals; low dose, 6/49; and high dose, 15/50). For female mice the incidence of necrosis was: controls, 3/48 animals; low dose, 5/50; and high dose, 9/50. Treated male mice (but not females) had a greater incidence of hepatic nuclear inclusion than controls (i.e., for controls, 2/49 animals; low dose, 5/49; and high dose, 9/50). The statistical significance of these data was not evaluated. Under the conditions of this study, rats did not develop hepatic lesions in response to exposure to PCE.

Hepatotoxity of PCE was investigated in *in vitro* and *in vivo* experiments by Stacey (1988). Hepatocytes isolated from male Sprague-Dawley-rats were incubated with PCE, and hepatotoxicity was monitored as a function of leakage of intracellular potassium ion (K+), lactate-dehydrogenase (LDH), and alanine-aminotransferase (ALT). Male Sprague-Dawley-rats were administered intraperitoneally with 15mmol/kg PCE. Hepatotoxicity was determined from plasma sorbitol-dehydrogenase (SDH) and ALT. Exposure to PCE alone did not cause any significant adverse effects on parameters tested, though signs of hepatotoxicity were observed *in vitro* and *in vivo* when PCE was coadministered with other solvents.

Dahlstrom-King and associates examined the *in vitro* cytotoxicity of PCE towards suspended hepatocytes isolated from male Sprague-Dawley rats (Dahlstrom-King et al., 1990). Alanine aminotransferase (ALT) release was monitored as a indicator of hepatocyte membrane integrity. ALT release

increased both as a function of exposure duration (over a 180 minute interval) and PCE concentration (5, 7.5, and 10 mM). The addition of of SKF525A decreased the cytotoxicity of PCE.

Renal Toxicity

Klaassen and Plaa administered PCE to dogs intraperitoneally and measured excretion of phenolsulfonephthalein (PSP), a substance used to test for renal function (Klaassen and Plaa et al., 1967). Control dogs excreted 56% of the PSP within 30 minutes; excretion of less than 39% was considered to be an indicator of kidney dysfunction. Kidney function was significantly affected after a single IP dose of 1.4 mL/kg PCE (statistical significance was not given). Plaa and Larson reported that all mice given a single IP dose of 2.5 mL/kg PCE exhibited swelling of the proximal convoluted tubule, and one animal (of six treated) developed necrosis of the proximal convoluted tubule (Plaa and Larson et al., 1965). Mice that received a single dose of 2.5 or 5.0 mL/kg PCE excreted protein in their urine. The statistical significance of these responses was not reported. Carpenter found that rats given 230 ppm PCE (8 hours/day, 5 days/week) for 21 days developed swelling and congestion of the kidneys (Carpenter et al., 1937). This response was exacerbated when the concentration was increased to 470 ppm PCE. Subchronic exposure of mice and guinea pigs to 400 ppm PCE, 7 hours/day for 169 times in 236 days caused swelling of the tubular epithelium along with an increase in kidney weight (Rowe et al., 1952).

Goldsworthy and coworkers examined the possible role of alpha-2-globulin (a2u) in protein droplet accumulation and renal cell proliferation following PCE exposure. Female Fischer 344 rats were administered PCE by gavage at a dose of 1000 mg/kg body weight for 10 days. The two parameters studied were not altered significantly compared with controls. Immunohistochemical staining showed a marked correlation between a2u presence and protein droplet formation in proximal convoluted epithelial cells (Goldsworthy et al., 1988a).

Green and associates administered 1500 mg/kg b.w. PCE (99.9% purity) by gavage for 42 days to F344 rats (Green et al., 1990). An accumulation of protein droplets containing alpha-2u-globulin was observed in P2 segments of renal proximal tubules. Other signs of renal toxicity noted were significant increases in urine volume, glucose, alkaline phosphatase, N-acetyl-beta-D-glucosaminidase concentrations relative to controls. No renal protein accumulation was noted in rats administered 400 ppm PCE via inhalation for 28 days. Microscopic evidence of proximal tubule protein accumulation was observed, however, following exposure to 1000 ppm PCE for 10 days.

The NCI cancer bioassay of PCE documented a high incidence of toxic nephropathy in mice and rats for all dose groups (toxic nephropathy was defined as degenerative changes in the proximal convoluted tubule, fatty degeneration, and necrosis of the tubular epithelium) (NCI, 1977). PCE was administered by gavage. In this study, mice received time-weighted-average (TWA) daily doses of 386 to 1972 mg/kg PCE; toxic nephropathy was observed in 82 to 100% of the animals. Rats received TWA daily doses of 471 to 949 mg/kg

PCE; 58 to 94% of these animals developed toxic nephropathy (see Table A-7 for specific data).

A bioassay sponsored by the NTP documented kidney casts, nephrosis, and tubular cell karyomegaly in mice (animals received 100 or 200 ppm of PCE 6 hours/day, 5 days/week for 103 weeks) (NTP, 1986). Casts occurred more frequently in treated male mice than in controls (incidence in controls, 3/49 animals; low dose, 9/49; and high dose, 15/50). The trend in female mice was not clearly dose related (incidence in controls, 4/48 animals; low dose, 4/49; and high dose, 15/50). Nephrosis developed at a greater incidence in treated female mice (control, 5/48 animals; low dose, 14/49; high dose, 25/50). For male mice, the incidence of nephrosis in the controls was 22/49 animals; low dose, 24/49; and high dose, 28/50. Karyomegaly of tubular cells was treatment-related. The incidence of this pathology in male mice was control, 4/49 animals; low dose, 17/49; high dose, 46/50. In female mice, the incidence of nephrosis was 0/48 animals, 16/49, and 38/50 in the controls, low-dose, and high-dose groups, respectively.

The same study reported a dose-related increase of renal tubular cell karyomegaly in rats of both sexes (NTP, 1986). Low-dose animals received 200 ppm of PCE; high-dose animals received 400 ppm (the exposure regime was the same as listed above for mice). The incidence of karyomegaly in male rats for the corresponding control group was 1/49 animals; low dose, 37/49; and high dose, 47/50. In female rats, the incidence was 0/50 animals, 8/49, and 20/50, respectively. Male rats exhibited a dose-related increase in renal tubular cell hyperplasia (controls, 0/49 animals; low dose, 3/49; and high dose, 5/50). Only one high-dose female rat had renal tubular cell hyperplasia.

Pancreas

Hamada and Peterson studied the effects of PCE on the electrolyte and protein concentration in bile duct-pancreatic fluid (BDPF) (Hamada and Peterson et al., 1977). The actual source of this fluid (bile duct and/or pancreas) is not known. Rats were given a single IP dose of PCE (1.3 mL/kg in corn oil). Animals were then fasted for 24 hours at which time BDPF was collected and analyzed. The PCE caused a significant increase in BDPF flow, a decrease in concentration of protein in the BDPF, and an increase in the concentration of chloride and potassium ($p<0.05$ for all parameters). The mechanism of enhanced BDPF is not known. Although Hamada and Peterson discussed possible mechanisms that may be analogous to secretion or cholinergic stimulation, they concluded that PCE (and other chlorinated aliphatic hydrocarbons) altered BDPF by an unknown mechanism, and that the toxicological significance of the reported effects is not known (Hamada and Peterson et al., 1977).

Eyes, Skin, and Lungs

Perchloroethylene is an eye and skin irritant. Application of PCE to the eye of rabbits caused abrasion of the epithelium and conjunctivitis. PCE was also extremely irritating when applied topically to the skin of rabbits (Duprat et al., 1976). However, Jakobsen and co-workers saw no visible sign of skin

irritation when guinea pigs were exposed to liquid PCE (Jakobsen et al., 1982).

The NCI reported a high incidence of pneumonia in control and exposed animals used in the bioassay of PCE (NCI, 1977). Because of the relatively high incidence of pneumonia observed in control animals, PCE probably did not contribute directly to the infection. In a separate study, chronic inhalation of PCE caused a dose-related incidence of passive congestion of the lungs in mice (NTP, 1986).

Reproductive System

The only indication that PCE has any effect on the reproductive system comes from the work of Rowe and fellow researchers (Rowe et al., 1952). Seven male guinea pigs were exposed to 1600 ppm, 7 hours/day for 8 exposures within 10 days. Microscopic examination of tissues revealed slight degenerative changes in the germinal epithelium of the testes. The implication is unclear since subsequent studies have not confirmed the finding.

Cardiovascular System

Perchloroethylene has been associated with sudden death from cardiac failure (Rowe et al., 1952; Reinhardt et al., 1973). It has been suggested that PCE (as well as a number of other solvents) may sensitize the heart to the effects of endogenously produced epinephrine (Price and Dripps, 1970; Reinhardt et al., 1973). If sensitization occurs, epinephrine-induced stimulation can lead to tachycardia and cardiac failure. This response is apparently precipitated by physical exertion and exposure to high concentrations of this agent.

Kobayashi and associates investigated the action of intravenously administered PCE on cardiac rhythm (Kobayashi et al., 1982). Rabbits were anesthetized with urethane, while cats and dogs were anesthetized with pentobarbital. A mean dose of 10 mg/kg of PCE administered with 0.7 μg/kg of epinephrine produced tachycardia in rabbits (although the most sensitive animals were effected by 5 mg/kg of PCE). Tachycardia also occurred in dogs given a mean dose of 13 mg/kg PCE with 4.2 mg/kg of epinephrine, while doses of 30 to 40 mg/kg PCE decreased left intraventricular pressure. Cats exhibited ventricular arrhythmias after 24 mg/kg of PCE was adminiwered in conjunction with 13 to 14 mg/kg of epinephrine.

In later work, Kobayashi et al. (1989) exposed dogs to PCE for approximately 2 minutes at ambient concentrations of approximately 2000 to 10,000 ppm. PCE decreased left ventricular contractility (dp/dt) in a concentration-dependent relationship over this range. PCE was the most potent of six organic solvents tested. A total of 26 exposures were conducted. Ambient PCE concentration was correlated to changes in peak dp/dt, and the regression line was fitted, resulting in the equation ($r=-0.85$, $p<0.01$):

$$\text{Change peak dp/dt (mmHg/s)} = -906.5 * \text{PCE concentration (\%)} +52.5$$

The threshold for observable effects for changes in left ventricular contractility was reported to be 2000 ppm PCE, whereas the regression equation suggests a threshold of approximately 600 ppm.

Rowe and co-workers speculated that death in some rats exposed to concentrations of 3000 ppm PCE or more in air was caused by cardiac failure; however, it is possible that cardiac failure occurred as a result of extreme CNS depression (Rowe et al., 1952). Reinhardt and colleagues studied the cardiotoxicity of PCE by exposing unanesthetized dogs to 5000 or 10,000 ppm (Reinhardt et al., 1973). Arrhythmias and cardiac failure were not observed and no evidence of sensitization was seen.

The PCE dose levels used in the aforementioned studies are not representative of typical human exposure levels. Furthermore, the early work of Kobayashi and associates utilized anesthetized animals, administered PCE intravenously, and used relatively large amounts of epinephrine, none of which readily facilitates extrapolation of results to humans (Kobayashi et al., 1982). The cardiotoxic potential of PCE requires additional experimental work before any conclusions can be drawn.

Effects of PCE, with hypoxia and/or ethanol, on the conduction system of the isolated perfused rat heart were investigated by recording ECG (Kawakami et al., 1988). With 100 μM PCE, significant PQ prolongation was only observed upon coadministration of hypoxia and ethanol. The authors concluded PCE has both direct and local effects on the heart conduction system, and that hypoxia and ethanol synergistically increase PCE effects on the atrioventricular conduction system.

Immunotoxicity

Kroneld and associates (1987) conducted *in vitro* experiments using isolated human peripheral blood lymphocytes. Lymphocytes were exposed to PCE at concentrations of 0.2, 1.6, 16, 160 μg/l. Significant decreases in ^{3}H-thymidine intake were noted at concentrations of 1.6 μg/l and greater.

Groups of 140 female CD-1 mice were exposed to 0, 25 or 50 ppm PCE for three hours (Aranyi et al., 1986). Challenge by inhaled *Streptococcus zooepidemicus* resulted in significantly increased mortality in animals exposed to 50 ppm PCE relative to controls. Pulmonary bactericidal activity against *Klebsiella pneumoniae* was significantly decreased in mice exposed to 50 ppm PCE. However, the significance of the effects observed is unclear because the mortality observed in the control animals in the 25 ppm experiment was greater than that of PCE-exposed animals in the 50 ppm experiment. Furthermore, exposure to 25 ppm PCE for 3 hours per day for 5 days did not result in changes in *Streptococcus* mortality or pulmonary bactericidal activity.

Miyano and associates (1987) reported PCE caused decreased cell viability in mouse spleen cell cultures. Lipopolysaccharide-stimulated B-lymphocyte transformation and Con A-stimulated T-lymphocyte transformation were also inhibited by PCE.

Central Nervous System

Acute exposure to PCE typically induces CNS depression. Initial depression can progress to loss of consciousness, anesthesia, and respiratory failure with prolonged or massive exposure. Single oral doses of 1623 mg/kg PCE produced reversible CNS effects in cats (Maplestone and Chopra, 1933), while a single dose of 6492 mg/kg caused lethal CNS depression (Lamson et al., 1929). Death from CNS depression resulted from single PCE doses of 4700 mg/kg (rat) and 6492 mg/kg (dog) (Smyth et al., 1969; Lamson et al., 1929). Over a 4-hour period, 2300 ppm PCE caused an impairment of muscular coordination in female rats, which contributed to a loss of 80 percent of avoidance and escape responses. Animals apparently developed some tolerance to PCE, because this effect did not persist when dosing was continued over a 2-week period (Goldberg et al., 1964).

Rats exposed to 6000 ppm PCE lost consciousness within a few minutes; decreasing the concentration to 3000 ppm required several hours to elicit the same effect (Rowe et al., 1952). The NTP study found that exposure of mice to 2917 ppm for 4 hours was lethal to all animals. Rats appear to be less sensitive to PCE, because a 4-hour exposure to 5163 ppm was required to produce 100 percent mortality (NTP, 1986).

Friberg et al. (1953) exposed groups of 15 mice (20 g b.w., strain not identified) to PCE at concentrations of 6800 or 12200 ppm. Degree of narcosis was assesses within four stages: mouse beginning to slide (stage 1), rolling over (stage 2), rolling over three times (stage 3), and complete immobilization (stage 4). At 6800 ppm, stages 1 and 2 were reached within about 1 minute, stage 4 within about 3 minutes. At 12200 ppm, stages 1 and 2 were observed in less than 3 minutes, and stage 4 within 6 minutes.

Korpela (1988) examined the effects of PCE on nerve cell membrane enzyme function in an *in vitro* assay. Synaptosomes isolated from cerebra of Sprague-Dawley rats were incubated with PCE at concentrations of 3, 9, and 30 mM. Acetylcholinesterase (AChE) and adenosine triphosphatase (ATPase) activities were significantly depressed at all three concentrations. The authors concluded this effect was detected at subanesthetic concentrations.

Karlsson and associates (1987) observed decreased DNA concentration in brains of gerbils exposed to PCE on a sub-chronic basis. Young adult Mongolian gerbils (*Meriones ungiculatus*) were continuously exposed for 3 months to 60 ppm PCE via inhalation, followed by a 4 month postexposure period. DNA concentrations were significantly decreased in the frontal cerebral cortex, but not in other areas of the brain. The authors suggested the changes were due to death of susceptible cells or to inhibition of DNA synthesis.

Kyrklund and associates (1987) exposed Mongolian gerbils continuously over a 3 month interval to 320 ppm PCE (containing 0.01% stabilizers). Changes in relative brain lipid concentrations were observed in the cerebral cortex and hippocampus. Linolenic acid and fatty acids derived from linolenic acid were

decreased. Several fatty acids derived from linoleic acid were increased. The general trend was one of increased desaturation.

In another study, male Sprague-Dawley rats were continuously exposed to PCE vapor at 320 ppm for 30 days (Kyrklund et al., 1988). Changes in brain lipid composition were noted relative to matched controls. Cholesterol, total phospholipids and the proportion of stearic acid was reduced. The proportion of docosapentaenoic, 22:5, and docosahexaenoic, 22:6 acids were increased, suggestin changes in desaturation of fatty acids.

Carpenter studied the effects on rats of chronic exposure to 70, 230, 470, or 7000 ppm of PCE (Carpenter et al., 1937). Although various pathological changes were observed at 230 ppm PCE and above, no CNS effects were reported. Savolainen and co-workers observed a slight decrease in brain RNA content and an increase in nonspecific cholinesterase in rats exposed to 200 ppm PCE (6 hours/day for 4 days) (Savolainen et al., 1977). A one-month study conducted by Honma and co-workers documented a dose-dependent decrease in dopamine content of the striatum in rats exposed to 200, 400, or 800 ppm PCE 12 hours/day (Honma et al., 1980). The decrease was not statistically significant. Norepinephrine content of the hypothalamus and serotonin levels of the cortex and hippocampus increased after exposure to PCE (all three concentrations). None of the increases were statistically significant. A significant decrease in acetylcholine (ACh) levels in the striatum was measured after exposure to 800 ppm ($p<0.05$). Drowsiness and other symptoms indicative of CNS depression were reported by Rowe and associates after rats were exposed to 1600 ppm PCE 7 hours/day, 5 days/week over a 25 day period (Rowe et al., 1952). At 2500 ppm PCE, 13 exposures within 18 days caused the death of most rats and guinea pigs from CNS depression. Rabbits that received the same treatment displayed signs of CNS depression but did not lose consciousness.

Briving and associates (1986) chronically exposed Mongolian gerbils to relatively low levels of PCE via inhalation. Six gerbils were exposed nearly continuously to 120 ppm PCE for 12 months. After completion of exposure, decreased taurine concentrations were found in the hippocampus and the posterior region of the cerebellar vermis. Hippocampus glutamine levels were elevated, whereas other amino acids and glutathione were not significantly different than controls.

TERATOGENICITY

The teratogenic activity of PCE has been studied in rats (Beliles et al., 1980; Nelson et al., 1980; Schwetz et al., 1974; Schwetz et al., 1975), mice (Schwetz et al., 1975) and rabbits (Beliles et al., 1980). Maternal exposure levels ranged from 100 to 1800 ppm PCE. Although some minor effects were seen in the progeny, PCE is not considered to be a teratogen. A summary of these studies is provided in Table 3-1.

Investigations of the teratogenic and/or developmental effects of PCE have most commonly shown evidence of maternal toxicity, rather than adverse effects on the progeny. Toxicity was evident at 300 ppm (Schwetz et al., 1974;

TABLE 3-1. TERATOLOGY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Concentration or dose	Exposure duration	Exposure period	Effect	Reference
Rat (Sprague-Dawley)	100 ppm	7 h/d	Days 14 to 20 of gestation	No observed fetal or maternal toxicity	Nelson et al., 1980
Rat (Sprague-Dawley)	300 ppm	7 h/d	Days 6 to 15 of gestation	Maternal toxicity: reduction in mean body weight (4 to 5%) Fetal toxicity: slight but significant increase in fetal resorptions (9 of 17 litters)	Schwetz et al., 1975
Mouse (Swiss-Webster)	300 ppm	7 h/d	Days 6 to 15 of gestation	Maternal toxicity: increase in relative liver weight Fetal toxicity: decrease in body weight, delayed ossification of skull bones, split sternebrae, increase in subcutaneous edema	Schwetz et al., 1975
Rat (Sprague-Dawley)	900 ppm	7 h/d	Days 7 to 13 of gestation	Maternal toxicity: decrease in feed consumption and weight Pup toxicity: diminished performance in some behavioral tests	Nelson et al., 1980
Rat (Sprague-Dawley)	900 ppm	7 h/d	Days 14 to 20 of gestation	Maternal toxicity: decrease in feed consumption and weight Pup toxicity: diminished performance on ascent test	Nelson et al., 1980

TABLE 3-1. (Continued)

Species	Concentration or dose	Exposure duration	Exposure period	Effect	Reference
Chicken embryo	5 to 10 mmole/egg	Injected into air space on days 2, 3, and 6 of incubation	Examined 14 d after incubation	Malformed embryos at 10 mmole; Estimated LD50 over 100 mmole	Elovaara et al., 1979

Schwetz et al., 1975) and 900 ppm (Nelson et al., 1980), while maternal death occurred at 1800 ppm (Nelson et al., 1980). Dams exposed to 300 ppm PCE 7 hours/day on days 6 to 15 of gestation had reduced body weights (rats) or an increase in liver weight (mice). The pups of these mice had lower body weights, and there was a slight increase in the number of runts. Some fetuses had subcutaneous edema or delayed ossification of the skull and sternebrae, as well as splits in the sternebrae. These pathologies probably reflect developmental delays and, as such, are considered to be reversible. Developmental delays are believed to result from maternal toxicity rather than from any direct teratogenic activity of PCE (Schwetz et al., 1975). The mechanism of maternal toxicity is unknown, but is thought to involve CNS depression (weight loss) and cytotoxicity (change in liver morphology). Because fetal health is often a reflection of the health of the mother, maternal toxicity is a significant concern. The loss of maternal weight, most probably due to decreased feed consumption from subclinical effects (ataxia and anesthesia), can have a great impact on the growth and maturation of the fetus. Maternal malnutrition can cause developmental retardation of the fetus (Doull et al., 1980). The hepatotoxicity observed in mice could also have a profound effect on the growth of the fetus. Maternal toxicity is also suspected of causing a small but significant increase in the number of resorptions in treated rats ($p<0.05$) (300 ppm PCE, 7 hours/day) (Schwetz et al., 1975).

Nelson and associates performed a series of behavioral and biochemical tests on the offspring of exposed rats (Nelson et al., 1980). There were no adverse effects to mothers or their pups following exposure of the mothers to 100 ppm of PCE (7 hours/day) on days 14 to 20 of gestation. Exposure at 900 ppm PCE for 7 hours/day during days 7 to 13 of gestation produced significant differences in neuromuscular coordination ($p<0.02$) and wire mesh ascent ($p<0.05$). Exposure at 900 ppm PCE for 7 hours/day during days 14 to 20 of gestation caused diminished performance in the wire-mesh ascent test, but increased the performance in the neuromuscular coordination test. A neurochemical analysis of whole brain (minus cerebellum) was performed on newborn and 21-day old pups. Twenty-one-day-old pups from dams exposed during either period had a significant decrease in acetylcholine ($p<0.05$). A significant decrease in dopamine ($p<0.05$) was measured in 21-day old pups from dams exposed during days 7 to 13 of gestation.

Beliles and associates exposed female rats and rabbits to PCE at ambient concentrations of 0, 100 or 500 ppm for 3 weeks preimpregnation and up to 30 days postgestation (Beliles et al., 1980). No evidence was found that PCE was teratogenic under the conditions employed.

Elovaara and co-workers injected 5 to 100 mmol of PCE into the air space of chicken eggs and studied the gross effects on the embryo (Elovaara et al., 1979). Malformations observed were exteriorization of viscera, as well as skeletal and eye abnormalities. These deformities occurred in six embryos of 61 examined.

Tests conducted in rodents have not clearly demonstrated that PCE is a teratogen. However, there is some evidence that inhalation exposure of

pregnant rodents to PCE can induce developmental delays and altered performance in behavioral tests of the offspring.

MUTAGENIC EFFECTS

Short-term assays have been conducted to evaluate the ability of PCE to permanently alter genetic information. Most of the tests of genetic activity have been microbial assays that measured forward or reverse mutations. Chromosomal effects have been studied in cultured mammalian cells.

The ability of short-term assays to detect mutagens is compromised by lack of knowledge of the mechanisms involved, by different sensitivity and predictive ability of each test, and by variations in protocols used by separate labs. Despite these problems, short-term assays provide supportive evidence in the evaluation of a compound's carcinogenic potential.

Microbial Assays

Greim and fellow researchers evaluated the mutagenic activity of PCE (purity >99.9%) in *Escherichia coli* K12 with and without metabolic activation (S-9) (Greim et al., 1975). The results were negative at four loci tested. Three of these loci are back mutation systems (gal^+, arg^+, and nad^+), while one measures a forward mutation that produces resistance to 5-methyl-DL-tryptophan. Only one PCE concentration was used (0.9 mM), and detailed data were not reported.

Cerna and Kypenova reported in an abstract that PCE of unspecified purity produced base-pair substitutions and frameshift mutations in *Salmonella typhimurium* without S-9 (Ames test) (Cerna and Kypenova, 1977). Concentrations of 0.01, 0.1, and 1.0 mg/mL of PCE produced a dose-dependent increase in the number of revertants. This response was significant only in TA100 (5% level of significance), a strain sensitive to base-pair substitutions. In a host mediated assay that used ICR mice and *S. typhimurium* strains TA1950, TA1951, and TA1952, PCE reportedly caused a significant increase in the number of revertants (level of significance was not given). The doses used were listed as LD_{50} and $1/2LD_{50}$, but exact quantities were not specified. The significance of this limited report is unclear.

Perchloroethylene (99.7% pure) was studied in the Ames test with strain *S. typhimurium* TA100 (Bartsch et al., 1979). Concentrations up to 4×10^{-3} M were not mutagenic in the presence of an S-9 liver fraction from mice pretreated with phenobarbital. Toxicity was observed at concentrations greater than 5×10^{-4} M.

Kringstad and associates evaluated the mutagenic activity of PCE in the Ames test (Kringstad et al., 1981). The PCE (99.0% pure) was tested at a single concentration (0.1 mg/plate) in *S. typhimurium*, strain TA1535. No source of exogenous metabolic activation (S-9) was used. A slight increase in the number of revertants was observed (31/plate after PCE compared to 19/plate in the ether controls). However, this response was considered negative.

The NTP reported the results of a series of Ames tests on PCE conducted by the Environmental Mutagen Test Development Program (NTP, 1986). *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) was incubated with technical-grade PCE in covered test tubes for 20 minutes. The test was conducted both with and without S-9 (S-9 fractions were prepared from the livers of male Sprague-Dawley rats and Syrian hamsters pretreated with Aroclor 1254). The greatest number of revertants was observed in strain TA100 (all doses, both with and without S-9); high doses (333 μg/plate) were toxic to TA1535 and TA1537 in the absence of S-9. However, PCE was judged not to be mutagenic in any of the strains, regardless of the concentration tested.

Shimada and co-workers studied effects of PCE vapors in Salmonella/microsome and hepatocyte primary culture DNA repair assays (Shimada et al., 1985). Vinyl chloride as a positive control induced reversions in *Salmonella typhimurium* strains TA100 and TA1535 and unscheduled DNA synthesis in cultured rat hepatocytes. Technical, low-stabilized and stabilized grades of PCE increased reversion and DNA repair rates. S-9 rat liver fractions did not affect the results. Since highly purified PCE was not genotoxic, the researchers concluded that stabilizers or impurities in PCE were responsible for the observed positive responses.

Callen and associates used *Saccharomyces cerevisiae*, strain D7, to study the frequency of gene conversion (trp5 and ilv1 loci) and mitotic recombination (ade2 locus) (Callen et al., 1980). The PCE (purity not given) was added directly to a cell suspension (log phase) and incubated in a closed vial for one hour. Samples were centrifuged, resuspended in buffer, and plated on a medium with glucose. Survival decreased as PCE dose increased from 0 to 4.9, 6.6, or 8.2 mM. At 8.2 mM, survival was greatly reduced and assessment of genetic activity was precluded. Exposure to 6.6 mM PCE elicited substantial increases in mitotic recombination (52.6 recombinants per 10^4 survivors versus 3.3 recombinants per 10^4 survivors in controls). The number of gene conversions (trp5) also increased at 6.6 mM (8.3 convertants per 10^5 survivors versus 1.4 x 10^5 convertants per 10^5 survivors in controls). Mitotic recombination and gene conversion are indicative of interaction of a substance with DNA. Since the increase in frequency of mitotic recombination was pronounced, this response indicates a mechanism of PCE genotoxicity.

Bronzetti and associates also studied the effect of PCE on the trp5, ade2, and ilv1 loci in *S. cerevisiae* (Bronzetti et al., 1983). Cells in the stationary phase were exposed for 2 hours to 5, 10, 20, 60, or 85 mM PCE. All results were negative. The PCE used was 99.5% pure, whereas the purity of that used by Callen and colleagues was not reported (Callen et al., 1980). When comparing the results of these two studies, consideration must be given to the possibility that cells in the stationary phase may be resistant to the mutagenic or toxic action of xenobiotics.

In addition, Bronzetti and fellow researchers conducted an intrasanguineous host-mediated assay that tested the ability of PCE to induce genetic effects in *S. cerevisiae* (Bronzetti et al., 1983). Yeast were exposed to PCE and its metabolites in the liver, lungs, and kidneys of mice. The PCE did not induce point mutations, mitotic recombination, or mitotic gene conversion.

Effects of PCE towards diploid strains of *S. cerevisiae* was further examined by Koch and colleagues (1988). Strain D7 late logarithmic-phase cells as well as stationary-phase cells combined with exogenous metabolic activating S9 fraction were used. No conclusions about genetic effects were possible, as PCE was highly toxic to the cells at the concentration tested (9 mM), decreasing survival rate of cells to less than 30%.

Drosophila

The NTP reported the results of an assay that tested the ability of PCE to induce sex-linked recessive lethal mutations in Drosophila (NTP, 1986). Males were exposed by injection or by feeding and were mated to a series of untreated females. Neither route of administration produced a statistically significant increase in sex-linked recessive lethal mutations.

TESTS OF DNA OR CHROMOSOMAL DAMAGE

Cerna and Kypenova did not observe any cytogenetic effects in mouse bone marrow cells following single or repeated IP injections of PCE (daily injections for five days) (Cerna and Kypenova et al., 1977). Cells were recovered for analysis 6, 24, or 48 hours after the last injection of PCE. No details were provided regarding doses used or the specific end points that were studied (the study was published only as an abstract). The NTP published the results of assays for PCE induced chromosomal aberrations and sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells, both with and without S-9 (NTP, 1986). The S-9 fractions were obtained from livers of male rats pretreated with Aroclor 1254. Data were reported in tables only, with no supportive discussion (such as the number of cells scored for each dose level). However, at least three dose levels were used (with and without S-9), as were both positive and negative controls. The PCE had little, if any, cytogenetic effect in either assay.

Perchloroethylene was not mutagenic in L5178Y/TK$^{+/-}$ mouse lymphoma cells, with or without metabolic activation. Cells were treated for 4 hours with 6.25, 12.5, 25.0, 50.0, and 100.0 nL/mL of PCE. Following incubation for 48 hours, cells were plated in medium supplemented with trifluorothymidine for selection of cells mutant at the thymidine kinase (TK) locus. No statistically significant increase in mutation frequency was observed at any dose level (NTP, 1986).

The non-mutagenicity of PCE in the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay was confirmed in a later study (McGregor et al., 1988). Cells were exposed to PCE for 4 h, then cultured for 2 days before plating in soft agar with or without trifluorothymidine. Significant responses were observed with a number of known mutagens such as bis(2-chloro-1-methylethyl)ether and heptachlor.

Effects of PCE on BALB/c-3T3 cells were studied by Tu and associates (1985). PCE tested at concentrations of up to 250 μg/ml was found to be cytotoxic but did not induce a statistically significant incidence of cell transformation

in this cell line. No exogenous metabolic activation system was added. The PCE tested was reported to be 97-99% pure.

Somatic mutations are thought to occur when a substance or one of its metabolites interacts with DNA. Alkylation of DNA is therefore one possible indicator of genotoxicity. Schumann and co-workers measured binding to hepatic macromolecules of mice after administration of radiolabeled PCE (Schumann et al., 1980). No radioactivity was detected bound to hepatic DNA. The specific activity of the tetrachloro[^{14}C]ethylene used in this study was too low to permit detection of low levels of DNA binding.

Mazzullo and coworkers administered ^{14}C-PCE (8.7 μmole/kg) IP to male Wistar rats and BALB/c mice (Mazzullo et al., 1987). *In vitro* DNA binding experiments were also conducted. Covalent binding to DNA, RNA and proteins was reported both *in vivo* and *in vitro*, and greatest binding was found to occur in mouse liver. Cytosolic and liver microsomal enzymes were most active in producing *in vitro* binding to nucleic acids and proteins. The addition of glutathione (GSH) to liver microsomes greatly enhanced binding. A CBI value of 76 for mouse liver DNA was calculated, and PCE was categorized as a weak-moderate initiator. Greatest RNA binding was found in rat kidney.

Herren-Freund and Pereira studied the tumor initiation effects of PCE. PCE (8.89 mmole/kg) was administered to rats 24 hours following a 2/3 partial hepatectomy (Herren-Freund and Pereira, 1986). After 7 days, phenobarbital (500 ppm) was given in the drinking water for 10 weeks. After an additional week the animals were sacrificed and liver tissue was stained for the presence of gamma-glutamyltranspeptidase (GGT) activity. GGT-positive foci incidence was 0.28 foci/cm^2. At a PCE dose of 17.8 mmole/kg a GGT-positive foci incidence of 0.29 was reported. It was concluded that PCE did not show evidence of initiation activity in this system. The purity of the PCE used in this study was not reported.

In a related study, Herren-Freund et al. (1987) examined the ability of trichloroacetic acid (TCA) and dichloroacetic acid (DCA) to act as tumor promotors in mouse liver. Both DCA and TCA were carcinogenic without prior initiation with ethylnitrosourea, resulting in hepatocellular carcinoma in 81 and 32% of the animals dosed with 5 g/liter TCA or DCA in drinking water for 57 weeks. Thus, in this study DCA and TCA acted as complete carcinogens in B6C3F1 mice.

Lundberg et al. (1987) studied the tumor promoting activity of PCE (99% purity) in male Sprague-Dawley rats that had undergone partial hepatectomy. A group of 6 rats were exposed to 1100 mg/kg b. w. of PCE. A second group of 7 rats were gavaged with 1100 mg/kg of PCE once per day, 5 days per week, for 7 weeks, commencing 5 days after intraparenteral administration of 30 mg/kg of diethylnitrosamine. No significant increase either in number of gamma-glutamyl-transpeptidase (GGT)-positive foci or total foci volume were found following PCE administration. It was concluded that PCE had no detectable hepatic tumor-initiating or tumor-promoting activity at the dose tested.

Milman and colleagues studied induction of GGT-positive rat liver foci by PCE (Milman et al., 1988). No significant initiating effect was observed in

livers of male Osborne-Mendel rats administered PCE at the maximum tolerated dose of 1000 mg/kg b.w. followed by promotion by phenobarbitol. However, when PCE was administered at the maximum tolerated dose by gavage (5 days/week for 7 weeks) in the promotion stage of the experiment, a significant increase in GGT-positive foci was observed. This was true with ($p<0.05$) or without ($p<0.01$) initiation with diethylnitrosamine.

Loury and co-workers examined the effects of PCE and other halogenated compounds with limited genotoxic potential on renal DNA synthesis (Loury et al., 1987; Goldsworthy et al., 1988b). Male and female F-344 rats were exposed to 1000 mg/kg PCE. Kidney cells obtained from these animals 12 to 24 hours after exposure were cultured and incubated with ^{3}H-thymidine. Replicative DNA synthesis was increased in PCE-exposed cells four-fold relative to non-exposed cells. The researchers suggested regenerative hyperplasia following PCE toxicity was involved in renal neoplasia formation.

Beliles and coworkers (1980) reported increased incidence of spermhead abnormalities in mice exposed to PCE.

Mutagenic Activity of Metabolites

Tetrachloroethylene oxide (PCE oxide) is believed to be the first intermediate formed by microsomal oxidation of PCE. A concentration-dependent mutagenic response was produced by PCE oxide (0.5, 1.3, 2.5, 5.0, and 25.0 mM) in *Salmonella typhimurium* TA1535 (without metabolic activation) but PCE oxide was not mutagenic to *E. coli* WP2 uvrA. The mutagenicity of this epoxide was also evaluated in a DNA-repair-deficient strain of *E. coli* (*E. coli* pol A 1^-). In the latter assay, genotoxicity is measured by comparing differential growth inhibition of the DNA-polymerase-deficient strain, pol A 1^-, with a polymerase-proficient strain, pol A 1^+. The PCE oxide gave positive results in this test; growth of the pol A 1^- strain was inhibited at all dose levels used (Kline et al., 1982).

Trichloroacetic acid (TCA) is the principal metabolite of PCE excreted in the urine of rodents; trichloroethanol has also been identified as a metabolite of PCE. In an Ames test conducted with metabolic activation, TCA (0.45 mg/plate) and trichloroethanol (7.5 mg/plate) were not mutagenic to *S. typhimurium* strains TA98 and TA100. There is some indication, however, that trichloroethanol can induce sister-chromatid exchange in cultured human lymphocytes (Gu et al., 1981).

The mutagenicity of PCE and its glutathione conjugate S-(1,2,2-trichlorovinyl)glutathione (TCVG) to *S. typhimurium* TA 100 was studied by Vamvakas and colleagues (1989). TCVG incubated with rat kidney particulate fraction containing high concentrations of gamma-glutamyl transpeptidase (GGT) and dipeptidases was highly mutagenic. PCE was not mutagenic in the absence of metabolic activation or under conditions supporting oxidative metabolism. TCVG was formed upon incubation of PCE with purified rat liver glutathione (GSH) S-transferases, GSH and rat kidney fractions, and the mixture was mutagenic in the Ames assay. TCVG was formed from PCE in isolated perfused rat liver and excreted in bile. Bile collected after addition of PCE to

isolated perfused liver was mutagenic in the presence of kidney particulate fractions. Serine borate, an inhibitor of GGT, or aminooxyacetic acid, an inhibitor of beta-lyase, reduced mutagenicity. Consequently, metabolism of PCE to a glutathione conjugate by the mercapturic acid pathway and beta-lyase could result in formation of a genotoxic metabolite.

CARCINOGENICITY IN ANIMALS

Two lifetime bioassays have been completed on PCE (NCI, 1977; NTP, 1986). Additionally, three other studies have addressed the question of PCE carcinogenicity (Rampy et al., 1978; Theiss et al., 1977; Van Duuren et al., 1979). Table 3-2 summarizes the data from the bioassay studies that resulted in significant increases in malignant neoplasms among the exposed animals.

The National Cancer Institute conducted a study in which B6C3F1 mice and Osborne Mendel rats were administered PCE in corn oil by gavage, 5 days/week for 78 weeks (NCI, 1977). Animals were then observed for 32 weeks (rats) or 12 weeks (mice). Mice were 25 days old at initial treatment; rats were 35 days of age. The time-weighted average daily doses of PCE were 536 and 1072 mg/kg for male mice, 386 and 722 mg/kg for female mice, 471 and 941 mg/kg for male rats, and 474 and 949 mg/kg for female rats.

Perchloroethylene caused a statistically significant increase in the incidence of hepatocellular carcinoma in mice of both sexes and both dosage groups ($p<0.001$) (Table 3.2). The time to first tumor development was considerably shorter in treated mice than in controls. Hepatocellular carcinomas were first detected at weeks 91 and 90 in untreated and vehicle controls respectively. However, in male mice, hepatocellular carcinomas were detected after 27 weeks (low-dose) and 40 weeks (high-dose). The first hepatocellular carcinomas were observed in female mice at week 41 (low-dose) and week 50 (high-dose). Noncarcinogenic hepatic lesions were not elevated. Exposed mice did not exhibit any significant increase in the incidence of renal tumors, although there was a high incidence of toxic nephropathy.

Median survival times of mice were inversely related to dose. Control males had median survival times of more than 90 weeks; this decreased to 78 weeks in low-dose males and 43 weeks in high-dose males. The median survival time of control females was also greater than 90 weeks. Median survival times of low- and high-dose females were 62 and 50 weeks, respectively (NCI, 1977).

Early mortality occurred in all groups of rats dosed with PCE. Half of the high-dose males had died by week 44; half of the high-dose females died by week 66. The median survival time of control animals was 88 to 102 weeks, depending on sex. The National Cancer Institute determined that there was a statistically significant association ($p<0.001$) between increased dosage of PCE and increased mortality (NCI, 1977). The early mortality observed in rats and its statistical association with dose of PCE indicate that the doses given to rats in this bioassay were inappropriately high. Because the optimum dosage was not used and because significant early mortality occurred, these results preclude any conclusions regarding the carcinogenicity of PCE in rats.

TABLE 3-2. SUMMARY OF TUMOR-INCIDENCE DATA FROM ANIMAL BIOASSAY STUDIES

Study	Species (strain)	Sex	Concentration or dose	Tumor responses		p-value
				Type[a]	Incidence	
NCI, 1977	Mice (B6C3F1)	M	0 mg/kg-d	HC	2/17	NA
			536 mg/kg-d	HC	32/49	p<0.001[b]
			1072 mg/kg-d	HC	27/48	p<0.001[b]
		F	0	HC	2/20	NA
			386 mg/kg-d	HC	19/48	p<0.001[b]
			772 mg/kg-d	HC	19/48	p<0.001[b]
NTP, 1986	Mice (B6C3F1)	M	0 ppm	HC	7/49	NA
			100 ppm	HC	25/49	p<0.001[b]
			200 ppm	HC	26/50	p<0.001[b]
		M	0 ppm	HAC	16/49	NA
			100 ppm	HAC	8/49	NS
			200 ppm	HAC	18/50	NS
		F	0 ppm	HC	1/48	NA
			100 ppm	HC	13/50	p<0.001[b]
			200 ppm	HC	36/50	p<0.001[b]
			0 ppm	HAC	3/48	NA
			100 ppm	HAC	6/50	NS
			200 ppm	HAC	2/50	NS

TABLE 3-2. (Continued)

Study	Species (strain)	Sex	Concentration or dose	Tumor responses Type[a]	Incidence	p-value
NTP, 1986	Rats (F344/N)	M	0 ppm	MLK	28/50	NA
			200 ppm	MLK	37/50	p - 0.046[c]
			400 ppm	MLK	37/50	p - 0.004[c]
		F	0 ppm	MLK	18/50	NA
			200 ppm	MLK	30/50	p - 0.023[c]
			400 ppm	MLK	29/50	p - 0.053[c]

[a]HC - hepatocellular carcinoma; HAC - hepatocellular adenoma; and MLK - mononuclear cell leukemia.

[b]Probability level, Fisher Exact Test (NCI, 1977; NTP, 1986).

[c]Probability level, Life Table Analysis (NTP, 1986).

NA - not available.

NS - not statistically significant.

Questions have been raised about the purity of PCE used in the NCI mouse and rat bioassays. The PCE was produced by Aldrich Chemical Co. and had a purity of 99%. However, epichlorohydrin (ECH) was apparently used as a stabilizer. It has been suggested that the presence of this contaminant may have directly contributed to tumor induction. The ECH is a direct-acting alkylating agent and is mutagenic (Kucerova et al., 1977; Bridges, 1978). Van Duuren and associates demonstrated that ECH was carcinogenic in mice when injected subcutaneously (Van Duuren et al., 1974). A subsequent study by Laskin and colleagues showed that ECH induced neoplastic lesions of the nasal cavity of rats (Laskin et al., 1980). Most of these tumors were carcinomas of the squamous epithelium. A study by Konishi and co-workers and Kawabata also showed that ECH-fed discontinuously to rats in drinking water at a concentration of 1500 ppm (and at a lifetime TWA dose of approximately 40.2 $mg/kg\text{-}d)^{-1}$ induced a significantly increased incidence of papillomas and squamous cell carcinomas of the forestomach above that of control animals (Konishi et al., 1980; Kawabata, 1981).

The exact quantity of ECH present in the PCE used in the NCI study is not known, but it has been estimated that high-dose male mice received 0.42 mg/kg-d (EPA, 1985a). This represents one percent of the dose that elicited squamous cell carcinomas in rats. Furthermore, ECH appears to initiate tumors by a localized tumorigenic reaction at sites where it is in direct contact with tissue, such as nasal or forestomach squamous-cell epithelium (EPA, 1984c). No animal in the NCI bioassay developed tumors at these sites. The ECH is among the weakest of the more than 50 suspect carcinogens evaluated by the EPA Carcinogen Assessment Group, having an estimated upper-bound carcinogenic potency, or effect per unit dose at low doses, to 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 via drinking water (EPA, 1984c). Using the methodology of the EPA, the equivalent potency to mice would 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 respectively (EPA, 1984c). The potency for ECH to mice is therefore estimated to be 0.058 $(mg/kg\text{-}d)^{-1}$. Using this potency estimate, the highest dosed animals (high-dose male mice) in the NCI bioassays would be expected to incur an increased cancer risk of $(0.42) \times (0.058) = 0.024$, or less than 2.5% (NCI, 1977). Therefore, it is unlikely that ECH contributed significantly to the observed increased tumor incidence in PCE-exposed mice in the NCI bioassay (NCI, 1977).

Rampy and fellow researchers exposed male and female Sprague-Dawley rats to PCE by inhalation (300 or 600 ppm) 6 hours/day, 5 days/week for 12 months (Rampy et al., 1978). Animals were subsequently observed for 18 months. Pathological changes in the liver or kidney were not observed. High-dose males had slightly greater mortality than did controls, but neither sex exhibited an increased incidence of tumors, regardless of dose. Interpretation of this study is limited by the duration of the exposure and by the fact that it was reported only as an abstract.

Theiss and co-workers studied the ability of PCE to induce lung adenomas in A/St male mice (Theiss et al., 1977). Animals 6 to 8 weeks old were given 80, 200, or 400 mg/kg of PCE in tricaprylin (intraperitoneally) three times a week. Each group received 14, 24, or 48 injections. Animals were sacrificed

24 weeks after the first injection and were examined histologically for the presence of pulmonary tumors. Treated animals did not exhibit a significant increase in the average number of lung tumors when compared to controls. The relevance and validity of these test results are of questionable significance, though, as this test has not produced positive results with several known animal carcinogens.

The ability of PCE to initiate and/or promote skin tumors in ICR/Ha Swiss mice was investigated by Van Duuren and associates (Van Duuren et al., 1979). In one group, 163 mg of PCE was applied once to surface skin. Fourteen days after this, phorbol myristate acetate, a promoter, was applied to the same area three times a week for 428 to 576 days. A second group received 54 mg of PCE by topical application three times a week for 440 to 594 days. The PCE did not show any initiating activity. It also gave negative results in the portion of the experiment that tested its ability to act as a complete carcinogen. It is difficult to interpret these data in relation to the carcinogenic action of PCE because the significance and sensitivity of skin application tests are not thoroughly understood.

The most definitive study of the carcinogenic potential of PCE was conducted by Battelle Pacific Northwest Laboratories for the National Toxicology Program (NTP, 1986). In this experiment, B6C3F1 mice and F344/N rats were exposed to 99.9% pure PCE by inhalation, 6 hours/day, 5 days/week for 103 weeks. Mice were exposed to concentrations of 0, 100, or 200 ppm; rats were exposed to concentrations of 0, 200, or 400 ppm. Treated male rats had lower survival rates than control animals (controls, 23/50 animals; 200 ppm, 20/50; 400 ppm, 12/50). Survival rates among female rats showed little variation (controls, 23/50 animals; 200 ppm, 21/50; 400 ppm, 24/50). Both exposure concentrations produced significant increases in mononuclear cell leukemia in female rats (incidence in controls, 18/50 animals; in rats receiving 200 ppm, 30/50; and in rats receiving 400 ppm, 29/50). Life Table analysis showed the significance of these increases to be $p = 0.023$ (200 ppm PCE) and $p = 0.053$ (400 ppm PCE). Treated male rats also developed mononuclear cell leukemia in greater numbers than controls (controls, 28/50 animals; 200 ppm, 37/50; 400 ppm, 37/50). Levels of significance (evaluated by Life Table analysis) are $p = 0.046$ (200 ppm PCE) and $p = 0.004$ (400 ppm PCE) (Table 3-2).

Renal tubular-cell adenomas are rare neoplasms with a historical occurrence at Battelle Laboratories of less than one percent (Appendix F, NTP, 1986). Renal tubular-cell adenocarcinomas are even less common, and have not been documented in any NTP studies (NTP, 1986). Male rats (at the 200 and 400 ppm PCE exposure levels) exhibited an increased incidence of both of these neoplasms (see Table 3-3). Although the increases were not statistically significant, they appeared to be dose-related. Tubular-cell hyperplasia was observed in eight treated males but only in one treated female rat. Tubular-cell karyomegaly developed in a majority of male rats but was less common in females.

Brain glioma is a rare tumor of neuroglial cells (the cells that compose the supporting structure of nervous tissue). Brain gliomas were observed in one male control rat and in four male rats that were exposed to 400 ppm PCE (NTP, 1986). This increase was not statistically significant. However, because the

TABLE 3-3. INCIDENCE OF RENAL TUBULAR CELL ADENOMAS AND ADENOCARCINOMAS IN MALE RATS EXPOSED TO PCE BY INHALATION

Neoplasm	Treatment		
	Control	200 ppm	400 ppm
Tubular cell adenoma	1/49	3/49	2/50
Tubular cell adenocarcinoma	0/49	0/49	2/50
Tubular cell adenoma or adenocarcinoma	1/49	3/49 (p = 0.259[a])	4/50 (p = 0.070[b])

[a]Data are from NTP, 1986.

[b]P-values are based on Life Table Tests (Appendix E, NTP, 1986).

historical incidence of these tumors is quite low (0.2% at Battelle Laboratories), the increased incidence in treated animals in this study is noteworthy.

In the NTP study, the survival of low-dose male mice (after week 74) and of high-dose male mice (after week 75) was significantly lower than controls ($p<0.001$) (NTP, 1986). The survival of high-dose female mice was significantly lower than controls after week 90 ($p<0.001$). Both concentrations of PCE produced a statistically significant increase of hepatocellular carcinomas in treated mice of both sexes ($p<0.001$) (3-2). The incidence of these carcinomas in male mice was as follows: controls, 7/49 animals; low-dose, 25/49; and high-dose, 26/50. The incidence of hepatocellular carcinomas in treated female mice was: controls, 1/48 animals; low-dose, 13/50; and high-dose, 36/50.

Hepatocellular adenomas occurred in both sexes of mice and at both concentrations of PCE (Table 3-2). The incidence of adenomas was not statistically significant. However, the combined incidence of hepatocellular adenomas and hepatocellular carcinomas was significant. In males, the combined incidence was: controls, 16/49 animals; low-dose, 31/49; ($p = 0.002$); and high-dose, 40/50 ($p<0.001$). In females, the incidence of hepatocellular adenomas and carcinomas was: controls, 4/48 animals; low-dose, 17/50 ($p = 0.001$); and high-dose, 38/50 ($p<0.001$).

Summary of Evidence of Carcinogenicity in Animals

The NCI bioassay of PCE found that administration of PCE by gavage was associated with a statistically significant increased incidence ($p<0.001$) of hepatocellular carcinoma (NCI, 1977). This increase was documented in low- and high-dose PCE-treated B6C3F1 mice of both sexes. A decrease in the time to first tumor development was also observed in treated mice of both sexes and both dose groups. Early mortality prevented an analysis of PCE's carcinogenic potential in rats. The NCI concluded that under the conditions of this study, PCE was a liver carcinogen to B6C3F1 mice of both sexes (NCI, 1977).

The final report of the NTP inhalation bioassay on PCE was released in 1986 (NTP, 1986). The NTP determined that, under the conditions of this study, there was "clear evidence of carcinogenicity" of PCE for male F344/N rats, "some evidence of carcinogenicity" of PCE for female F344/N rats, and "clear evidence of carcinogenicity" of PCE for both sexes of B6C3F1 mice. In rats, these conclusions were based on an increased incidence of mononuclear cell leukemia in males and females. Male rats also developed renal tubular cell neoplasms (a rare type of tumor). The evaluation of carcinogenicity in mice was based on an increased incidence of hepatocellular adenoma and hepatocellular carcinoma in males, and an increased incidence of hepatocellular carcinoma in females.

In 1979, IARC reviewed the NCI study on PCE as well as the animal carcinogenicity studies of Rampy and associates (1978) and Theiss and colleagues (1977). Only two short-term assays were evaluated (Cerna and Kypenova, 1977; Greim et al., 1975). The IARC determined that at that point

there was "limited evidence" that PCE was carcinogenic in mice (IARC, 1979). The IARC re-evaluated the evidence of carcinogenocity of PCE, concluding that there was sufficient evidence that PCE is carcinogenic to animals (IARC, 1987).

4. TOXIC EFFECTS IN HUMANS

To assess correctly the health risks from a chemical, consideration of human toxicity data is essential. Unfortunately, information on human toxicity for many substances is limited or is anecdotal in nature. For PCE, however, there have been some controlled inhalation exposure studies to define occupational limits. In addition, epidemiological studies have been conducted to explore the relationship between exposures to PCE vapors and potential health effects. This section presents a brief overview of the health effects of PCE exposures. This discussion is followed by a review of different epidemiological studies dealing with PCE. Finally, human data on the toxic effects of PCE on specific organs and systems are examined.

GENERAL TOXICITY

Acute exposure to PCE can produce skin irritation and burns, as well as irritation of the eyes and respiratory tract. Central nervous system (CNS) depression is the most immediate effect of exposure, but high concentrations can also cause loss of consciousness and respiratory failure. Liver and kidney toxicity can result from single exposures (Stewart et al., 1961a; Stewart, 1969; Hake and Stewart, 1977), but the concentration and duration are typically greater than those that produce transient CNS effects. Chronic occupational exposure to PCE has caused headache, dizziness, hangover, intoxication, diminished cognitive abilities, and a decreased performance in the Romberg and Flanagan Coordination Tests (Stewart et al., 1961b; Stewart et al., 1970; Stewart et al., 1974). Extended exposure to PCE (2 1/2 months to several years) has also promoted changes in kidney and liver function, cirrhosis, and toxic hepatitis (Coler and Rossmiller, 1953; Meckler and Phelps, 1966; Hake and Stewart, 1977).

In experimental studies, human volunteers have been exposed to PCE by inhalation at various concentrations and for various durations. Because subjects were allowed to leave exposure chambers when they felt discomfort, observed adverse effects have been restricted to the respiratory tract and CNS (Carpenter, 1937; Rowe et al., 1952; Stewart et al., 1961b; Stewart et al., 1970; Stewart et al., 1974; Hake and Stewart, 1977). Accidental exposure to PCE has occurred primarily as a result of its use as an industrial solvent. Although specific exposure levels have not always been determined, concentrations have been high enough to cause liver and kidney dysfunction (Coler and Rossmiller, 1953; Hake and Stewart, 1977; Koppel et al., 1985).

EPIDEMIOLOGIC EVIDENCE FOR CARCINOGENICITY IN HUMANS

Epidemiologic studies of PCE exposure have been reviewed by Reichert and by the EPA (Reichert, 1983; EPA, 1985a). Blair and associates analyzed the death certificates of 330 union laundry and dry-cleaning workers (out of a cohort of 10,000) (Blair et al., 1979). Of 330 decedents, 279 had worked solely in dry-cleaning establishments (while union members). The solvent(s) used by the dry cleaners were not identified. Length of union membership ranged from one

to 25 years, with a mean of 13 years. The number of expected deaths from cancer was 67.9 (based on proportionate mortality of the U.S. population) while 87 deaths from cancer were observed. The authors also compared the number of years of union membership with cause of death. With the exception of nonwhite males, length of union membership was nearly identical for cancer and noncancer deaths. Increased mortality from cancers of the respiratory tract, cervix, and skin was documented ($p<0.05$). When all malignancies were evaluated together, the number of observed deaths was also significantly greater than expected ($p<0.05$). The authors noted that the excess of cervical cancer may be related to the typically low wages and socioeconomic class of this occupational group. Although an excess of liver cancer and leukemia was also observed, these increases were not statistically significant.

The increases in cancer deaths among the study group probably contributed to a lower than expected relative frequency of deaths from other causes. It is noteworthy that death from circulatory disease was significantly lower than expected ($p<0.005$) (Blair et al., 1979). The factors contributing to this phenomenon are not known. Causes of death were not determined separately for laundry and dry-cleaning workers in this study. The actual solvent(s) used were not identified, and smoking history was not documented. The lack of control for smoking proved a significant deficiency of the study because lung cancer was a major contributor to the total number of cancers. Although this study identifies a potential occupational hazard, data provided are not adequate to evaluate the carcinogenic potential of PCE.

In an expanded study, Blair and associates reported on mortality among 5365 dry cleaning union members (Blair et al., 1990). A Standardized Mortality Ratio (SMR)[1] was used to compare the number of observed deaths to the number of expected deaths by cause. Overall mortality was less than expected (SMR = 0.9) but slightly raised for cancer mortality (SMR = 1.2). Statistically significant excesses of cancer of the esophagus (SMR = 2.1) and cervix (SMR = 1.7) and non-significant excesses for cancer of the larynx (SMR = 1.6), lung (SMR = 1.3), bladder (SMR = 1.7), and thyroid (SMR = 3.3) were reported. Non-significant increases in lymphosarcoma and reticulosarcoma (SMR = 1.7) and Hodgkin's disease (SMR = 2.1) were also noted. No excess mortality from kidney cancer was observed. Mortality from lymphatic and hematopoietic cancers was greatest among workers presumed to have the highest exposures. Lack of PCE exposure data and lack of accounting for potential confounding factors such as economic status, tobacco, or alcohol use prevents any firm conclusion as to the association of PCE exposure and excess cancer.

Katz and Jowett analyzed the mortality patterns of 671 white female laundry and dry-cleaning workers (Katz and Jowett, 1981). Data were obtained from the death certificates of individuals who died in the period 1966 to 1977. Occupational codes listed on the certificates did not distinguish between the two types of work. Data on the duration of employment were not available, nor were the investigators able to determine to which solvent(s) the individuals

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$$* \ \text{SMR} = \frac{\text{observed deaths in study population}}{\text{expected deaths in study population}} \times 100$$

were exposed. Smoking history was not known. Cause-specific proportionate mortality ratios were calculated for 25 causes of death. A significant increase in risk of death from cancer of the kidneys ($p<0.05$) and genitals ($p<0.01$) was documented. An excess risk from skin and bladder cancer was also found; however, neither increase was statistically significant. Individuals in the group under study had a greater risk of death from cancer of the cervix, ischemic (obstructive) heart disease, and diabetes mellitus. However, when the effect(s) of low-wage occupations were accounted for, only the risk for diabetes mellitus remained statistically significant ($p<0.05$).

Other studies of laundry and dry-cleaning workers have also reported an increased risk of death from cervical cancer (Blair et al., 1979; Kaplan, 1980); however, these investigators have not compared mortality data by low-wage occupation. Although not definitive, the findings of Katz and Jowett suggest that factor(s) other than (or in addition to) solvent exposure are important contributors to cervical cancer (Katz and Jowett, 1981).

Kaplan completed a retrospective mortality study of 1597 dry-cleaning workers exposed to PCE for at least one year (prior to 1960) (Kaplan, 1980). By the end of the study period, 1028 of the cohort were alive, 285 had died, and the status of the remaining 254 was not known. Although a considerable effort was made to determine the history of solvent exposure, the solvent history of approximately half of the dry-cleaning establishments was unknown. Of those shops with known solvent history, none had used trichloroethylene; individuals who had worked in shops that had used carbon tetrachloride were eliminated from the study. However, prior to 1960 (the period of interest in this study), the majority of dry cleaners used petroleum solvents (NIOSH, 1980). In keeping with this information, Kaplan decided that employment in a shop with unknown solvent history probably involved exposure to petroleum solvents. Similarities in the physical properties and physiological effects of gasoline, which has been associated with kidney cancer in rats (Kitchen, 1983), and petroleum solvents suggest that use of these solvents may contribute to an increased risk of cancer. The inability of Kaplan to quantify solvent exposure adds an important confounding variable to the study (Kaplan, 1980). The mean exposure concentration of individuals to PCE was calculated to be 22 ppm for dry-cleaning machine operators and 3.3 ppm for all other jobs. These values are based on a NIOSH survey (NIOSH, 1980) cited by Kaplan (1980).

Kaplan found an elevated SMR (182) for malignant neoplasms of the colon (11 observed deaths, 6.77 to 6.98 expected deaths) (Kaplan, 1980). In discussing this observation, Kaplan pointed out that those individuals of high socioeconomic status are at greater risk for cancer of the colon than individuals of low socioeconomic status (Kaplan, 1980). Because dry-cleaning workers generally receive low wages, the study cohort may have overrepresented individuals of low socioeconomic status, and therefore, included a disproportionate number of individuals who are at low risk for colon cancer. If this risk trend is valid, the elevated SMR reported for colon cancer may actually be an underestimate of PCE risk.

In addition to colon cancer, SMR's for malignant neoplasms of the rectum (158), pancreas (152), respiratory system (140), urinary organs (198), and "other and unspecified sites (major)" (156) were observed (Kaplan, 1980).

Although Kaplan did not evaluate SMR's for statistical significance, a review of this study by the EPA included an evaluation of significance of these malignant neoplasms (EPA, 1985a). The SMR's for cancer of the rectum, pancreas, respiratory system, urinary organs, and "other and unspecified sites (major)" were not significant at the $p<0.05$ level. However, cancers of the respiratory system, urinary organs, and "other and unspecified sites" were of borderline significance ($0.10<p<0.05$).

For nonneoplastic diseases, elevated SMR's were reported for diseases of the blood and blood-forming organs (290) and for diseases of the stomach and duodenum (211) as well as hernia and intestinal obstruction (125) (Kaplan, 1980). These values are based on data from only two to four individuals, and cannot be considered definitive. They do suggest the need for additional study to determine if a relationship does exist between PCE or solvent exposure and these diseases.

Although the relatively small cohort in this study limits conclusions about the carcinogenic potential of PCE, the study (Kaplan, 1980) results suggest a relationship between colon cancer and solvent exposure. Because the NTP study of PCE found hyperplastic and neoplastic changes in the kidneys of treated rats (NTP, 1986), the increase in the SMR from malignant neoplasms of the urinary organs raises the possibility that occupational exposure to solvents may increase the risk of cancer in these organs. Since petroleum products have also been linked to kidney cancer in rats (Kitchen, 1983), and because it is probable that some of the cohort were exposed to petroleum solvents (as well as to PCE), the possible contribution of PCE to an increased risk of urinary organ cancer cannot be ascertained.

An additional problem in this study was the inability of investigators to collect data on (and thus control for) smoking history. Since smoking is associated with an elevated risk of many types of cancers (including lung and kidney), its contribution to the elevated SMR's reported by Kaplan needs to be evaluated (U.S. DHEW, 1979).

A group of Danish laundry and dry-cleaning workers were identified from the Danish Occupational Cancer Register by Lynge and colleagues (1990). From cancer incidence data for a 10-year period, a significant excess risk was found for primary liver cancer among 8567 women (standardized incidence ratio 3.4, 95% confidence interval 1.4-7.0). No case of primary liver cancer was observed among 2033 men, for whom the expected value was 1.1. Excess alcohol consumption did not appear to account for the excess primary liver cancer risk for women. However, no data was available on actual exposures of the study group to PCE or other chemicals.

Duh and Asal studied the cause(s) of mortality among 440 laundry and dry-cleaning workers from Oklahoma who died during 1975 to 1981 (Duh and Asal, 1984). This study had the same problem as the studies of Blair and co-workers and Katz and Jowett: smoking histories were not available and separation of the two groups by occupation was not possible (Blair et al., 1979; Katz and Jowett, 1981). Therefore, duration or characterization of individual exposure was not reported. However, Duh and Asal noted that the two groups of workers probably experienced substantially different solvent exposure.

The NIOSH reported that, although 75% of dry-cleaning establishments in the U.S. use PCE, Oklahoma may be unique in that petroleum solvents account for more than 50% of total solvents used (NIOSH, 1980).

A Standardized Mortality Odds Ratio (SMOR)[2] revealed elevated SMOR's for all digestive diseases (1.5), cirrhosis of the liver (1.3), and homicide (3.8). The SMOR's less than 1.0 were reported for diabetes mellitus (0.7), ischemic (obstructive) heart disease (0.8), emphysema (0.8), and suicide (0.2). A SMOR less than 1.0 suggests that laundry and dry-cleaning workers may be at low risk for these diseases. Analysis of deaths due to cancer showed an increase in the SMOR for cancers of the respiratory system (1.8), lung (1.7), and kidney (3.8). Deaths from breast cancer were considerably less than expected (SMOR = 0.1).

Brown and Kaplan conducted a retrospective, cohort-mortality study of workers employed in the dry-cleaning industry to evaluate the carcinogenic potential from occupational exposure to PCE (Brown and Kaplan, 1987). The study cohort consisted of 1,690 members of four labor unions (located in Oakland, Detroit, Chicago, and New York City). Individuals selected for the study had been employed for at least one year prior to 1960 in dry-cleaning shops using PCE as the primary solvent. Complete solvent-use histories were not known for about half of the shops included in the study. Because petroleum solvents were widely used by dry cleaners prior to 1960, most of the cohort had known or potential exposure to solvents other than PCE (primarily, various types of Stoddard solvents). The investigators also identified a subcohort of 615 workers who had been employed only in establishments where PCE was the primary solvent. The PCE exposure in shops included in the study was evaluated independently (Ludwig et al., 1983). The geometric mean of time-weighted-average exposures was 22 ppm PCE for machine operators, and approximately 3 ppm for other workers.

Brown and Kaplan calculated person-years-at-risk (PYAR) for each worker (Brown and Kaplan, 1987). The PYAR were then combined into five-year calendar periods and five-year age groups by the life-table-analysis system (Waxweiler et al., 1983). The PYAR values were also evaluated by length of employment and by time lapsed since first employment in a shop that used PCE. The expected number of deaths was calculated by multiplying PYAR (by age and calendar period) by the U.S. mortality rates. Risk of death due to a specific cause was calculated by means of a SMR.

Among the (main) cohort, the number of observed deaths from all causes (considered together) was less than expected (493 observed, 575.5 expected; SMR = 86) (Brown and Kaplan, 1987). No deaths occurred from liver cancer, although 3.5 were expected. There were also fewer deaths due to diseases of the circulatory system and nervous system (SMR = 70 and 73, respectively). However, observed deaths from all types of neoplasms were higher than expected

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[2] SMOR was defined by Duh and Asal as a method that compares the number of deaths caused by specific cause to the number of deaths due to other causes in the exposed population (the odds) to the expected odds derived from a comparison population (Duh and Asal, 1984).

(142 observed, 122.9 expected; SMR = 116). Elevated SMR's from malignant neoplasms of the intestine (136) and pancreas (172) were documented. Malignant neoplasms of the urinary tract caused a significant excess of deaths (12 observed, 4.7 expected; SMR = 255). Of these urinary tract cancers, kidney cancer caused four deaths (2.0 expected; SMR = 200), while eight deaths from bladder cancer were observed (2.7 expected; SMR = 296). Mortality from calculi of the urinary system (a nonmalignant disease) was also greater than expected (2.0 observed deaths, 0.3 expected; SMR = 667). Although Brown and Kaplan note that there may be an association between calculi and malignant disease of the urinary tract (Brown and Kaplan, 1987), the association is speculative. An elevated SMR for cancer of the cervix (196) and a decreased SMR due to cancer of the breast (87) were attributed to factors associated with the low socioeconomic status of the cohort (Hoover et al., 1975). The subcohort (workers employed only in shops where PCE was the primary solvent) had only one death from urinary tract cancer (1.3 deaths expected). All deaths from urinary calculi (2) occurred in this group.

In summary, a statistically significant excess of deaths from urinary tract cancer was observed in those workers potentially exposed to both PCE and petroleum solvents. Individuals employed in shops where PCE was the primary solvent did not have an increased risk of mortality from kidney or bladder cancer. Although these findings do not rule out PCE as the causative agent of urinary tract cancer, the data suggest that other factors or agents may have contributed to the development of neoplastic disease.

The possible relationship between exposure to petroleum solvents and kidney cancer has already been noted (Kitchen, 1983). An excess risk of bladder cancer has been associated with cigarette smoking (Matanowski and Elliot, 1981). Brown and Kaplan were not able to document smoking history; however, they calculated the possible effects of smoking on the risk for bladder cancer (based on Axelson, 1978) (Brown and Kaplan, 1987). They concluded that the three-fold increase in bladder cancer among the cohort could not be attributed to smoking.

The currently available epidemiologic studies add limited information to the understanding of the health hazards associated with occupational exposure to PCE. Although there is some indication that use of dry-cleaning solvents poses a health risk, the contribution of individual solvents to the overall problem is far from clear. Until studies are completed that include a thorough analysis and quantification of PCE exposures, epidemiological studies will not be useful for the assessment of the human health risks of PCE.

TOXICITY TO MAJOR ORGANS AND SYSTEMS

Information on human toxicity following exposure to PCE has been obtained from case reports of accidental exposures, as well as from a limited number of experimental studies. Human health effects from short- or long-term exposures are similar to those observed in animals. Perchloroethylene initially affects the CNS, and larger doses cause various degrees of hepatic and renal damage. Table 4-1 summarizes human health effects resulting from experimental inhalation exposures to PCE.

TABLE 4-1. HUMAN HEALTH EFFECTS FROM EXPERIMENTAL INHALATION EXPOSURE TO PCE.

Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
20	5 d/wk for 5 wk	7.5 h/d	No change in EEG[a]	Stewart et al., 1974
75 to 80	Single exposure	1 to 4 min	Slight eye irritation	Stewart et al., 1961b
100	Single exposure	7 h	Headache; sleepiness; transient eye, nose, and throat irritation; abnormal modified Romberg test scores[b]	Stewart et al., 1970
100	5 d/wk for 5 wk	7.5 h/d	Increase in delta-wave activity on EEG[a]	Stewart et al., 1974
100 to 120	Single exposure	4 to 6 min	Soft palate irritation	Stewart et al., 1961b
106	Single exposure	1 h	Transient eye irritation; congestion of frontal sinuses	Rowe et al., 1952
150	5 d/wk for 5 wk	7.5 h/d	Lower scores in Flanagan test[c]	Stewart et al., 1974
200	Single exposure	6 to 30 min	Normal Romberg test scores[b]	Stewart et al., 1961b
216	Single exposure	45 min to 2 h	Eye irritation; sinus congestion; inebriation	Rowe et al., 1952
210 to 244	Single exposure	30 to 187 min	Lightheadedness; difficulty maintaining normal Romberg test[b]	Stewart et al., 1961b

TABLE 4-1. (Continued)

Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
280	Single exposure	2 h	Lightheadedness; eye irritation; sinus congestion; transient nausea	Rowe et al., 1952
475	Single exposure	130 min	Eye irritation; sinus congestion; slight feeling of elation	Carpenter, 1937
600	Single exposure	10 min	Eye and nose irritation; dizziness; diminished motor coordination; some loss of inhibition	Rowe et al., 1952
911	Single exposure	95 min	Lassitude; inebriation; exhilaration; mental fogginess	Carpenter, 1937
1060	Single exposure	1 to 2 min	Eye and upper respiratory tract irritation	Rowe et al., 1952
2000	Single exposure	7.5 min	Exposure terminated because all subjects felt faint	Carpenter, 1937

[a]EEG: electroencephalogram.

[b]Romberg test: measurement of ataxia.

[c]Flanagan test: measurement of coordination.

Liver

The earliest reports of PCE-induced liver damage are associated with its use as an anti-helminth in the 1920's and 1930's. Hundreds of thousands of individuals were treated with PCE for hookworm infestations. The PCE was typically given as a single oral dose of 0.12 mL/kg (maximum of 5 mL)(Reichert, 1983). The PCE reportedly produced hepatic necrosis on some occasions. Damage was transient and recovery took place within 1 to 2 weeks (Hall and Schillinger, 1925; Lambert, 1933). Meckler and Phelps documented a case in which an individual developed hepatitis after a massive inhalation exposure to PCE (of undetermined concentration) (Meckler and Phelps, 1966). The individual's liver remained enlarged 6 months after the exposure. Interestingly, in a separate case of oral overexposure reported by Koppel and co-workers, an individual did not have any measurable liver or kidney damage after ingestion of 8 to 10 mL of relatively pure PCE. Clinical measurements of organ function that were within normal limits included SGOT, SGPT, alkaline phosphatase, red and white blood-cell counts, and serum creatinine. However, the individual was hospitalized for treatment within 1 hour of ingestion, which probably averted organ damage (Koppel et al., 1985).

Stewart and co-workers have investigated several cases of acute overexposure to PCE that caused liver damage. The earliest report documented the clinical effects of occupational exposure to the vapor of a petroleum-based solvent mixture that contained approximately 50% PCE (Stewart et al., 1961). The individual was exposed to this mixture for about 3.5 hours, which caused a loss of consciousness. Simulation of exposure conditions gave an estimated concentration of 250 ppm, with levels that reached 1000 ppm for the last 30 minutes. Nine days after the incident, urinary urobilinogen and serum bilirubin levels were elevated, an indication of liver impairment. On the 18th day after exposure, a slight elevation of SGPT was measured (Stewart et al., 1961a). In a separate incident reported by Stewart, a worker was overcome by PCE vapors (of unknown concentration) (Stewart, 1969). The exposure lasted about 10 minutes and produced hepatic dysfunction. Clinical measurements of liver function were normal shortly after exposure. A slight increase in SGOT levels was measured on the third and fourth days following exposure, and urinary urobilinogen levels were elevated on the ninth day. Elevated serum enzymes were also measured by Hake and Stewart after a massive overexposure to PCE (estimates of the exposure concentration were not made) (Hake and Stewart, 1977). The individual was found lying in a pool of PCE, some of which was probably absorbed dermally as well as through the lung. Recovery was complete within 21 days.

Eight of nine firemen exposed to PCE vapors for 3 minutes (unknown concentration) had elevated SGOT levels. Hepatomegaly and splenomegaly were also found in one individual. Normal function was regained within 22 to 63 days (Saland, 1967).

The effects of chronic occupational exposure to PCE vapor were studied in seven individuals exposed for 2 to 6 years (Coler and Rossmiller, 1953). Short-term tests indicated that exposure levels ranged from 232 to 385 ppm. The authors assumed that these measurements were representative of levels that workers were exposed to 8 hours/day, 5 days/week. Of the 7 individuals, 3 had

abnormal liver-function tests, and 1 was diagnosed as having cirrhosis; none of the four individuals consumed alcohol. Consequently, liver toxicity in humans appears to occur at a concentration similar to that of mice, approximately 200 ppm.

Kidneys

In animals, kidney damage generally occurs at exposure levels greater than those that cause liver toxicity. This trend seems to hold true for humans as well. Several cases of overexposure great enough to cause loss of consciousness have not produced measurable kidney damage (Stewart et al., 1961a; Stewart, 1969; Patel et al., 1973; Patel et al., 1977). In 1 case of overexposure (where an individual was unconscious in a pool of PCE for 12 hours), kidney damage was measured by proteinuria and hematuria. These effects lasted for 20 days and for 8 days, respectively (Hake and Stewart, 1977). Kroneld and colleagues conducted *in vitro* experiments using isolated human uroephithelial cells (Kroneld et al., 1987). Viability of human uroepithelial cells decreased with PCE concentrations increasing from 0.2 to 16 μg/l.

Lungs

Pulmonary edema has been documented in only one instance. Exposure to PCE was probably massive (>1500 ppm) because the individual was comatose and required mechanical ventilation upon admission to the hospital. Recovery was complete within 4 days (Patel et al., 1977).

Boulet reported a case of an 18-year old student who developed intermittent dyspnea, cough, wheezing, and tightness of the chest after two months occupational exposure to PCE (Boulet, 1988). The symptoms increased in severity over a 6-month period. Progressive dyspnea with a fall in FEV_1 was observed following PCE challenge (concentration and duration not specified). FEV_1 values returned to within 10% of normal after 6 hours.

Blair and associates reported mortality from emphysema was significantly increased (SMR = 2.0) among dry cleaning union members (Blair et al., 1990). However, the lack of PCE exposure data and failure to consider potential confounding factors prevents any conclusion as to an association between PCE exposure and emphysema.

Skin and Eyes

Dermal contact with PCE causes localized irritation; prolonged exposure can cause erythema, first- and second-degree burns, and blistering (Gold, 1969; Stewart et al., 1961b; Hake and Stewart, 1977). The PCE vapor is also irritating to the eyes (Carpenter, 1937; Rowe et al., 1952). Exposure of human volunteers to 106 ppm produced transient eye irritation; this became more pronounced when the concentration was increased to 216 ppm (Rowe et al.,

1952). Stewart and colleagues has also reported eye irritation when humans were exposed to 100 ppm for a period of 7 hours (Stewart et al., 1970).

Redmond and Schappert reported a patient developed dermatitis at points of contact with a uniform and hat. Five additional cases were found among workers at the same facility. The outbreak was traced to dry cleaned and sealed garments found to contain 0.8 to 32 ppm PCE. After substitution of PCE with water-based detergents as cleaning agents, no further cases were observed (Redmond and Schappert, 1987).

Connective Tissue

Sparrow described a patient who had a connective tissue disorder with similarities to a syndrome observed in vinyl chloride workers (Sparrow, 1977). The individual in question was exposed to PCE vapor during his work at a dry cleaners. No measurements of concentration were reported, but at least once a week (over a 4-year period), exposure was high enough to cause dizziness and sleepiness. The individual displayed pathological changes in the skin of the hands, acrocyanosis, and polymyopathy. Abnormalities in the immune system and in hepatic function (mild hepatitis) were also documented. The patient may have been abnormally sensitive to PCE, perhaps related to an existing abnormality in the immune system. Indications that the individual did have an abnormal immune system are suggested by intermittent alopecia areata (since childhood), vitiligo (an apparently autoimmune condition characterized by destruction of melanocytes), and an absence of immunoglobulin A (IgA). This appears to be a unique case report, whose information is confounded by the abnormal medical history of the individual. Although PCE exposure may have contributed to the disease, it is not clear if PCE was the sole causative agent.

Central Nervous System

Acute exposure to PCE generally causes temporary CNS effects such as dizziness, headache, and confusion. However, massive single exposures can cause loss of consciousness (Patel et al., 1973; Patel et al., 1977; Hake and Stewart, 1977), and have been fatal in at least two instances (Lukaszewski, 1979; Levine et al., 1981). Protracted exposure produces symptoms similar to those observed after short-term exposure, although the effects apparently persist, even after exposure is terminated.

Rowe and associates did not observe any CNS effects in humans exposed to 106 ppm (single exposure of unspecified duration) (Rowe et al., 1952). Minor CNS effects were produced by 216 ppm (45 minutes to 2 hours), and a 10-minutes exposure to 600 ppm significantly affected motor coordination. Eleven volunteers exposed for a single 7-hour period to 100 ppm experienced headache, dizziness, and somnolence (Stewart, 1970). Three of the 11 had abnormal scores on the Romberg test, which measures ataxia. Tests of coordination, visual inspection, visual acuity, and depth perception were normal. Carpenter noted only minor and transient CNS effects in individuals exposed to 500 ppm

for 2 hours (Carpenter, 1937). When the same subjects were exposed to 911 ppm, they complained of lassitude, exhilaration, and inebriation.

In a study conducted by Stewart and co-workers, individuals were exposed to 20 to 150 ppm of PCE, 7.5 hours/day over a 5-week period (Stewart et al., 1974). No alterations in the EEG were noted at 20 ppm, but some aberrant EEG tracings were seen after 100 ppm. A decrease in Flanagan Coordination Test scores was observed following exposure to 150 ppm. In a subsequent experiment, individuals were repeatedly exposed to 0, 25, or 100 ppm of PCE, 5.5 hours/day over 11 week (Stewart et al., 1977). CNS effects were observed by measuring the subjects' response on the Romberg, Michigan Hand-Eye Coordination, and Flanagan Coordination Tests, and on the EEG. In contrast to the 1970 study, exposure to 100 ppm did not produce abnormal scores on the Romberg Test. PCE at 100 ppm did cause a significant decrease in Flanagan Coordination Test scores.

Coler and Rossmiller documented subjective complaints of malaise, dizziness, headache, lightheadedness, and intoxication in individuals regularly exposed to concentrations of 232 to 385 ppm PCE (Coler and Rossmiller, 1953). Gold described a case history of an individual exposed to PCE vapors, 6 to 7 days a week for 3 years (Gold, 1969). The individual was hospitalized after exhibiting confusion, disorientation, agitation, and an inability to concentrate. A neurological examination revealed a normal EEG. However, his performance on psychological tests that required concentration was "poor", and he showed "marked confusion." These problems persisted over a 12-month follow-up period, although there was no further exposure to PCE (the individual was lost to follow up at this time). Gold concluded that there was suggestive evidence of both cerebral and cortical damage, and basal ganglia involvement (Gold, 1969). However, it was not possible to obtain conclusive evidence of any neurological damage. Gregersen and colleagues examined 65 Danish workers exposed to various organic solvents for neurotoxic effects (Gregersen et al., 1984). Although only 15% of the cohort were exposed to PCE (approximately 100 ppm), the results are intriguing. Individuals were examined for intelligence, given a neuropsychological exam, and were subjected to a number of neuropsychological tests. The authors concluded that solvent exposure was correlated with acute neurotoxic symptoms, as well as longer-lasting symptoms of intellectual impairment. A relationship between exposure and signs of peripheral neuropathy was also observed.

McMullen reported on a case in which an individual was exposed to PCE vapor (500 ppm) for an undetermined period of time (McMullen, 1976). CNS depression was observed; the effects were described as resembling alcohol intoxication. No clinical measurements of sensory or organ function were made, but the individual apparently recovered within 6 hours.

Freed and Kandel reported a case study of a 68-year old male previously diagnosed with progressive dementia due to Alzheimer's disease (Freed and Kandel, 1988; Kandel and Freed, 1989). Complete alopecia and loss of fingernails also occurred around the onset of memory impairment. Neuropsychological testing revealed impairments in short-term memory, which improved to normal over a period of months. The subject had used PCE as a dry cleaner for over 30 years. The shop had been cited by the state of California

in 1981 for unacceptable vapor levels. A new ventilation system was then installed, following which his condition reportedly partially improved. A serum sample was analyzed and PCE was detected at 745 ppb. The authors suspected the memory impairment was associated with long-term PCE exposure.

Seeber studied neurobehavioral effects associated with long term human exposure to PCE (Seeber, 1989). Based on monitoring of ambient PCE in dry cleaning shops, 57 workers were classified as having low PCE exposures (time weighted average of 83.4 mg/m^3, or 12 ppm) and 44 as having high exposures (time weighted average of 363.8 mg/m^3, or 53 ppm). Age, sex, and preexposure intelligence were considered. PCE exposed workers showed significantly impaired functioning on tests of perceptual function, attention, and intellectual function relative to control subjects. The significance of these findings are limited by the lack of significant differences between the high and low exposure groups.

Benes and associates exposed human volunteers to PCE (concentration and purity not specified) for three 90 minute intervals during a single day. The average peak blood concentration of PCE for 14 subjects was 3.0 mg/l. Mean errors of omission in a test of acoustical discrimination of spatial localization of click sounds, "microsleeps" and sleepiness were determined to be significantly different after PCE exposure compared with pre-exposure observations (Benes et al., 1986).

Reproductive System

Bosco and co-workers studied the health status of 67 women employed in dry cleaning shops (Bosco et al., 1987). Urinary excretion of trichloroacetic acid over a 24-hour period was monitored as a indirect indicator of PCE exposure. Among 40 of the dry cleaners a mean urinary TCA concentration of 4.0 μg/l was reported, while 13 control subjects had a mean of 1.6 μg/l. No significant differences in pregnancies, live births, birth weight, spontaneous abortions, birth defects per live birth or still births per live birth were noted between dry cleaners and the control group. However given the very small study size, no conclusions can be drawn from the study. Furthermore, monitoring alveolar air and blood concentrations of PCE are much better indicators of PCE exposure than urinary TCA, and such monitoring was not conducted.

Kyyronen and associates studied the outcome of pregnancies among 419 dry cleaning workers (Kyyronen et al., 1989). PCE exposure was found to be significantly associated with spontaneous abortion (odds ratio 3.6, $p<0.05$).

Zielhuis and associates surveyed 68 women, employed in the dry cleaning profession and not using oral contraceptives, to determine the prevalence of menstrual disorders (Zielhuis et al., 1989). No data on actual PCE exposures was available. These results were compared to those of 76 laundry workers presumed to be without significant occupational PCE exposure. Odds ratios ranged from 3.6 for premenstrual syndrome to 0.8 for polymenorrhea. While odds ratios for 7 out of 8 menstrual disorders studied were elevated, the

small study size and lack of PCE exposure data prevent any conclusions to be drawn from the study.

Ahlborg and co-workers assessed adverse pregnancy outcomes among women employed in laundry or dry-cleaning positions (Ahlborg et al., 1990). Effects studied were spontaneous abortion, perinatal death, congenital malformations and birth weights of less than 1,500 g. Pregnancy outcomes were assessed by national registries. Exposures were roughly estimated by questionnaire. The adjusted odds ratio for PCE exposure during the first trimester was calculated as 1.1 after consideration of confounding factors. The small study size and the low odds ratio do not allow any positive or negative association to be drawn between PCE exposures and adverse pregnancy outcomes.

Cardiovascular System

Some organic solvents have been associated with cardiac arrest due to ventricular fibrillation. It is thought that these compounds sensitize the heart to epinephrine-induced arrhythmias. There is suggestive evidence that PCE has this effect in animals (see Section 3), and there is one report on PCE-induced cardiac arrhythmias in humans. Abedin and associates observed that occupational exposure to PCE probably caused dizziness and premature ventricular contractions in one case (Abedin et al., 1980). Although PCE may not have been the only factor in this response, removal from exposure to PCE alleviated the symptoms.

Hara and colleagues found no significant differences in the prevalence of electrocardiographic abnormalities between 200 dry cleaning workers and a control group (Hara et al., 1985). However, 31 workers with direct exposures and 24 workers with indirect exposures to PCE were found to have increased wall thickness to radius ratios and decreased fractional shortening of the left ventricle internal dimension relative to controls. A greater effect was seen in directly-exposed workers than in indirectly-exposed workers. Decreased circumferential fiber shortening mean velocity was found in directly exposed workers relative to controls. The significance of this study is difficult to evaluate due to the small sample size, the possibility of confounding factors, and the lack of PCE exposure data.

TERATOGENIC EFFECTS

There is no published information on the teratogenicity of PCE in humans.

MUTAGENIC EFFECTS

Ikeda and co-workers examined lymphocytes from individuals exposed to PCE for 3 months to 18 years (Ikeda et al., 1980). Chromosomal aberrations, sister-chromatid exchanges, and alterations of the mitotic index were the cytogenetic effects studied. The exposure level in 1 group of workers was 92 ppm (geometric mean), while a second group was exposed to 10 to 40 ppm (the authors did not give TWA exposure concentrations). Although a control group

was included, the criteria used to select and/or match controls was not reported. Exposed individuals did not have a significantly greater frequency of chromosomal aberrations or sister-chromatid exchanges, nor were there any substantial differences in the mitotic index.

Seiji and associates followed up with additional studies on sister chromatid exchanges in peripheral lymphocytes of workers exposed to PCE (Seiji et al., 1990). PCE exposures were monitored over 8 hour work days. No significant increase in SCE frequency was found for the PCE-exposed group (N=27) over control workers (N=26).

Trichloroethanol is a metabolite of PCE isolated from the urine of humans. Gu and associates reported a slight increase in the number of sister-chromatid exchanges per cell in human lymphocytes exposed to 178 mg/L of trichloroethanol (Gu et al., 1981). Neither of these studies is adequate to assess the mutagenic potential of PCE and its metabolites in humans.

SUMMARY OF EVIDENCE OF HUMAN CARCINOGENICITY

Evaluation of the carcinogenic potential of a chemical is based on the results of short-term assays of mutagenesis, pharmacological data (e.g., distribution and metabolism), lifetime animal bioassays, and epidemiological evidence. Several agencies and groups have developed systems of classification for evaluating evidence on the carcinogenic activity of a substance. The International Agency for Research on Cancer (IARC) separates strength of evidence of carcinogenic activity into 4 groups: sufficient evidence, limited evidence, inadequate evidence, and no evidence of carcinogenicity (IARC, 1982). Inclusion in any of these categories is based on data from short-term assays, as well as animal and human studies (if available).

EPA uses a weight of evidence approach, and uses 6 categories to characterize the overall evidence of carcinogenic potential to human: group A - Known Human Carcinogen (based on sufficient human evidence), group B - Probable Human Carcinogen (further separated into B1 and B2, based on limited human evidence or sufficient animal evidence, respectively), group C - Possible Human Carcinogen (based on limited animal evidence), group D - Not Classified (due to inadequate animal evidence), and group E - No Evidence of Carcinogenicity for Humans (EPA, 1984a).

In the absence of sound 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 those noted in controls, a decrease in latency (time to tumor development), development of rare tumors, and an increase in the number of tumors in individual animals.

In 1982 and 1987, the IARC evaluated available information on PCE and determined that there was "inadequate" evidence to conclude that PCE is carcinogenic to humans (IARC, 1982). An analysis of animal data gave sufficient evidence of carcinogenicity (IARC, 1987); evidence for any activity of PCE in short-term tests was "inadequate" to judge its carcinogenic

potential (IARC, 1982). Thus, IARC placed PCE in category 2B (possible human carcinogen), indicating there was sufficient evidence of carcinogenicity in animals and inadequate evidence in humans.

The *EPA Health Assessment Document for Tetrachloroethylene (Perchloroethylene)* has also analyzed the evidence of carcinogenicity of PCE (EPA, 1985a). This evaluation included an extensive review of short-term test results, data from animal tests, and several epidemiological studies. The EPA concluded that the evidence for the carcinogenicity of PCE in animals is "limited," and that the epidemiological data were inconclusive. Perchloroethylene was placed in group C, a possible human carcinogen (EPA, 1985a). However, a re-evaluation of the evidence for PCE carcinogenicity was conducted by the EPA. The EPA Carcinogen Assessment Group classified PCE within Group B2 as a probable human carcinogen (EPA, 1986), while the Science Advisory Board Halogenated Organics Subcommittee has concluded that a classification could not adequately be made under current EPA guidelines and that PCE should be placed on a continuum between B2 and C categories (EPA, 1987). As a result, the "C" classification based on the 1985 EPA report is still in effect, subject to a ruling by the EPA administrator.

5. QUANTIFICATION OF PCE'S CARCINOGENIC POTENCY

The term carcinogenic "potency" is used herein to mean the quantitative increase in tumorigenic risk per unit dose for very low exposure levels. Risks are predicted using the "linearized" multistage dose-response extrapolation model that has been generally used for cancer risk assessment by the California Department of Health Services (CDHS, 1985) and the U.S. Environmental Protection Agency (EPA) (EPA, 1985b; Anderson et al., 1983).

The following carcinogenic potency assessment proceeds in 5 steps: (1) selection of those cancer bioassay data sets suitable for dose-response assessment; (2) determination of the empirical relationship between applied dose and cancer risk for each data set selected; (3) determination of the relationship between administered dose and the amount of PCE metabolized for animals and humans; (4) expression of carcinogenic potency in terms of rate of metabolite formation; and (5) expression of carcinogenic potency in terms of estimated human applied dose, taking PCE metabolism into account.

SELECTION OF BIOASSAY DATA INDICATIVE OF PCE CARCINOGENICITY

The bioassays used in this potency assessment are the National Cancer Institute (NCI) study of mice exposed to PCE by gavage (NCI, 1977) and the National Toxicology Program (NTP) studies of mice and rats exposed to PCE by inhalation (NTP, 1986). The EPA used the gavage study as the basis of a carcinogenic potency assessment in 1985 (EPA, 1985a) and used the inhalation study to update this assessment in 1986 (EPA, 1986). In each of these studies PCE caused a significantly increased tumor incidence (see Section 3). These bioassay data sets represent the only long-term exposure studies with well-defined, exposure-response data that indicate a positive carcinogenic response for PCE in animals. Human epidemiological data suitable for dose-response assessment are not available.

Brief reviews of the administered dose and associated tumor response for the NCI gavage study with mice and for the NTP inhalation studies with rats and mice are given below. This information is summarized in Table 5-1, along with other information derived later, which also is tabulated here for convenience. From the doses (D) administered in those bioassays corresponding lifetime, time-weighted average (TWA) applied doses (A), are derived using certain standard assumptions (noted below).

As discussed in Section 2 PCE is extensively metabolized in mammals. The metabolites (rather than PCE) have been suggested to be responsible for almost all acute, chronic, and carcinogenic effects (e.g., Green et al., 1990, Mazzullo et al., 1987, Odum et al., 1988, Pegg et al., 1979, Schumann et al., 1980; Vamvakas et al., 1989). Postulated toxic metabolites of PCE that have been identified include trichloroacetyl chloride, trichloroacetic

TABLE 5-1. DOSE-RESPONSE DATA FOR SELECTED CANCER BIOASSAYS

Study species (strain)	Sex and weight (kg)	Administered dose or conc., D	TWA Applied dose, A. (mg/kg-d)	Tumor	
				Type	Incidence[a]
NCI, 1977		0	0		2/20
Mice	Male	536[b]	215.0[d]	hepatocellular carcinoma	32/48
(B6C3F1)	0.030	1072[b]	430.1[d]		27/45
		0	0		0/20
	Female	386[b]	154.9[d]	hepatocellular carcinoma	19/48
	0.025	772[b]	309.7[d]		19/45
NTP, 1986		0	0		7/49
Mice	Male	100[c]	146.6[e]	hepatocellular carcinoma	25/47
(B6C3F1)	0.037	200[c]	293.2[e]		26/50
		0	0		1/44
	Female	100[c]	153.9[e]	hepatocellular carcinoma	13/42
	0.032	200[c]	307.8[e]		36/47
		0	0		16/49
	Male	100[c]	146.6[e]	hepatocellular adenoma/carcinoma	31/47
	0.037	200[c]	293.2[e]		40/50

TABLE 5-1. DOSE-RESPONSE DATA FOR SELECTED CANCER BIOASSAYS

Study species (strain)	Sex and weight (kg)	Administered dose or conc., D	TWA Applied dose, A. (mg/kg-d)	Tumor	
				Type	Incidence[a]
		0	0		4/44
	Female	100[c]	153.9[e]	hepatocellular adenoma/carcinoma	17/42
	0.032	200[c]	307.8[e]		38/47
NTP, 1986		0	0		28/50
Rats	Male	200[c]	143.0[f]	mononuclear-cell leukemia	37/48
(F344/N)	0.44	400[c]	286.0[f]		37/50
		0	0		18/49
	Female	200[c]	159.0[f]	mononuclear-cell leukemia	30/50
	0.32	400[c]	318.1[f]		29/50

[a] Tumor-incidence denominator excludes animals dying before the occurence of the first corresponding tumor type observed in each study.

[b] Average administered daily gavage dose, D, in mg/kg-d, for a 5 d/wk exposure over 78 wk of a 90-wk bioassay.

[c] Average administered inhalation exposure in ppm 6 h/d, 5 d/wk over 2-years.

[d] Time-weighted average (TWA) dose: $A = D\ (5/7) \times (78/90)\ (90/104)^3$ (following Anderson et al. 1983).

[e] Total respired dose averaged over time: $A = D\ (6/24)\ (5/7)\ (0.0345)(\text{body weight}/0.025)^{2/3}$ $(6.78\ \text{mg PCE/m}^3\ \text{ppm})$ /body weight.

[f] Total respired dose averaged over time: $A = D\ (6/24)\ (5/7)\ (0.105)\ (\text{body weight}/0.113)^{2/3}$ $(6.78\ \text{mg PCE/m}^3\text{-ppm})$/body weight (following Anderson et al. 1983).

acid, and trichloroethanol; the formation of the reactive epoxide tetrachloroethylene oxide is thought to be the first step in the metabolism of PCE (Bouse et al., 1975; Greim et al., 1975). A role for metabolites formed in the glutathione conjugation pathway in some of the observed toxic, mutagenic and carcinogenic effects of PCE has been proposed. Other minor metabolites have also been identified and/or proposed. If a reactive metabolite is indeed responsible for the carcinogenic effects of PCE, then the total metabolized dose may be useful as a surrogate measure of the actual delivered dose. Since the metabolized dose is likely to be smaller than the administered dose, using the metabolized dose to calculate cancer potency would result in a higher potency value than would the administered dose. Using available data, carcinogenic potencies have been estimated on the basis of metabolized dose (denoted M), in addition to those based on the administered dose or "applied" dose (denoted A) (EPA, 1985a, 1985b, and 1985c; EPA Draft, 1986; CDHS, 1985). From pharmacokinetic data an empirical relationship was obtained between administered dose and the total metabolized dose.

PHARMACOKINETIC ANALYSES

In two previous risk assessments, EPA calculated the metabolized dose (M) as a function of the administered dose (D) for the NCI and NTP bioassay data using a simple steady-state pharmacokinetic (SSPK) approach (EPA, 1985a; EPA Draft, 1986). In using this approach the EPA did not take into account: (1) bioassay duration relative to expected lifespan; (2) male/female animal weight differences; (3) the treatment of species specific data on metabolic elimination pathways; (4) the scaling of certain kinetic parameters across species; and (5) the treatment of available human metabolic data. Therefore, a reanalysis of the relevant pharmacokinetic data was undertaken as part of the current potency assessment for PCE. This reanalysis, prepared to a great extent by staff at the Lawrence Livermore National Labs (LLNL), used the same basic SSPK approach that had EPA used. These two approaches are based on metabolic data which may not have been gathered under steady-state physiological conditions at ambient exposure levels.

Physiologically-based pharmacokinetics (PB-PK) is designed to consider physiological processes, biochemical kinetics, and tissue levels of the chemical (or its metabolites) under investigation, based on available biological data. This approach represents the body by compartments, into and out of which compounds are transferred by diffusion and blood flow. This can aid in cross-species comparison of metabolizing enzymes, metabolism pathways, organ volume and blood flow, absorption and distribution phenomena, differences in body size and composition, transport characteristics, and differences in the relative routes of detoxification and excretion. Results from PB-PK models of PCE metabolism for bioassay and environmental contexts are available and a detailed description of the approach has been published (EPA Draft, 1986; Hattis et al., 1987; Chen and Blancato, 1987; Travis et al., undated; Reitz and Nolan, 1986). Results using such approaches are presented below.

The use of metabolized, rather than applied, dose adds and/or emphasizes several uncertainties relevant to the analysis of the experimental dose-response data, including uncertainty in: 1) identifying the active carcinogenic species; 2) parameter estimates based on experimental animal data; 3) extrapolating kinetic constants between species; 4) some key constants estimated for humans based on limited human metabolism data; 5) the application of the non-steady state PB-PK model (which purportedly adjusts variables to steady-state conditions); 6) the detection of all human PCE metabolites; 7) the relative metabolic rates at environmental exposure levels; and 8) regarding the extent to which inter-individual variability relates to pharmacokinetic parameters, particularly for humans. The problem of inter-individual variability is, of course, common to many problems in predictive regulatory toxicology, not just pharmacokinetic analysis. For risk assessments using toxic endpoints other than carcinogenesis, this is generally dealt with by incorporating a safety factor. With regard to the other sources of uncertainty mentioned, an attempt is made here and in other reports (EPA, 1985a, 1985b, and 1985c; EPA Draft, 1986) to assess available metabolism data for internal consistency. Reviews of the uncertainties in pharmacokinetic modeling for perchloroethylene have recently been prepared (Hattis et al., 1990; Bois et al. 1990).

The alternative to using estimates of metabolized PCE dose in this analysis is to rely on the applied dose. This latter approach would not consider possible interspecies differences in: 1) absorption of the administered dose; 2) both the qualitative and quantitative metabolism of the PCE; and 3) relative rates of detoxification. However, the applied dose approach can be adjusted for absorption differences. The decision to use the applied dose or the metabolized dose model involves a trade-off between the additional parameter uncertainties associated with a physiologically-based pharmacokinetic model and a potential systematic error associated with an applied dose model. The optimum approach hinges on scientific/policy judgments concerning the quality of data underlying (1) identification of carcinogenic mechanism of PCE and (2) pharmacokinetic parameter estimates. The problems and benefits associated with both approaches are discussed below for the different bioassays.

METHODOLOGY

Evaluation Of Cancer Potency

To calculate potential risks associated with PCE exposure, the staff of CDHS used a linearized multistage model, which provides a reasonably health-protective risk estimate due to its property of being linear at low exposure levels. The lifetime probability of developing a tumor (p) induced by an average daily intake of chemical (d) is often assumed to be (CDHS, 1985; Anderson et al., 1983):

$$p(d) = 1\text{-}\exp\text{-}(q_0 + q_1 d + q_2 d^2 + \ldots + q_j d^j)$$

with constraints $q_i > 0$ for all i.

The q_i are parameters of the model which are taken to be constants and are estimated by maximum likelihood techniques from the data. The parameter q_0 represents the background lifetime incidence of the tumor. The q_1 value or some upper bound is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1*, the upper 95% confidence bound of q_1, as estimated by maximum likelihood techniques. When the dose is given in mg/kg-d, the parameters q_1 and q_1* are given in units $(mg/kg\text{-}d)^{-1}$. Details of the estimation procedure are given by Crump and colleagues (Crump et al., 1977). In some cases, experiments are terminated at a predetermined time regardless of the lifespan of the animal, e.g., at 12 months, 18 months, or 24 months. Although exposure may have occurred up to the termination of the experiment, the shortened lifespan may have prevented the expression of cancer. To estimate potency in animals (q_{animal}) from such experiments of duration Te, rather than the natural lifespan of the animals (T), it is assumed that cancer incidence increases with the third power of age:

$$q_{animal} = q_1^* \times (T/Te)^3$$

Following Gold et al. (1984) and the EPA (Anderson et al., 1983), the natural lifespan of mice and rats is assumed to be two years, so that for experiments lasting Te weeks in these rodents:

$$q_{animal} = q_1^* \times (104/Te)^3.$$

To estimate risk at low doses or exposure levels, the potency value is multiplied by the average daily dose.

Dose Adjustments

Several adjustments need to be made to the experimental exposures to calculate the lifetime daily exposure levels. Thus, for inhalation exposures, the reported dose must be multiplied by:

H/24: where H is the hours of exposure per day. This converts the exposure period to a time weighted average for 24 hours daily continuous exposure.

D/7: where D is the number of days exposed per week. This converts the dosing schedule to a time weighted average for a seven day/week continuous exposure.

L_e/L: where L_e is the length of the experimental exposure and L is the lifespan of the animal (the longer of L_e or 24 months). This converts the experimental protocol to a continuous lifetime exposure.

NCI (1977) Mouse Study: Gavage

Applied Dose and Tumor Incidence

In the NCI study, PCE in corn oil was administered to male and female B6C3F1 mice, beginning at about 5 weeks of age, by gastric intubation for 5 days/week for 78-weeks, followed by 12 additional weeks of pre-sacrifice observation (NCI, 1977). Incidence of hepatocellular carcinomas increased with dose for both males and females (see earlier discussion in Section 3). Table 5-1 gives the average administered daily dose, D, for the male and female vehicle control, low- and high-dose groups along with corresponding tumor incidence data (see EPA, 1985a). Note that D is the time-weighted average (TWA) dose (in units of mg/kg). In table 5-1, in contrast to Table 3-2 tumor-incidence data are given as the number of tumors found in animals which survived at least until the appearance of the first carcinoma in each study (which appeared at week 24 for female mice and at week 41 for male mice in the NCI study). The mortality-adjusted incidence data are used here in preference to unadjusted incidence data to partially correct for the influence of competing mortality risks.

Metabolized Dose

The pharmacokinetic data of Buben and O'Flaherty (1985) for PCE metabolism in mice were used to calculate the metabolized dose from the applied dose. In this study, PCE metabolism was examined in 3- to 5-month-old, male Swiss-Cox mice during subchronic administration of PCE by gavage in corn oil for 5 days/week for 6 weeks, exposure conditions similar to those of the NCI study. Seven different animal groups received dose levels ranging from 20 to 2000 mg/kg. Metabolism was estimated by measuring the daily excretion of the sole urinary metabolite detected, trichloroacetic acid (TCA). Dose-dependent metabolism was observed to be described by the Michaelis-Menten equation:

$$M_u = D \frac{V_{max,u}}{D + K_m} \qquad (5\text{-}1)$$

where M_u = TWA yield of urinary metabolite, mg/kg-d;
D = TWA daily administered dose, mg/kg-d;
$V_{max,u}$ = apparent maximum rate of urinary metabolite production, mg/kg-d; and
K_m = apparent Michaelis constant, mg/kg-d.

The value of M_u is essentially equivalent to the dose rate in mg PCE metabolized, since the molecular weights of PCE and TCA differ by less than 1.5%. In the Buben and O'Flaherty study, $V_{max,u}$ was estimated to be 136 mg/kg-d, while K_m was estimated to be 660 mg/kg-d (Buben and O'Flaherty, 1985).

If for different species the dose estimated from the urinary metabolites, M_u, were a constant proportion of total metabolized dose (which we shall denote as M), then it would make no difference whether M or M_u were used to derive a relationship between cancer risk and applied dose, D. But this proportion does not appear to be constant for different species. In B6C3F1 mice, for example, the mass-balance metabolic study by Schumann and co-workers reported that the relative amounts of urinary metabolites to total PCE found after either a 6-hour inhalation at 10 ppm or a single, oral dose of 500 mg/kg of radiolabeled PCE, were 80.0% and 82.3%, respectively (Schumann et al., 1980). These results were based on the assumption that metabolism was complete by 72 hours post-exposure and that cagewash radioactivity was attributable solely to urine content. For Sprague-Dawley rats, a metabolic study by Pegg and associates found that urinary metabolites comprised only 58.9% and 57.1% of the total PCE metabolized after exposures of 10 ppm for 6 hours and 1 mg/kg by injection, respectively, of radiolabeled PCE (and only 49.9% and 45.7% after the higher exposures of 600 ppm for 6 hours and 500 mg/kg by injection, respectively) (Pegg et al., 1979). Since both studies assumed complete metabolism by 72 hours post-exposure, the estimates of M would be decreased to a greater extent for rats than for mice. Thus, these two studies indicate that M_u was a greater fraction of M for the mice than for the rats. In addition, M_u was found to decrease with an increase in dose for rats.

Based on the proportion M_u/M indicated by Schumann et al. (1980), and the relationship between applied dose and urinary metabolite production indicated by Buben and O'Flaherty (1985), M_u and $V_{max,u}$ in mice are assumed to be 80% of M and V_{max}. Thus, the parameter V_{max} (which allows us to estimate the total amount of PCE metabolized) is 170 mg/kg-d (= 136/0.80, using the $V_{max,u}$ estimated by Buben and O'Flaherty, 1985). This assumes that PCE metabolism is quantitatively similar for B6C3F1 and Swiss-Cox mice. A single, oral dose of 500 mg/kg of PCE administered to B6C3F1 mice was found by Schumann et al. (1980) to yield an average metabolized dose of 85.02 mg/kg. This is 16% greater than the value of 73.26 mg/kg predicted by Equation 5-1 using a $V_{max,u}$ estimate of 170 mg/kg. Using a $V_{max,u}$ of 136 mg/kg yields an average metabolized dose of 58.6 mg/kg. These results did not agree with the PCE metabolism data obtained by Mitoma et al. (1985) for groups of four male 4-6 week-old B6C3F1 mice. Mitoma and co-workers reported that only 22 to 37% of radiolabeled gavage doses (900 and 225 mg/kg, respectively) administered after 4 weeks of similar, daily, non-labeled dosing, was metabolized. Fitting the Michaelis-Menten equations (5-4 and 5-5 below) to these two data points results in parameter estimates of V_{max} = 366 mg/kg and K_m = 765 mg/kg.

The advantage of using the data from Mitoma and associates in estimating Michaelis-Menten metabolic parameters is that the same strain of mouse and a similar exposure protocol were used in the 1977 NCI mouse bioassay (Mitoma et al., 1985; NCI, 1977). The drawbacks, on the other hand, are (1) that the mice studied by Mitoma and colleagues were quite young and therefore perhaps not representative of mice in the 90-week NCI bioassay, (2) the metabolite recovery period used by Mitoma and co-workers was only 48 hours, and (3) total radioactivity recovered by Mitoma and associates accounted for

only about 80% of the radiolabeled PCE administered. Thus, some models have chosen not to use the data from Mitoma and colleagues (1985) for PCE. However, it should be kept in mind that, for mice administered comparable dose levels, a 3- to 4-fold difference in the rate of PCE metabolism has been reported (22 to 82%). This leads to a two-fold difference in the calculated V_{max} (170 mg/kg-d to 366 mg/kg-d) in mice.

For gavage studies the apparent Km is unaffected by the transformation from M_u to M (see Equation 5-5 below). Under the assumptions stated above, it is possible to relate administered dose to the metabolized dose for the 1977 NCI mouse bioassay using the equation

$$M = \frac{1}{0.80} * \frac{D\,V_{max,u}}{D + K_m} \tag{5-2}$$

To convert M to a lifetime TWA, equation 5-2 needs to be adjusted for the specific exposure scenario being considered.

The EPA assumed that cumulative age-specific cancer rates increase as the third power of age (EPA, 1980; Anderson et al., 1983). Thus, given a bioassay duration of L_e and a natural test-species lifespan of L, the cancer potency value calculated using a multistage-risk-extrapolation model would be increased by the factor $(L/L_e)^3$ (EPA, 1980; Anderson et al., 1983). Equivalently, the lifetime TWA dose that would yield the corrected potency value would be the actual bioassay TWA dose multiplied by the factor $(L_e/L)^3$. In the 1977 NCI study, for example, the mice used were 5 weeks old at start, and their age at the end of the bioassay was 95 weeks; the L_e value used was 90 weeks (NCI, 1977). The median survival time for (control) mice was 104 weeks, i.e., L = 104 wk. For the NCI (1977) data, a partial lifetime-exposure-correction factor of $(90/104)^3$ was used. This is close to the value of $(95/108)^3$, which reflects the actual age at bioassay termination and assumes an experimental lifespan for B6C3F1 mice of about 25 months. The EPA used this approach to derive potencies for trichloroethylene (EPA, 1985b), but not for PCE (EPA, 1985a; EPA Draft, 1986).

Thus, the daily metabolite doses (M) in the NCI (1977) study were derived from corresponding values of applied dose, D, using the following modification of Equation 5-2:

$$M = \frac{D\,V_{max}}{D + K_m} * \frac{5}{7} * \frac{78}{90} * \left[\frac{90}{104}\right]^3 \tag{5-3}$$

where M and D are in units mg/kg-d, V_{max} and K_m were assumed to be 170 mg/kg-d and 660 mg/kg-d, respectively.

NTP (1986) Rat Study: Inhalation

In the 1986 NTP study, groups of fifty 8- to 9-week-old male and female F344/N rats were exposed to PCE by inhalation at 0, 200, or 400 ppm for 6 hours/day, 5 days/week over 2 years (NTP, 1986). The exposure concentration values for males and females appear in Table 5-1, along with the corresponding incidence of mononuclear-cell leukemia (MLK), the only tumor type observed to be significantly increased in rats at either the low or high dose levels. Again, the incidence values in the denominators appearing in Table 5-1 represent animals surviving at least until the appearance of the first MLK (which was at week 53 for males and at week 60 for females (NTP, 1986)).

The amount of PCE metabolized in the NTP study can be estimated by fitting the Michaelis-Menten equation to the data obtained from the metabolism study in Sprague-Dawley (SD) rats conducted by Pegg et al. (1979). This assumes F344 and SD rats metabolize PCE similarly. In the Pegg and associates study, rats exposed via inhalation to 10 or 600 ppm of radiolabeled PCE for 6 hours were found to have metabolized a total of 1.87 and 36.4 mg/kg of PCE, respectively, by 72 hours post exposure. Of these totals, 1.10 (or 59%) and 18.2 (or 50%) mg/kg, respectively, were in the form of urinary metabolites. Thus, both urinary and total PCE metabolite production reportedly demonstrated saturable kinetics that may be represented by Equation 5-1. When only 2 dose-response points, say D_1, M_1 and D_2, M_2, are available, as in the data from Pegg et al., the corresponding Michaelis-Menten parameter estimates are completely specified by the following equations:

$$V_{max} = \frac{D_2 - D_1}{\frac{D_2}{M_2} - \frac{D_1}{M_1}} \tag{5-4}$$

$$K_m = \frac{M_2 - M_1}{\frac{M_1}{D_1} - \frac{M_2}{D_2}} \tag{5-5}$$

Thus, V_{max} and K_m are estimated to be 24.6 mg/kg and 214 ppm based on the urinary metabolite data for a 6-hour exposure to PCE, and 53.0 mg/kg and 273 ppm based on the corresponding total metabolite data (Pegg et al., 1979). In its updated PCE potency assessment, the EPA used the former values based on urinary metabolism to derive the total metabolized doses for the NTP (1986) rat inhalation study (EPA Draft, 1986). The CDHS estimates of V_{max} and K_m are based on the total amount of metabolites generated by rats inhaling PCE as measured by Pegg et al. (1979). The V_{max} values expressed above in mg/kg of metabolites produced following a single 6-hour exposure were also used to estimate corresponding steady-state values (in mg/kg-d) for a repeated exposure of 6 hours/day.

Before estimating the rate of metabolite formation for the 1986 NTP rat bioassay, adjustments must be made to account for the interrupted exposure pattern (5 days/week) used in the NTP (1986) rat inhalation study, and to account for the fact that the rats used by Pegg et al. (1979) were lighter in weight compared to those used in the NTP study. The weight differences can be expected to result in corresponding differences in metabolic capacity and were taken into account in this analysis.

In the context of pharmacokinetic modeling, physiological parameters such as maximum enzymatic reaction rates and related metabolic clearance rates are generally assumed to vary with basal metabolic rate in proportion to body surface area (or, approximately, to body weight raised to the two-thirds power), rather than to body weight per se (Gehring et al., 1978; Dedrick and Bischoff, 1980; Anderson et al., 1980; Andersen et al., 1980; Calabrese, 1983; Ramsey and Andersen, 1984; EPA, 1985a; EPA Draft, 1986; NAS, 1986). Accordingly, given an estimated Vmax in mg/hour for an animal of weight w_1, the corresponding predicted value for animals of weight w_2 would be $V_{max}(w_2/w_1)^{2/3}$ or, for V_{max} expressed in mg/kg-hour, $V_{max}\ (w_2/w_1)^{2/3}$. 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-Menten constant, K_m, for a given compound is generally assumed to be independent of body size (in the absence of data indicating otherwise) when this constant is expressed as the reactant concentration in units mg/L blood (Ramsey and Andersen, 1984; EPA Draft, 1986; NAS, 1986). Since blood weight is proportional to body weight for animals of widely varying weights (Adolph, 1949; 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. Under this assumption, however, apparent K_m values expressed in terms of an inhaled air concentration (e.g., ppm), as opposed to a blood concentration, would not be expected to be constant for animals of widely varying size unless pharmacokinetic steady-state conditions applied. Upon initial exposure or after an exposure of short duration (relative to the half-life of metabolism/elimination), the maximum rate at which an inhaled toxicant reaches an organ is related to the rate of pulmonary uptake. Since weight-normalized breathing volume decreases as body weight increases, an apparent inhalational K_m in ppm for animals of a given weight is expected to be higher for heavier animals (Anderson et al., 1980). That is, the heavier the animal the higher the air concentration required to achieve a specified target organ concentration (corresponding to the actual K_m).

Respiration rate is observed to be proportional to body surface area (or, approximately, to body weight to the two-thirds power) (Guyton, 1947; Adolph, 1949; EPA, 1980b; Anderson et al., 1983; Calabrese, 1983). Accordingly, for brief, nonsteady-state and/or discontinuous exposure scenarios such as those present in the Pegg et al. (1979) and Schumann et al. (1980) metabolic studies and (to a certain extent) in the 1977 NCI and the 1986 NTP bioassays, the assumed corresponding predicted value of Km for animals of weight, w_2, would be $K_m\ (w_2/w_1)^{1/3}$ ppm, given an estimated apparent inhalational Km in ppm for an animal of weight, w_1. Under this assumption, lifetime TWA equivalent values for total PCE metabolized dose, M, in the

1986 NTP rat study is derived from corresponding values of applied dose, D, using the following modification of Equation 5-3:

$$M = \frac{D\, V_{max} \left[\frac{W_1}{W_2}\right]^{1/3}}{D + K_m \left[\frac{W_2}{W_1}\right]^{1/3}} \qquad (5\text{-}6)$$

where M is in mg/kg-d and D is in ppm, V_{max} is 52.982 mg/kg-d and K_m is 273.32 ppm, and in which w_1 and w_2 are the weights of the Pegg et al. (1979) rats (0.25 kg) and the 1986 NTP rats (0.44 or 0.32 kg), respectively. Values of D and M derived from Equation 5-6 for the 1986 NTP bioassays for male and female rats are listed in Table 5-2, along with corresponding tumor-incidence data.

NTP (1986) Mouse Study: Inhalation

In the 1986 NTP study, groups of fifty 8- to 9-week-old male and female B6C3F1 mice were exposed to PCE by inhalation at 0, 100, or 200 ppm for 6 hours/day, 5 days/week over 2 years (NTP, 1986). These exposure concentrations appear in Table 5-1, along with the corresponding incidence data for hepatocellular carcinoma (HC) and hepatocellular adenoma or carcinoma (HAC), the only tumor types observed to be significantly increased in the mice at either the low- or high-dose levels. Again, the incidence-rate denominators appearing in Table 5-1 represent animals surviving at least until the appearance of the first HC (which was at week 60 for males and week 67 for females; cf. NTP, 1986). Since one female mouse was missexed in the control group, the tumor-incidence-rate denominator used for that group appearing in Table 5-1 is 44 (NTP, 1986). In a draft form of its updated carcinogenic risk assessment for PCE, the EPA used a denominator value of 46 for the control female mice (EPA Draft, 1986). The latter value is not consistent with the data published by the NTP (1986).

Lifetime TWA values for the total metabolized dose (M) of PCE for the 1986 NTP mouse bioassay are derived from Equation 5-6. The mouse data of Schumann et al. (1980) on PCE metabolism following inhalation or injection provide insufficient information for estimating V_{max} and K_m using Equation 5-6, because only one exposure level was used for each route. The value of V_{max} for the total amount of metabolites formed was assumed to be 170 mg/kg-d, estimated from the PCE oral administration data of Buben and O'Flaherty (1985). The value of K_m was estimated from the rat inhalation data of Pegg et al. (1979) and was calculated to be 126 ppm ($= 273.32 \times (w_1/0.25\ kg)^{1/3}$) where w_1 represents the body weight of one mouse and 0.25 kg is the approximate weight of one rat in the study by Pegg et al. (1979). The values for mice of differing weights were estimated using Equation 5-6, where w_1 and w_2 are the weights of the mice from the Schumann et al. study (0.0245 kg) and the 1986 NTP mice (0.037 or 0.032 kg). One of the assumptions made in using this approach is that (lacking evidence to the

TABLE 5-2.

METABOLIZED DOSE CALCULATIONS FOR SELECTED CANCER DATA SETS

Study species (strain)	Sex and weight (g)	Experimental applied dose or conc., D[a]	Average Daily Metabolized Dose (M) in mg/kg-d: LLNL[b]	EPA equiv.[c]	EPA equiv. PB-PK[d]	Reitz & Nolan equiv. PB-PK[e]	Hattis et al. equiv. PB-PK[f]
NCI, 1977 Mice (B6C3F1)	Male 0.030	0 mg/kg	0	0	--	--	0
		536 mg/kg	30.6	30.6	--	--	77.6
		1072 mg/kg	42.2	42.2	--	--	108.8
	Female 0.025	0 mg/kg	0	0	--	--	0
		386 mg/kg	25.2	25.2	--	--	64.9
		772 mg/kg	36.8	36.8	--	--	93.5
NTP, 1986 Mice (B6C3F1)	Male 0.037	0 ppm	0	0	0	0	0
		100 ppm	43.3	35.0	13.5	65.4	44.5
		200 ppm	61.4	53.1	19.5	93.8	74.6
	Female 0.032	0 ppm	0	0	0	0	--
		100 ppm	46.7	35.0	13.5	67.5	--
		200 ppm	65.8	53.1	19.5	96.9	--
NTP, 1986 Rats (F344/N)	Male 0.44	0 ppm	0	0	0	--	0
		200 ppm	11.8	15.7	14.9	--	14.1
		400 ppm	17.2	21.2	18.6	--	22.2
	Female 0.32	0 ppm	0	0	0	--	--
		200 ppm	14.0	15.7	14.9	--	--
		400 ppm	20.0	21.2	18.6	--	--

[a]For the NCI study, average administered daily gavage dose, D, in mg/kg-d, for a 5 d/wk exposure over 78 wk of a 90-wk bioassay; for the NTP study, average administered inhalation exposure in ppm for a 6 h/d, 5 d/wk exposure over a 2-y bioassay.

[b]LLNL refers to the Lawrence Livermore National Labs' approach used herein.

[c]Here M = M* W/F, where M* is the "estimate of dose metabolized", which for NCI study is given in Table 9-9 of EPA (1985a) and the NTP study given in Table 4-3 of EPA (1986). W is $(78/90)(5/7)(90/104)^3$ for mice in the NCI study, and W is (5/7) for mice and rats in the NTP study. F equals 0.80 and 0.54 for mice and rats, respectively.

[d]These values for the NTP mouse and rat studies are derived from information in Tables A-4 and A-3, respectively, in EPA (1986). Metabolized doses appear to be low for mice (by a factor of about 3) due to the underestimated mouse alveolar ventilation rate (EPA, 1986; see p. 4-15 and p. A-17).

[e]Derived from the values for amount metabolized ("AM" in mg/day) given on page 6 of Reitz and Nolan (1986).

[f]Derived using the Mitoma (1985) data by Hattis et al. (1987).

contrary), there are no differences in the metabolism of PCE between oral and inhalation exposure. Thus, after scaling for differences in body size, it is possible to estimate the V_{max} and K_m for PCE metabolism based on both a mouse oral study and a rat inhalation study.

The Schumann et al. (1980) data on metabolism following PCE inhalation by mice for 6 hours at 10 ppm may be used to check the estimates of V_{max} and K_m. They found that a total of 14.5 mg/kg PCE was metabolized by mice within 72 hours after being exposed to 10 ppm PCE for 6 hours. The 14.5 mg/kg PCE value is 16% greater than the predicted value of 12.5 mg/kg using Equation 5-6 with a D of 10 ppm, a V_{max} of 170 mg/kg, and a K_m of 126 ppm. For this case w_2 and w_1 are equivalent and the factor 5/7 relating to the NTP exposure scenario is deleted.

Results of Metabolized Dose Calculations

Metabolized PCE doses described in detail here, referred to as the LLNL approach are summarized and compared in Table 5-2 to values calculated by the EPA (1985a; 1986), Reitz and Nolan (1986) and Hattis and associates (1987) using alternative approaches to metabolic analysis. Their values have been adjusted to correspond to a lifetime TWA total metabolized dose. The discussion is representative of the type of analysis used in estimating the apparent target tissue dose.

For the NTP inhalation study, the metabolized doses estimated for mice in either exposure group range 5-fold. However, the actual range may be smaller, due to an underestimate of the mouse alveolar ventilation rate by the EPA (1986), which would reduce the estimate of the amount of PCE metabolized (Table 5-2). This 5-fold range indicates the magnitude of some of the uncertainties involved in the modeling and parameter selection from limited data. The importance of data selection is discussed extensively by Hattis et al. (1990).

CARCINOGENIC POTENCY EXTRAPOLATION BASED ON ANIMAL BIOASSAY DATA

Carcinogenic "potency" refers to a quantitative expression of increased tumorigenic response per unit dose at very low dose levels. The following carcinogenic potency assessment is based on a quantitative analysis of animal bioassay data sets, assuming that PCE is carcinogenic to both animals and humans at low environmental dose levels. The rationale for using this assumption for PCE is well-established and is discussed in detail elsewhere (EPA, 1980; Anderson et al., 1983; CDHS, 1985). Arguments against using this assumption for PCE focus on the possibility that the carcinogenicity of PCE in bioassays conducted at high doses may be caused primarily by increased cellular and/or subcellular proliferation, i.e., by tumor promotion or some epigenetic mechanism, rather than by initiation or some genotoxic mechanism, and that associated dose-response relationships contain a threshold below which carcinogenic effects do not occur (e.g., Schumann et al., 1980; Stott et al., 1982; Buben and O'Flaherty, 1985; Elcombe et

al., 1985; Green and Prout, 1985; Prout et al., 1985; Mirsalis et al., 1985; Odum et al., 1988).

Low-dose potency extrapolation from dichotomous tumor-response information in selected animal-bioassay data sets was performed as described by Crump and Watson (1979) to numerically fit parameters q_i of the multistage dose-response extrapolation model:

$$\textit{Probability of Cancer } (R) = 1 - e^{-\sum_{i=1}^{g} q_i d^i} \quad \text{with } q_i \geq 0 \text{ for all } i. \qquad (5\text{-}7)$$

in which g is the number of exposed groups in the bioassay, and d is the dose level at which the risk function is evaluated. Following EPA (1980), Anderson et al. (1983) and CDHS (1985), q_1 or some upper statistical confidence bound is defined as the low-dose "potency" parameter since at low doses an estimated extra risk may be obtained by multiplying q_1 by the dose. The input to this program for each data set consisted of the values for total lifetime TWA applied dose, A, or metabolized dose, M, (in mg/kg-d) and corresponding tumor incidence data given in Table 5-1. For each data set GLOBAL86 was used to calculate a maximum likelihood estimate (MLE) and a one-tailed 95% upper confidence limit (UCL) for the linear parameter q_1. The MLE estimates of q_1 that relate A and M to predicted tumor risk are denoted $q_1(A)$ and $q_1(M)$, respectively, and the corresponding UCL estimates are denoted $q_1^*(A)$ and $q_1^*(M)$, respectively. When dose is given in units of mg/kg-d, the units of q_1 and q_1^* are $(mg/kg\text{-}d)^{-1}$. Calculated values of $q_1(A)$, $q_1^*(A)$, $q_1(M)$ and $q_1^*(M)$ corresponding to each of the 8 bioassay data sets considered here are given in Table 5-3.

The male and female rat potency values of 0.064 and 0.04 $(mg/kg\text{-}d)^{-1}$ calculated from the 1986 NTP rat-leukemia data are somewhat larger than those calculated from both the 1977 NCI and the 1986 NTP mouse data. The maximum potency value of 0.064 $(mg/kg\text{-}d)^{-1}$ is about 9 times larger than the smallest calculated potency value of 0.0073 $(mg/kg\text{-}d)^{-1}$ derived from HC incidence data for female mice in the 1986 NTP bioassay. In contrast, the calculated UCL potency values corresponding to the applied dose approach ($q_1^*(A)$) span only a 2-fold range (0.0026 to 0.0064 $(mg/kg\text{-}d)^{-1}$). Thus, the metabolized dose tends to indicate a different sensitivity between rats and mice while the applied dose indicates a more similar susceptibility to the carcinogenicity of PCE.

Time-to-tumor data are readily available for the NTP mouse and rat bioassay data, as well as for the NCI mouse bioassay data. Thus, it is 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. In the NTP bioassays, survival was only slightly decreased in the PCE-exposed groups, so the multistage time-to-tumor model is not used as the basis for potency extrapolation in the present analysis. However, for comparative purposes a time-dependent version of the multistage model, WEIBULL82 (Howe and Crump, 1983; Crump and Howe, 1984) was used to derive

TABLE 5-3

CANCER POTENCIES ESTIMATED FROM SELECTED DATA SETS

	Administered Dose		Metabolized Dose Time-Independent Analysis		Metabolized Dose Time-Dependent Analysis	
Data Set	MLE $q_1(A)$	95% UCL $q_1^*(A)$	MLE $q_1(M)$	95% UCL $q_1^*(M)$	MLE $q_1(M)$	95% UCL $q_1^*(M)$
Oral						
NCI (1977) B6C3F1 Mice						
Male - Hepatocellular Carcinoma	0.0046	0.0064	0.024	0.032	-----	-----
Female - Hepatocellular Carcinoma	0.0023	0.0030	0.017	0.022	-----	-----
Inhalation						
NTP (1986) B6C3F1 Mice						
Male - Hepatocellular carcinoma	0.0025	0.0035	0.011	0.015	0.012	0.022
Female - Hepatocellular carcinoma	0	0.0028	0	0.0073	0	0.0072
Male - Hepatocellular adenoma/ carcinoma	0.0043	0.0059	0.0062	0.024	0.0032	0.026
Female - Hepatocellular adenoma/ carcinoma	0.00044	0.0039	0	0.0098	0.0088	0.012
NTP (1986) F344/N Rats						
Male - Mononuclear-cell leukemia	0.0022	0.0040	0.037	0.064	0.043	0.071
Female - Mononuclear-cell leukemia	0.0015	0.0026	0.024	0.040	0.024	0.040

MLE = maximum-likelihood estimate; 95% UCL = one-tailed 95% upper confidence limit.

potency estimates from the NCI and NTP bioassay data. These potency values are given in the last two columns of Table 5-3. Note that the time-dependent potency values are similar to values based on the time-independent form of the multistage model (also shown in Table 5-3). These time-dependent values differ from time-independent values by no more than a factor of 1.5. The largest potency value, 0.071 $(mg/kg/day)^{-1}$ for MLK in male rats, represents an increase of about 11% over the corresponding q_1.

CARCINOGENIC POTENCY IN TERMS OF HUMAN APPLIED DOSE

Assuming that the above calculated values of $q_1^*(M)$ are adequate to approximate expected tumor incidence among animals subjected to very low levels of effective dose M, three issues need to be addressed: first, the application of these calculated potency values across exposure routes; second, the extrapolation of potency from animals to humans; and third, determination of the relationship between applied dose, D, and metabolized dose, M, for humans. Each of these issues will be addressed in turn.

Exposure Route

Various physiological processes, such as the "first pass effect" involving liver-mediated activation or detoxification, may result in differing values of metabolized dose, given the same applied dose but administered via different exposure routes. If biotransformation of PCE to a reactive metabolite is solely responsible for the carcinogenic effects of PCE, the actual exposure route and administered dose may not be as important as the determination of the metabolized dose. Different routes of exposure can allow differing amounts of PCE to reach the sites of metabolism or the target tissues, and thus effect the calculation of the metabolized dose. In this document it is assumed that a given dose of PCE is equipotent regardless of exposure route, as long as the distribution of this dose among susceptible target tissues is not affected by the exposure route. Comparison of the potency estimates for both the applied dose and the metabolized dose for the mouse gavage study and the mouse inhalation study indicate that the route of exposure may not be an important factor for PCE (Table 5-3) (NCI, 1977; NTP, 1986). However, for the purposes of risk assessment of inhaled PCE, the range of the inhalation studies will be used.

Interspecies Dose Equivalence

Once a potency value is estimated in animals using one of the techniques described above, human potency can be estimated. Following the suggestion of Mantel and Schneiderman, the EPA and the California Department of Health Services assume that mg/(surface area) is an equivalent measure of lifetime TWA dose between species for carcinogens (Mantel and Schneiderman, 1975; EPA, 1980b, 1984a; Anderson et al., 1983; CDHS, 1985). Specifically, for purposes of carcinogen-risk assessment, it is assumed that dose in units of milligram per unit surface area produces the same disease of effect in different species in the absence of information indicating otherwise.

Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency value (q_{animal}) by the ratio of human (bw_h) to animal body weights (bw_a) raised to the one-third power when animal potency is expressed in units $(mg/kg\text{-}d)^{-1}$:

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

Body weights are typically assumed to be 70 kg for humans, 0.35 kg for rats and 0.030 kg for mice, but each experiment is interpreted with respect to the actual weight recorded during the bioassay.

Given this assumption and a lifetime metabolized dose rate for an animal of M_a mg/kg-d, then the equivalent human TWA dose rate would be M_a/f, where the dose-equivalence factor f is here given by:

$$f = \left[\frac{W_a}{W_h}\right]^{1/3} \qquad (5\text{-}8)$$

in which W_h and W_a are the weights of humans (generally assumed to be 70 kg) and the test animal, respectively.

In the absence of definitive empirical data, however, it is simply not known whether a mg/(surface area) or a mg/kg 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. For comparative purposes extrapolation of potency values from animals to humans is here carried out using both a dose per body weight (BW) and a dose per body surface area (SA) extrapolation method.

In terms of ingested dose or any form of internal dose, the surface area adjustment results in the conclusion that mice respond similarly to humans at approximately 12.7 times the human dose and that rats respond similarly to humans at approximately 5.5 times the human dose. In terms of inhaled concentration, use of surface area adjustment results in smaller interspecies difference. This occurs because conversion of inhaled concentration to mg/kg-day for rodents and conversion of mg/kg-day to inhaled concentration for humans tends to conteract the surface area adjustment. Thus, converting from inhaled concentration to mg/kg-day which is adjusted for surface area and converted back to inhaled concentration, results in the conclusion that mice respond similarly to humans at 2.9 times the human exposure and that rats respond similarly to humans at 2.6 times the human exposure.

The use of a surface area correction is to account for interspecies differences in pharmacokinetics and pharmacodynamics. When an internal dose, calculated on the basis of pharmacokinetics, is used, the surface area correction accounts for pharmacodynamic differences between species. These pharmacodynamic differences may also be viewed as differences in sensitivity to the internal dose. Use of a correction factor for sensitivity is similar

to use of an uncertainty or safety factor. As in the cases of methylene chloride and trichloroethylene, DHS staff choose to use the surface area correction, in addition to adjusting for internal pharmacokinetic dose, to take into account interspecies differences in sensitivity. There are two major reasons to justify the use of a surface area correction for pharmacodynamic differences for PCE. First, interspecies differences in response to the calculated internal dose appear to exist. Second, comparison of cancer potencies between mice and rats calculated for the internal dose indicates roughly a 4-fold difference in response.

Human Metabolism

To estimate the carcinogenic potency of PCE in humans from $q_1^*(M)$, additional assumptions need to be made regarding human metabolism. The assumptions and extrapolations reveal additional uncertainties to the potency calculation when the metabolized dose is used.

Several studies have examined the metabolism of PCE in humans following a controlled inhalation exposure to this compound. As discussed in Section 2, several PCE metabolites have been identified in human urine, the major one being trichloroacetic acid (TCA). There is a possibility that other metabolic products of PCE in humans have not been identified. Some human exposure studies have sought to identify total urinary trichloro-compounds produced after inhalation exposure to PCE using a nonspecific, Fujiwara-reaction analytical method (e.g., Ikeda and Imamura, 1973), but the actual products measured by this technique are in doubt (EPA, 1985a). The studies used here to estimate the extent of human PCE metabolism consist of four relatively recent studies that quantified only the cumulative amount of TCA produced for an observation period less than one estimated half-life (typically about 3 days), during and after a controlled PCE inhalation exposure (Ogata et al., 1971; Fernandez et al., 1976; Monster et al., 1979; Bolanowska and Golacka, 1972).

Before reviewing the above studies, three issues concerning how the results of these studies may be used to estimate the degree to which PCE in humans is metabolized during a steady-state (continuous) exposure will be discussed. These issues are: the excretion half-life of PCE in humans, estimation of total PCE metabolism and use of continuous versus peak exposures.

Excretion Half-life in Humans

The first of these issues involves the decay half-life of urinary metabolite formation. That is, the time required for half of the TCA formed from PCE metabolism to be excreted in the urine. Because the metabolism rate of PCE in humans is very slow, it is unlikely that the studies discussed herein were actually able to quantitatively determine the total amount of PCE metabolized following exposure. (Exposure time, t, for each of the studies discussed is listed in Table 5-4). To extrapolate from the existing data, the remaining TCA produced after the end of urine collection had to be

TABLE 5-4.

COMPARISON OF HUMAN PCE METABOLISM AFTER INHALATION EXPOSURES

	Administered Dose				Urinary metabolite[a]		Estimated total metabolites[b]				
	Exposure period	Concentration	Urine collection	Inspired Dose[c]	U(T)	U(E)	M_t	M_6	M_p		
Study	(h)	(ppm)	(h)	(mg)	(mg)	(mg)	(mg)	mg/kg	mg/kg	P[d]	P_{corr}[e]
Ogata et al., 1971 (n=4)	3	87	67	594.6	4.3	8.6	14.8	0.42	0.38	2.5	3.3
Fernandez et al., 1976 (n=2)	8	150	72	2734	24.6	35.4	61.0	0.65	0.63	2.2	3.5
Monster et al., 1979 (n=6)	4	72	70	656.1	6.0	10.5	18.1	0.35	0.31	2.8	4.0
Monster et al., 1979 (n=6)	4	144	70	1312	11.0	20.0	34.5	0.67	0.60	2.6	3.8
Bolanowska and Golacka, 1972 (n=2)	6	56.4	23.5	771.1	3.5	14	24	0.34	0.25	3.1	3.8
Weighted mean (weight = n, the sum of n = 20)										2.6	3.7
S.D. of mean										±0.05	±0.06
99% Upper (2-tailed) confidence limit of mean (df=19)										2.8	3.9

[a] U(T) = observed cumulative urinary metabolite (trichloroacetic acid only) production by any time T after exposure. See text for derivation of U(T) values. U(E) = estimated total urinary metabolites if collection period were extended to infinity.

[b] Estimated total metabolites produced if the monitoring period were extended so time = infinity.

M_t = total amount of metabolites formed.

M_6 = amount of metabolites formed in 6 hrs.

M_p = predicted total metabolite dose produced if the collection period were extended to infinity, and assuming that $M = D\,V_{max}/(D + K_m)$, where V_{max} = 8.099 mg/kg and K_m = 273.32 ppm.

[c] Assumes an alveolar ventilation rate of 5.6 L/min = 2.016 m^3/6-h, and 6.78 mg PCE/m^3 per ppm.

[d] P = the percent of respired dose that is metabolized.

[e] P_{corr} = where P is corrected to reflect a steady-state, low level exposure. The correction factor is based on equation 5-9.

approximated. This was done by integrating the TCA production rate as a function of time from the termination of collection to time equals infinity, where this function is assumed to represent a simple first-order decay process. This is the same assumption used by the EPA (EPA, 1985a; EPA 1986) in assessing the results of the Bolanowska and Golacka (1972) human-exposure study. The EPA assumed a half-life for urinary TCA excretion of 100 hours, apparently based on a presumed empirical range of approximately 65 to 144 hours for this process (EPA, 1985a, where the TCA decay half-life is stated to be 144 hours). The lower end of this range is derived from studies indicating a terminal decline in TCA excretion with a half-life of from 65 to 90 hours in volunteers exposed to 72 and 144 ppm PCE for 4 hours (Monster et al., 1979). The upper value of 144 hours is derived from the study of Ikeda and Imamura (1973) as the mean of measured total urinary trichloro-compound decay half-lives of 13 subjects occupationally exposed to PCE, where the observed range was reported to be 123 to 190 hours. In this study, however, TCA itself was not measured, but total trichloro-compounds were measured using the nonspecific type of Fujiwara reaction method whose dependability has been questioned (EPA, 1985a). The values based on the studies by Monster and co-workers indicate that the rate of PCE release from adipose tissue, the most highly retentive tissue type for PCE, is an exponentially decaying function of time with a half-life of about 71.5 hours (Monster et al., 1979; Guberan and Fernandez, 1974). Thus, a value of 100 hours may overestimate the expected late-stage decay half-life of TCA excretion in humans. Accordingly, for the purpose of the metabolized dose analysis performed herein, a value of 75 hours was used as the presumed approximate late-stage TCA excretion half-life for humans.

Urinary Metabolites as a Fraction of Total Metabolites

The second issue addresses the uncertainties of estimating total metabolism of PCE strictly from TCA measurements. It involves developing the relationship in humans of total PCE metabolized to urinary metabolites, and estimating the proportion of TCA metabolites excreted in the urine as compared to total elimination by all routes. For the purposes of this analysis it was assumed that 58% of the metabolized dose was found in the form of urinary TCA following low levels of exposure to PCE. The value of 58% is the average fraction of total metabolites found in the form of urinary metabolites produced by rats exposed to 1 mg/kg PCE by intubation (yielding 57.1% urinary metabolites) or 10 ppm PCE for 6 hours (yielding 58.9% urinary metabolites), in the study by Pegg et al. (1979). Rat, as opposed to mouse, data are used as the basis for this assumption only because of the greater similarity in body weight between rats and humans. However, this assumption may greatly underestimate the fraction of PCE metabolized in humans. Since humans produce lower levels of trichloro urinary metabolites than do rats, they may produce more nonurinary metabolites or more urinary metabolites that are not trichloro- compounds. As indicated in studies below and in Section 2, only TCA has been used to estimate urinary excretion of PCE metabolites. The production of other urinary metabolites in humans, although documented, has not been adequately investigated as to their relative importance in the metabolism of PCE.

Continuous Versus Peak Exposures

The third and final issue concerns the assumptions about a continuous human-exposure scenario. Results of the human studies considered below were compared to predictions of PCE metabolism based on extrapolation from the data on PCE metabolism in rats exposed to 10 or 600 ppm PCE for 6 hours (Pegg et al., 1979), using the assumptions made in Equation 5-6. The V_{max} calculated from this rat study was 53.0 mg/kg. Using Equation 5-6, the equivalent human V_{max} would be the latter value multiplied by $(0.25/70)^{1/3}$, or 8.10 mg/kg for a 6-hour exposure to PCE. Similarly, the corresponding rat inhalational apparent Km value of 273 ppm would be divided by $(0.25/70)^{1/3}$ to yield an equivalent (initial-exposure) human Michaelis-Menten constant of 1790 ppm. Using these parameter values, the predicted metabolized dose, M (in mg/kg), as a function of applied dose, D (in ppm), is presented in Table 5-4 for each of the various studies considered below.

The brief exposure periods used in the human experiments were conducted at relatively high concentrations compared to the average ambient concentrations of PCE identified in air. At lower doses, a greater percent of the dose is likely to be metabolized. Using the Michaelis-Menten relationship, the maximal metabolized dose per unit applied dose occurs as D approaches 0 in the equation $M = D(V_{max}/K_m)$. Thus, an estimate of the maximum factor by which the ratio M/D is increased at very low doses is given by:

$$\lim_{D \to 0} \frac{M_0}{M} = \frac{D\frac{V_{max}}{K_m}}{\frac{D\,V_{max}}{D + K_m}} = 1 + \frac{D}{K_m} \qquad (5\text{-}9)$$

This factor is used in Table 5-4 to adjust the percent, P, of respired dose, I, that is metabolized to yield a new percentage value, P_{corr}, which is corrected to reflect the steady-state, very low-level exposure conditions that relate to the human environmental exposure scenario being modeled. In this factor, K_m was assumed to be 273 ppm, based on the Pegg and associates (1979) rat metabolism data (this value was not adjusted upward to account for initial rapid uptake prior to steady-state, since P_{corr} is meant to reflect steady-state exposure conditions).

Having addressed these issues and assumptions regarding human metabolism, the four studies used for calculating the rate and extent of human PCE metabolism will now be reviewed.

Ogata and Co-workers (1971)

Urinary excretion of TCA was measured by Ogata and co-workers in four volunteers exposed to 87 ppm PCE for 3 hours (Ogata et al., 1971). From the graphical information presented in this study, the amount of TCA excreted over the 67-hour collection period was calculated from the area under the time versus TCA excretion-rate curve, and found to be about 4.3 mg. This

value is presumed to be excess excretion over background, but a pre-exposure TCA excretion rate was not provided in this study. At 67 hours, the observed excretion rate was about 0.040 mg/hour, so that remaining excretion from that time on is estimated to be (0.040 mg/hour x 75 hours)/ln(2) = 4.3 mg. Total metabolized dose, M_3, (assuming that urinary metabolites in humans represent 58% of total metabolites, as was seen in the rat study by Pegg et al., 1979), from this 3-hour exposure was (4.3 + 4.3)/0.58 = 14.8 mg, which when multiplied by (6/3) yields an approximate equivalent 6-hour metabolized dose, M_6, of 0.42 mg/kg (assuming a 70-kg average subject weight).

Fernandez and Co-workers (1976)

Urinary excretion of TCA was measured by Fernandez and colleagues in two volunteers exposed to 150 ppm PCE for 8 hours (Fernandez et al., 1976). This study reported an average total TCA excretion of 24.6 mg over the 72-hour collection period applied. Urine was not collected during the exposure period, thus the total metabolites produced were underestimated. During the period from 48 to 72 hours post-exposure the reported TCA excretions for the 2 subjects were 3.63 and 3.70 mg, equivalent to an average excretion rate of about 0.15 mg/hour. It was estimated from the data that by 72 hours the average excretion rate had declined to about 0.10 mg/hour, so that remaining excretion from that time on is estimated to be (0.10 mg/hour x 75 hours)/ln(2) = 10.8 mg. Total metabolized dose, M_8, (assuming a proportion similar to rats), from this 8-hour exposure was (24.6 + 10.8)/0.58 = 61.0 mg, which multiplied by (6/8) yields an approximate equivalent 6-hour metabolized dose, M_6, of 0.65 mg/kg (again assuming a 70-kg average subject weight).

Monster and Co-workers (1979)

Urinary excretion of TCA in the Monster and colleagues study was measured in six volunteers at rest exposed to 72 ppm PCE for 8 hours and again in these same volunteers at a later date exposed at rest to 144 ppm PCE for 4 hours (Monster et al., 1979). (Data from this study that were obtained from volunteers subjected to physical activity routines are not used for the present analysis). From the published graphical urinary excretion information, it was estimated that average total TCA excretions of 6.0 and 11.0 mg were observed among the 72 ppm and 144 ppm exposure groups, respectively, over the 70-hour collection period applied. (It appears that urine was not collected during the exposure period. These estimates take into account the background 0.025 mg/h TCA excretion rate observed in this study.) During the 24-hour period from 46 to 70 hours post-exposure, the reported TCA excretions were about 1.0 and 2.0 mg (and fairly constant over the period 22- to 70-hours post-exposure) for the 72 ppm and 144 ppm exposure groups, equivalent to average terminal excretion rates of about 0.042 and 0.083 mg/hour. Thus, the corresponding remaining excretions from 70 hours on are estimated to be (0.042 mg/hour x 75 hours)/ln(2) = 4.8 mg and (0.083 mg/hour x 75 hours)/ln(2) = 9.0 mg, respectively. Corresponding

total metabolized doses, M_4, from these 4-hour exposures are (6.0 + 4.5)/0.58 = 18.1 mg and (11.0 + 9.0)/0.58 = 34.5 mg, respectively, which multiplied by (6/4) yields corresponding approximate equivalent 6-hour metabolized doses, M_6, of 0.35 and 0.67 mg/kg (using the reported average subject weight of 77 kg).

Bolanowska and Golacka (1972)

In evaluating the human metabolism study of Bolanowska and Golacka (1972), the EPA assumed that five subjects were exposed to 390 mg/m^3 PCE for 6 hours and were followed for 20 hours; the EPA did not take into account the background TCA excretion rates observed in the study. Actually, urinary excretion of TCA was measured from only 2 volunteers exposed to 391 and 374 mg/m^3 PCE for 6 hours (interspersed with two 30-minutes "rest" periods). Urine from these subjects was collected for a total of 22 and 25 hours, respectively, including the exposure time. Background TCA excretion rates for the two subjects are given as approximately 0.035 and 0.010 mg/h. Taking these background rates into consideration, it is estimated from the graphical urinary excretion information given in this study that average total TCA excretions of 2.3 and 4.7 mg were observed from these subjects over the (approximately) 23.5-hour average collection period. During the final hours of urine collection, excretion rates for these subjects were approximately 0.045 and 0.15 mg/hour (where 0.15 mg/hour represented a weighted average of 9 hours at 0.14 mg/hour followed by 3 hours at 0.19 mg/hour). Thus, the corresponding remaining excretions from about 24 hours on are estimated to be (0.045 mg/hour x 75 hours)/ln(2) = 4.9 mg and (0.15 mg/hour x 75 hours)/ln(2) = 16.2 mg. Corresponding total metabolized doses, M6, from these 6-hour exposures are thus 12 mg ((2.3 + 4.9)/0.58) and 36 mg ((4.7 + 16.2)/0.58), yielding an average value of about 0.34 mg/kg for this study (assuming an average subject weight of 70 kg).

Metabolic Parameters Estimated from Different Human Data Sets

Table 5-4 summarizes the metabolic data obtained from the studies discussed herein and presents comparisons between the predicted and the observed total metabolite dose for each experiment. The internal consistency of the general approach used in the metabolic analyses is supported by the observation that the predicted total metabolite doses for a 6-hour exposure to PCE for humans are quite similar to the corresponding empirically derived values, M_6, as shown in Table 5-4 (correlation coefficient $r = 0.99$, degrees of freedom = 3, $p < 0.01$).

To calculate the average percentage, P, of PCE metabolized on the basis of the studies considered here, the inspired dose I had to be estimated from the PCE concentrations specified in these studies. Although PCE retention values were given in the Ogata et al. (1971) study, the basis for their calculations was not provided. Fernandez and associates used an estimated alveolar ventilation rate of 5.6 L/minute (equivalent to 340 L/hour or approximately 4.8 L/kg-hour for a 70 kg person) in their calculation of retained dose, a value close to those currently used in proposed PB-PK

models for human metabolism (Fernandez et al., 1976; EPA Draft, 1986; NAS, 1986). The 5.6 L/minute value is also close to the effective value used by Monster and co-workers, who assumed that alveolar retention was approximately equal to 60% of the minute volume (which averaged 10 ± 1.8 L/minute among their 6 subjects) (Monster et al., 1979).

To calculate inspired dose, I, in mg as a function of applied concentration in ppm for the purpose of the present analysis, the alveolar ventilation rate of 5.6 L/minute was assumed, and 1 ppm PCE = 6.78 mg/m^3. This 5.6 L/minute estimate has been used in several pharmacokinetic models, but underestimates the average breathing rate of humans (20 L/minute) by almost 3-fold. The resulting values of P for the 5 data sets range from about 2 to 3%, with a weighted average of 2.6%, where the values of n, the number of people studied in each experiment, are the weights (see Table 5-4). On the basis of the predictive model adjusted for continuous human exposure discussed above, corrected values, P_{corr}, for the average percent PCE metabolized range from about 3 to 4%, with a weighted average of 3.7%. The upper 99% confidence limit on the average P_{corr} was calculated to be approximately 4%, assuming a normal error distribution of the mean and 19 degrees of freedom associated with the sample estimate (i.e., 20 persons studied, minus one). The UCL was approximated in this way because needed data for each individual studied could not be obtained for most of the studies considered. The calculated UCL value of 4% is also equal to the highest value of P_{corr} calculated here, derived from the study of Monster et al. (1979).

It should be emphasized here that these results are based on relatively brief experimental exposures to relatively high concentrations of PCE in air. Linear extrapolation of animal studies from high experimental exposure concentrations to low ambient concentrations suggests that close to 100% of the PCE may be metabolized at ambient levels by mice and rats. At ambient levels humans would be expected to metabolize PCE to a much greater extent than that reported in brief, high exposure studies. The Pegg and associates inhalation (and oral) studies showed that for rats metabolism increases with decreasing PCE exposure (Pegg et al., 1979). In these studies, rats were exposed to either 10 or 600 ppm PCE for 6 hours. With a 60-fold decrease in concentration, there was a 2.7-fold increase in percent PCE metabolized. These acute exposure experiments were conducted at concentrations 10,000- to 100,000-fold greater than ambient levels (0.001 ppm). Assuming an inverse linear relationship between percent metabolism and concentration, rats and mice would be predicted to metabolize a greater percentage of the PCE at concentrations below 0.1 ppm. Similar estimates can also be made for humans by applying the relationship of percent metabolism and concentration established for rats. The fraction of applied dose metabolized in humans, based on measured urinary trichloro compounds is estimated to be 4%, based on human experiments where exposure concentrations averaged 102 ppm. This exposure concentration is 100,000 times greater than ambient levels. Using the same linear relationship between metabolism and exposure conditions described above, extrapolating from the limited human exposure data to ambient PCE concentrations implies that humans would be expected to metabolize more than 60% of the PCE absorbed. This 15-fold increase in the

expected extent of PCE metabolism at ambient concentrations indicates some uncertainty in the use of the PCE pharmacokinetic data for risk assessment.

Using a PB-PK modeling approach, Bogen and McKone estimated that the maximum plausible rate of PCE metabolism in humans at steady-state for a given, extremely low PCE concentration (C_{in}) in air is a physiologically defined fraction (f_m^{**}) of the alveolar input rate, $C_{in}Q_a$ mg/hour, where Q_a is the alveolar ventilation rate in L/hour (Bogen and McKone, 1987). This fraction is defined by:

$$f_m^{**} = \left[\frac{Q_A/Q_1}{P_b} + 1\right]^{-1} \quad (5\text{-}10)$$

in which Q_1 is the blood flow to liver (metabolizing) tissue in L/hour and Pb is the PCE blood/air partition coefficient for humans. This relationship reportedly holds for continuous, steady-state infusion by other exposure pathways as well. Note that Equation 5-10 assumes that the rate of PCE metabolism becomes infinite as PCE concentration in air approaches zero. Using the parameter specifications of $Q_a = 3.8Q1$ and $P_b = 10.3$ taken from recent studies of PCE pharmacokinetics (Reitz and Nolan, 1986; Ward et al., 1987), it follows that for PCE, $f_m^{**} = 73\%$. Thus, the physiological upper bound on metabolism of inspired PCE is predicted to be near 73%, i.e., 0.7 $C_{in}Q_a$ at any given extremely low concentration C_{in}.

Using a PB-PK approach and an analysis of the data of Ikeda and co-workers and Ohtsuki and colleagues on PCE metabolism among occupationally exposed humans, it was estimated that the actual fraction of PCE metabolized at steady-state for extremely low levels of PCE absorption (by any route) is likely to be between 2% and 50% (Ikeda et al., 1972; Ohtsuki et al., 1983; Bogen and McKone, 1987). This conclusion is consistent with predictions of other investigators who used dynamic PB-PK modeling (EPA Draft, 1986; Hattis et al., 1987).

Pharmacokinetic models do not account for individual differences in metabolism and storage. A high variability of body burden of PCE was found for different people tested (Guberan and Fernandez, 1974; Hake and Stewart, 1977; Stewart et al., 1970). The body burden was found to depend on such factors as age, sex, exercise or workload, body mass and adipose tissue mass, pulmonary dysfunctional states, and individual differences in the intrinsic capacity to metabolize PCE. The pathways of PCE metabolism are speculative at present, but it is known that cytochrome P-450 is involved. The presence and basal activity of the enzyme class of cytochrome P-450 are determined genetically, but such systems are also readily altered by components of the diet. Certain phenolic food additives such as BHA and BHT, for example, influence enzyme levels involved in the detoxication and conjugation as well as the activation in animals. Epidemiologic evidence relating enzyme induction to components of human diet are not available at this time, but such induction is likely. Thus, although there is no definitive evidence, a large variability in metabolism in humans appears likely, but cannot be accounted for in the calculation of the metabolized

dose other than through the incorporation of generic safety factors. None of the available pharmacokinetic models has incorporated a safety factor to account for variability in humans due to dietary or genetic predisposition.

Hattis et al. (1986, 1987) chose to estimate human metabolic constants from the urinary excretion data of Ohtsuki et al. (1983) and Ikeda et al. (1972). These values are based on chronic occupational studies instead short-term experimental studies of Ogata et al. (1971), Monster et al. (1979), Fernandez et al. (1976), and Bolanowska and Golacka (1972). Underestimation of the half-life would lead to underestimation of human metabolism and therefore human risk from activated metabolities. Hattis (1987) indicated that the bottom line range of values for the percentage metabolism of PCE of 3-4% is too narrow to be credible. Using the data of Ohsuki et al. (1983). Hattis (1986, 1987) estimated a value of 4.9% and a "plausible upper limit" of 24.6%.

As indicated in the above discussion there is a large range of estimates of the human metabolized dose of PCE. Many investigators have predicted that humans metabolize 2 to 10% of an inhaled PCE dose based on available scientific data (EPA, 1985; EPA, 1986, Hattis, 1987; Rietz and Nolan 1986). As indicated above a plausible range of human metabolism is 2 to 50% of an inhaled PCE dose. Upper limits of metabolism have been reported to range from 25 to 73% (Hattis, 1987; Bois et al., 1990; Bogen and McKone, 1987, 1988). This wide range of values highlights the limited understanding of PCE metabolism in humans.

In humans, metabolism that produces urinary trichloro compounds accounts only for a small percentage of the administered dose following exposure to high concentrations, whereas 60-80% of the PCE inhaled is excreted unchanged through the lungs during the exposure. This implies that 20-40% of the absorbed PCE may be stored (presumably in adipose tissue) and released over time. The difficulty in tracing the fate of these residual amounts of PCE has prevented the construction of a mass balance relationship between PCE absorbed and the PCE metabolized or exhaled unmetabolized by the lungs from human experiments. The residual PCE could be metabolized by the trichloro pathway over time, or by an unrecognized pathway producing metabolites such as CO_2 and oxalic acid that could not be easily recognized as products of metabolism without using radiolabeled compounds.

Data on the amount of PCE metabolized at ambient concentrations (less than 1 ppb) are not available. However, several studies indicate that PCE metabolism increases as the concentration decreases. Pharmacokinetic models generally assume that PCE is metabolized at the same rate at different concentrations unless an ambient absorption factor is added. In light of all these considerations staff at the DHS choose to use the estimate of 25% metabolized by humans as the basis for the risk assessment. Choice of a 25% metabolism factor takes into account much of the uncertainty regarding use of a PBPK model.

PCE Carcinogenic Potency as a Function of Human Applied Dose (Taking PCE Metabolism Into Account)

To estimate the carcinogenic potency of PCE in humans for a given steady-state applied dose (in mg/kg-day) administered by ingestion or inhalation, the value of 25% derived above was used as the fraction of applied dose that is metabolized in humans. Under this assumption, extrapolated potency is reexpressed in Table 5-5 as a function of human applied dose for each bioassay data set considered here under both the body weight (BW) and surface area (SA) assumptions for extrapolating equipotent doses between species.

Corresponding values of $q_1^*(A)$ based on the BW and SA approaches are listed in Table 5-5. The $q_1^*(A)$ values for the BW approach range from 0.0018 to 0.016 $(mg/kg\text{-}d)^{-1}$, a 9-fold range, while the $q_1^*(A)$ values for the SA approach range from 0.024 to 0.11, a 5-fold range. So the SA values are somewhat more homogeneous than those based on the BW approach. Using the SA approach, the maximum $q_1^*(A)$ value is 0.11 $(mg/kg\text{-}d)^{-1}$ based on HC incidence in male mice in the 1977 NCI bioassay. This value is less than 7 times greater than the largest value obtained using the BW approach (based on MLK in male rats).

Table 5-6 compares the 95% UCL potencies from Table 5-5 with corresponding values based on different approaches to calculating dose than the SSPK approach used to generate the potency values appearing in Table 5-5.

In interpreting the values presented in Table 5-6, 4 points should be kept in mind.

1) The EPA approach to the NCI bioassay data implies that following ingestion humans metabolize 20% of the PCE dose (the "EPAI" method in Table 5-6), whereas following inhalation exposure humans are assumed to metabolize a somewhat lesser amount of PCE than mice or rats (the "EPAR" method in Table 5-6) (EPA 1985a; NCI, 1977). In the LLNL and Hattis (HATT) methods a 25% rate of metabolism for humans was used. In the EPAR and Reitz and Nolan (RNPK) methods the metabolism rates ranged from 2 to 4%.

2) All approaches based on the use of metabolic data involving respiratory exposure to PCE (namely, the "EPAR", "EPPK", "RNPK", and "LLNL" methods referred to in Table 5-6) were standardized to reflect a single assumption regarding human alveolar respiration (estimated at 5.6 L/minute). This was done for the purpose of presenting meaningful comparisons in Table 5-6.

3) Both the "EPAI" and "EPAR" methods were adjusted in the context of the 1977 NCI bioassay data sets to incorporate the partial lifetime factor of $(104/90)^3$ used in the "LLNL" method, again to facilitate a meaningful comparison of alternative potency calculations.

4) If the assumption is made that humans (and animals) metabolize some fraction, f_m (besides the 25% value assumed in this analysis), of all

TABLE 5-5.

PREDICTED UPPER 95% UPPER CONFIDENCE LIMITS ON POTENCY (MG/KG-D) SUMMARY (ASSUMING HUMANS METABOLIZE 25% OF ABSORBED PCE).

Study species strain	Tumor type[a]	Sex and weight (kg)	Metabolized dose[b] $= q_1^*(M)$		Human applied dose[c] $= q_1^*(A)$	
			BW[d]	SA[e]	BW[d]	SA[e]
NCI, 1977 Mice B6C3F1	HC	M 0.030	0.032	0.42	0.008	0.11
		F 0.025	0.022	0.31	0.0055	0.078
NTP, 1986 Mice B6C3F1	HC	M 0.037	0.015	0.19	0.0038	0.048
		F 0.032	0.0073	0.095	0.0018	0.024
NTP, 1986 Mice B6C3F1	HAC	M 0.037	0.024	0.30	0.006	0.075
		F 0.032	0.0098	0.13	0.0025	0.033
NTP, 1986 Rats F344/N	MLK	M 0.44	0.064	0.35	0.016	0.088
		F 0.32	0.040	0.24	0.01	0.06

[a]HC = hepatocellular carcinoma, HAC = hepatocellular adenoma or carcinoma, MLK = mononuclear-cell leukemia.

[b]Human equivalent lifetime, time-weighted-average metabolized dose, M, in mg/kg-d, LLNL method.

[c]Human equivalent lifetime, time-weighted-average applied dose, D, in mg/kg-d, is here assumed to be equal to M/0.25 when D is very small; thus, $q_1(D) = 0.25q_1$ (M) for very small D.

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

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

TABLE 5-6

TUMORIGENIC POTENCY OF PCE: SUMMARY OF VALUES BASED ON DIFFERENT APPROACHES TO DOSE CALCULATION.

Study/ Species/ Sex	Tumor type[a]	Calc. method[b]	95% UCL potency[c] values			
			As a function of metabolized dose[d] (M)		As a function of human applied dose[e] (A):	
			$q_1^*(M)$ in (mg M/kg-d)$^{-1}$		$q_1^*(D)$ (mg D/kg-d)$^{-1}$	
			BW[f]	SA[g]	BW[f]	SA[g]
NCI, 1977 Mouse Male	HC	LLNL	0.032	0.42	0.008	0.11
		EPAI	0.032	0.42	0.0064	0.085
		EPAR	0.032	0.42	0.00066	0.0088
		APPL	--	--	0.0064	0.085
Mouse Female	HC	LLNL	0.022	0.31	0.0055	0.078
		EPAI	0.022	0.31	0.0044	0.062
		EPAR	0.022	0.31	0.00046	0.0064
		APPL	--	--	0.0030	0.042
NTP, 1986 Mouse Male	HC	LLNL	0.015	0.19	0.0038	0.048
		EPAR	0.018	0.22	0.00037	0.0046
		EPPK	0.048	0.59	0.0047	0.058
		RNPK	0.0099	0.12	0.00040	0.0049
		HATT	0.013	0.16	0.0033	0.04
		APPL	--	--	0.0035	0.043
Mouse Female	HC	LLNL	0.0073	0.095	0.0018	0.024
		EPAR	0.0093	0.12	0.00019	0.0025
		EPPK	0.025	0.32	0.0024	0.031
		RNPK	0.0050	0.064	0.00020	0.0026
		HATT	0.0074	0.094	0.0018	0.024
		APPL	--	--	0.0028	0.036
Mouse Male	HAC	LLNL	0.024	0.30	0.006	0.075
		EPAR	0.029	0.36	0.00060	0.0074
		EPPK	0.077	0.95	0.0075	0.093
		RNPK	0.016	0.20	0.00064	0.0079
		HATT	0.022	0.28	0.0055	0.070
		APPL	--	--	0.0059	0.073
Mouse Female	HAC	LLNL	0.0098	0.13	0.0025	0.033
		EPAR	0.013	0.17	0.00026	0.0034
		EPPK	0.033	0.43	0.0033	0.042
		RNPK	0.0067	0.087	0.00027	0.0035
		HATT	0.012	0.15	0.003	0.038
		APPL	--	--	0.0039	0.051

TABLE 5-6 (Continued)

Study/ Species/ Sex	Tumor type[a]	Calc. method[b]	95% UCL potency[c] values: As a function of metabolized dose[d] (M): $q_1^*(M)$ in (mg M/kg-d)$^{-1}$ BW[f]	SA[g]	As a function of human applied dose[e] (D): $q_1^*(D)$ (mg D/kg-d)$^{-1}$ BW[f]	SA[g]
Rat Male	MLK	LLNL	0.064	0.35	0.016	0.088
		EPAR	0.051	0.28	0.0016	0.0085
		EPPK	0.057	0.31	0.0056	0.030
		HATT	0.048	0.26	0.012	0.065
		APPL	--	--	0.0040	0.022
Rat Female	MLK	LLNL	0.040	0.24	0.010	0.060
		EPAR	0.037	0.22	0.0011	0.0068
		EPPK	0.041	0.25	0.0040	0.024
		HATT	0.037	0.20	0.0093	0.050
		APPL	--	--	0.0026	0.016

[a]HC - hepatocellular carcinoma, HAC - hepatocellular adenoma or carcinoma, MLK - mononuclear-cell leukemia.

[b]Methods of calculation used are as follows (see text for more detailed discussion):

LLNL - simple steady-state pharmacokinetic method, corresponding values taken from Table 5-5, assuming a 25% metabolism rate for humans.

EPAI - EPA-equivalent ingestion method: assumes that M = 20% of ingested PCE is metabolized

EPAR - EPA-equivalent respiratory method; assumes that for humans M = 0.0207A or M = 0.0306A for mouse or rat data, respectively.

RNPK - Reitz & Nolan-equivalent PB-PK method; assumes that for humans M = 0.04A and AVR = 5.6 L/min.

EPPK - EPA-equivalent PB-PK method; assumes that for humans M = 0.0977A and AVR = 5.6 L/min.

HATT - Hattis et al. - equivalent PB-PK method; assumes that for humans M = 0.25A, the plausible upper limit for metabolism.

APPL - applied dose method; corresponding values taken from Table 5-3, where they are labelled $q_1^*(A)$.

[c]95% UCL = one-tailed 95% upper confidence limit.

[d]Human equivalent lifetime, time-weighted-average metabolized dose, M, in mg/kg-d, defined as a function of D as described above.

[e]Human equivalent lifetime, time-weighted-average applied dose, D (or A, in the context of the APPL method described above).

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

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

respired or ingested PCE at very low levels of applied PCE dose, the appropriate upper-limit potency value to use would be calculated as $q_1^*(D) = f_m q_1^*(M)$ based on the values of $q_1^*(M)$ in Table 5-6. Such alternative assumptions are plausible, given that there are no animal or human experimental data that specifically address the issue of what fraction of an extremely small applied PCE dose (e.g., continuous exposure to one ppb in air) is metabolized.

A total of 144 alternative potency values appear in Table 5-6, with 64 values representing potency expressions, $q_1^*(M)$, in terms of metabolized dose and 80 values representing potency expressions, $q_1^*(D)$, in terms of applied dose (or PCE potentially available for uptake). The $q_1^*(M)$ values range from 0.0050 to 0.42 (mg M/kg-d)$^{-1}$, or an 84-fold range, and the $q_1^*(D)$ values range from 0.00019 to 0.085 (mg D/kg-d)$^{-1}$, or a 447-fold range. Clearly, then, the model, study, route of exposure, and tumor type taken to calculate the appropriate dose in dose-response assessment is a significant factor in cancer-risk extrapolation for PCE.

Recommendation

The staff of CDHS recommends that the metabolized PCE dose be used while taking into account the uncertainties of the dose calculation. For PCE there are several basic areas of uncertainity. 1) The structure of models used to calculate the "carcinogenic" dose assumes that the metabolic pathway leading to the production of the carcinogenic metabolite has been identified and is correlated to the production of trichlorinated metabolites. However, the carcinogenic mechanism of PCE has not been established. 2) Assumptions and interpretation of experimental data utilized by various investigators in building models may result in a fairly wide range of conclusions. For example, it has been reported that the ratios of low dose human to bioassay rodent metabolism spanned a 30-fold range depending on the data chosen or estimated from the literature (Hattis et al., 1990). As indicated in Table 5-6, the 95% UCL potency values based on the human applied metabolized dose (A), that is, exposure estimates calculated using metabolized dose, can vary up to 14-fold for a single endpoint for a specific species using the BW conversion. 3) The extent to which PCE is metabolized in humans is unclear due to the lack of metabolic data on humans exposed to PCE under well-controlled, long-term experimental conditions. Thus, a mass balance for human exposure to PCE has not been constructed. 4) Interspecies and intraspecies differences in their sensitivity to the effects of the internal dose need to be considered.

These uncertainties regarding human metabolism, the carcinogenic pathway, parameter variability, and the relative sensitivity of a heterogeneous population to the effects of PCE can be taken into account. The first and second concerns can be alleviated for the most part by utilizing a moderate upper bound on metabolism (25%). The third concern can be alleviated by considering the range of values and choosing a best value that uses appropriate parameters. The fourth concern can be taken into account through the utilization of the surface area correction. DHS staff have chosen to utilize the metabolized dose approach with a surface area correction.

The carcinogenic risk potency range is taken from the 1986 NTP rat and mouse studies. The 1977 mouse studies are not used due to shortcomings described in Section 4 and the superiority of the 1986 studies. The range includes the LLNL and HATT approaches which are based on 25% metabolism. These represent the two major pharmacokinetic models available for PCE, one based on a steady-state approach (LLNL) and one based on the PB-PK approach (HATT). These values, listed in Table 5-6, are expressed as a function of human applied dose (D) and range from 0.0025 to 0.093 $(mg/kg\text{-}day)^{-1}$. These values have been converted to units of $(ppb)^{-1}$ in Table 5-7. For these conversions it was assumed the human was a 70 kg individual breathing 20 m^3/day, and the PCE conversion factor was 1 ppb = 6.78 $\mu g/m^3$. The potencies were converted as follows:

$$0.0025\ (mg/kg\text{-}day)^{-1} \times 6.78\ ((mg/m^3)/ppm) \times (ppm/1000\ ppb) \times 20\ (m^3/day) \times (0.4\ \text{AVR correction}) \div 70\ kg = 1.9 \times 10^{-6}/ppb$$

$$0.093\ (mg/kg\text{-}day)^{-1} \times 6.78\ ((mg/m^3)/ppm) \times (ppm/1000\ ppb) \times 20\ (m^3/day) \times (0.4\ \text{AVR correction}) \div 70\ kg = 7.2 \times 10^{-5}/ppb$$

The range of risks, reflecting incorporation of metabolic data, is 0.2 to 7.2 x 10^{-5} per ppb. In contrast, the range of risks produced using an applied dose approach was 3.1 to 14.4 x 10^{-5} per ppb. For the male mouse the estimated unit risk is reduced over three-fold when the metabolic data are incorporated.

As evident in Table 5-7, humans are predicted to be less sensitive than rodents to the effects of PCE by 2- to 6-fold. Chronic studies in mice and occupational studies in humans indicate that mice and humans may have similar sensitivity to PCE, so a much larger species difference in carcinogenic response does not appear justified.

Considering the quality of the cancer bioassays and the uncertainty of human metabolism the best value of the 95% UCL is 5.4 x 10^{-5} per ppb (8.0 x 10^{-6} per $\mu g/m^3$), the value associated with the male mouse HAC using the Hattis et al. (1987) method. This result is comparable to that derived by Bois and colleagues using a Monte Carlo simulation approach to evaluate the parameters used in the pharmacokinetic and multistage models (Bois et al., 1990). Their upper-bound risk level was 4.6 x 10^{-5} per ppb (6.8 x 10^{-6} per $\mu g/m^3$), which is within the DHS range of upper-bound values and is close to the best value suggested by DHS. The best value is within an order-of magnitude of the 6.4 x 10^{-6} per ppb (0.95 x 10^{-6} per $\mu g/m^3$) value recommended by the EPA (derived from USEPA, 1986, 1990). The EPA value is lower because they did not take into consideration the recent analyses addressing the uncertainties involved in the human metabolism data; the EPA assumed humans metabolized about 4% of the PCE absorbed.

Even among metabolized dose approaches the best value does not represent the largest values to be calculated. Using the EPPK approach with an estimated human metabolism of 20% generates a value of 7.4 x 10^{-5} per ppb. Using the RNPK approach with a 25% estimate of human metabolism instead of 4% yields estimates comparable to the best value derived using the HATT approach. As indicated earlier under the heading of "Metabolic Parameters Estimated from

TABLE 5-7

TUMORIGENIC UNIT RISK OF PCE: RANGE OF VALUES

Species/ Sex[a]	Tumor Type[b]	Method[c]	Cancer Potency Estimate (Unit Risk 95% UCL)[d] $(ppb)^{-1} \times 10^{-5}$ Rodent Risk[e]	Human Risk[f]
Mouse Male	HC	LLNL	7.8	3.7
		EPAR	9.3	0.4
		EPPK	24.8	4.5
		RNPK	5.1	0.4
		HATT	6.7	3.1
Mouse Female	HC	LLNL	3.9	1.9
		EPAR	4.8	0.2
		EPPK	12.9	2.4
		RNPK	8.3	0.2
		HATT	11.4	1.9
Mouse Male	HAC	LLNL	12.4	5.9
		EPAR	15.0	0.6
		EPPK	39.8	7.2
		RNPK	8.3	0.6
		HATT	11.4	5.4
Mouse Female	HAC	LLNL	5.1	2.6
		EPAR	6.7	0.3
		EPPK	17.1	3.3
		RNPK	3.5	0.3
		HATT	6.2	3.0
Rat Male	MLK	LLNL	36.2	6.8
		EPAR	28.9	0.7
		EPPK	21.5	2.3
		HATT	18.1	5.1
Rat Female	MLK	LLNL	15.1	4.7
		EPAR	14.0	0.5
		EPPK	15.5	1.9
		HATT	14.0	3.9

[a]Derived from the NTP inhalation study (NTP, 1986).

[b]Abbreviations are for hepatocellular carcinoma (HC), hepatocellular adenoma or carcinoma (HAC), and mononuclear-cell leukemia (MLK).

[c]Methods of calculation used were (see text for a more detailed discussion):

- LLNL - Simple steady-state pharmacokinetic method (corresponding values taken from Table 5-5)
- EPAR - EPA equivalent ingestion method (assumes M = 0.0207 * A for humans and M = 0.0306 * A for mice or rats)
- RNPK - Reitz & Nolan equivalent PB-PK method (assumes M = 0.04 * A for humans)
- EPPK - EPA equivalent PB-PK method (assumes that M = 0.0977 * A for humans)
- HATT - Hattis et al. equivalent PB-PK method (assumes that M = 0.25 * A based on a plausible upper limit for human metabolism)

[d]95% UCL = one-tailed 95% upper confidence limit.

[e]Risk to animals based upon metabolized dose, as calculated by the formula:

Rodent Risk = q_1*(M for BW)[mg/kg/day]$^{-1}$ x 6.78 [(μg/m^3)/ppb] ÷ 1000 [μg/mg] x BR [m^3/day] x 0.4 [AVR correction] ÷ BW [kg], where BR is 0.105 for rats and 0.034 for mice, and AVR correction is 0.4 for rats and 0.6 for mice.

[f]Risk to humans based upon metabolized dose, as calculated by the formula:

Human Risk = q_1*(A for SA)[mg/kg/day]$^{-1}$ x 6.78 [(μg/m^3)/ppb] ÷ 1000 [μg/mg] x 20 m^3/day x 0.4 [AVR correction] ÷ 70 kg.

Different Human Data Sets," 50% is a possible upper limit of PCE metabolism in humans, while 73% is the physiological upper limit of human metabolism of PCE. Using a metabolic rate of 50% for humans would double the range of risks while use of 75% would triple the current range. However, DHS staff believe that 25% adequately reflects the uncertainty in the metabolized dose approach.

Based on this estimate, an upper bound of 54 excess cancer cases is predicted for every million individuals exposed over their lifetime to 1 ppb (6.78 $\mu g/m^3$) PCE. Based on the same estimate, the unit risk for a lifetime continuous exposures to 1 $\mu g/m^3$ of PCE is estimated to be 8.0×10^{-6}. The calculation represents the upper bound on risk and not the actual risk, which may be insignificant. Based on the CDHS potency evaluation of the annual average of 0.43 ppb of PCE in the South Coast Air Basin and assuming that there are about 10 million residents in that area, an excess number of additional lifetime cancer cases of 233 might result from PCE exposure. Based on these findings, *CDHS concludes that at ambient concentrations, PCE may cause or contribute to an increase in mortality or serious illness due to the induction of cancer and thus pose a hazard to human health.*

APPENDIX A

DOSE-RESPONSE INFORMATION FOR ACUTE, SUBCHRONIC, AND CHRONIC TOXICITY IN ANIMALS (EXCLUDING TERATOGENIC, MUTAGENIC, AND CARCINOGENIC EFFECTS)

Animal dose-response data should be evaluated together with applicable human data to establish safe exposure limits for PCE to prevent acute, subchronic and certain (specifically, noncarcinogenic and nonmutagenic) chronic toxicological endpoints. Here, dose-response data are reviewed according to exposure period (e.g., acute, subchronic, and chronic), route of exposure, and species for different toxic effects to major body organs and systems in animals.

Acute Exposures

Most of the studies reviewed did not clearly identify a no-observed-adverse-effect level (NOAEL) for acute exposures. At the upper bound of acute, oral toxicity, LD50 values range from 3005 mg/kg to over 10,000 mg/kg (rats). Representative LD50 values are presented in Table A-1. Other acute responses to oral and inhalation exposures of PCE are shown in Tables A-2 and A-3, respectively. Even at doses as low as 10 mg/kg, ventricular arrhythmias were observed in rabbits, and cardiovascular effects were noted in dogs at an average dose of 13 mg/kg. Table A-4 summarizes the acute responses to intravenous (IV) and intraperitoneal (IP) administrations. It is difficult to evaluate these studies with regard to their implications for predicted human toxicity because neither the doses nor the routes of administration are typical of human exposure.

Subchronic and Chronic Exposures

Carpenter and Rowe and co-workers conducted studies that identified subchronic and chronic exposure levels in the rat and guinea pig at which no effects were observed (Carpenter, 1937; Rowe et al., 1952). Carpenter exposed rats to 70 ppm of PCE 8 hours/day, 5 days/week for 7 months (Carpenter, 1937). Blood samples were analyzed for glucose and calcium. Periodic counts of white cells, polymorphonuclear neutrophil leukocytes, lymphocytes, and eosinophils were made. Urine was evaluated for bilirubin and albumin. In all instances, values were within normal limits. At the end of the exposure period, animals were sacrificed and examined for histological and/or pathological changes. No damage to organs or to the peripheral and central nervous systems were observed.

Rowe and associates exposed female guinea pigs to 100 ppm of PCE 7 hours/day for a total of 13 exposures in 17 days (Rowe et al., 1952). Animals were observed for changes in behavior, appearance, growth, mortality, and final body weight. Tissues were also examined microscopically. No evidence of adverse effects was reported. However, a separate group of guinea pigs that were administered PCE by the same dose regime 132 times over 185 days displayed some evidence of toxicity. Females had a significant increase in liver weight ($p = 0.01$), and animals of both sexes had some abnormal deposition of fat in the liver.

A recently completed bioassay of PCE used 100 ppm as the lowest concentration administered to mice (6 hours/day, 5 days/week for 103 weeks) (NTP, 1986). Although the results have been discussed previously (see Section 4), it is important to note that numerous adverse effects were observed. Therefore, it appears that 70 ppm may be a NOAEL for inhalation exposure of rats (based on the 1937 Carpenter study), but NOAELS for mice and guinea pigs have not been identified.

Hayes and co-workers studied the subchronic toxicity of PCE administered in the drinking water of rats (Hayes et al., 1986). Animals received 14, 400, or 1400 mg/kg-d in water daily, for 90 days. The primary effect was a significant decrease in body weight of high-dose males and of females given 400 to 1400 mg/kg-d ($p<0.05$). Although a loss of body fluids was not considered to be a factor in the lowered body weights, the actual cause of weight loss was not determined. Blood and serum were analyzed for a number of parameters. The only consistent effect was a significant elevation ($p<0.05$) of 5'-nucleotidase in high-dose animals of both sexes, as well as in males that received 400 mg/kg-d. The authors suggest that this may be indicative of cholestasis (suppression of bile flow). However, no other serum indicators of hepatotoxicity were observed. A dose-dependent increase in liver and kidney/body weight ratios was found, but no statistical difference in liver and kidney/brain weight ratios occurred. No adverse hematological or pathological effects were noted in low-dose animals (14 mg/kg-d). The observed NOAEL in rats for ingestion of PCE in drinking water is 14 mg/kg. Tables A-5, A-6, A-7, and A-8 summarize the responses to subchronic and chronic administration of PCE.

TABLE A-1. MEAN LETHAL DOSES (LD50) OF TETRACHLOROETHYLENE TO LABORATORY ANIMALS

Route	Species	Dose (mg/kg)	Reference
IP[a]	Rat (female)	3005	Hayes et al., 1986
IP	Dog	3400	Klaassen and Plaa, 1967
IP	Rat (male)	3835	Hayes et al., 1986
Oral	Rat	3980 to 4680	Withey and Hall, 1975
IP	Mouse	4700	Klaassen and Plaa, 1966
Oral	Mouse	6400 to 8000	Von Oettingen, 1964
Oral	Mouse	8115	Wenzel and Gibson, 1951
Oral	Mouse	8571	Dybing and Dybing, 1946
Oral	Rat	8850	Lewis and Sweet, 1984
Oral	Rat	13,000	Smyth et al., 1969

[a]Intraperitoneal administration.

TABLE A-2. ACUTE ORAL TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Dose (mg/kg)	Effect	Reference
Cat	812	No observed effects	Maplestone and Chopra, 1933
Cat	1623	Drowsiness; unsteadiness	Maplestone and Chopra, 1933
Rabbit	2158	50% Increase in serum lipoprotein; transient elevation in serum-enzyme activities	Fujii, 1975
Rat	4700	Death	Smyth et al., 1969
	6492 to 8115	Death in 2 to 9 h	Lamson et al., 1929
Dog	6492	Death in 5 h	Lamson et al., 1929
Cat	6492	Death in 36 h	Lamson et al., 1929
Rabbit	8115	Death in 17 to 24 h	Lamson et al., 1929

TABLE A-3. ACUTE INHALATION TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Concentration (ppm)	Exposure period	Effect	Reference
Mouse	200	4 h	Moderate fatty infiltration of liver	Kylin et al., 1963
Mouse	400	4 h	Moderate to massive fatty infiltration of liver	Kylin et al., 1963
Mouse	800		Decrease in hepatic ATP levels; increase in total hepatic lipids and triglycerides	Ogata et al., 1968
Mouse	1600	4 h	Massive fatty infiltration of liver; statistically significant increase in liver fat content	Kylin et al., 1963
Rat	2000	Various exposure durations	CNS depression; loss of consciousness; possible cardiac failure; respiratory failure	Rowe et al., 1952
Rat	2300	4 h	Ataxia; 80% loss of avoidance and escape response	Goldberg et al., 1964
Mouse	2917	4 h	100% Mortality	NTP, 1986
Rat	3000	"Several hours"	Loss of consciousness	Rowe et al., 1952
Mouse	3700	24 min	Anesthesia	Gehring, 1968
Mouse	3700	470 min	Liver dysfunction	Gehring, 1968
Rat	5163	4 h	100% Mortality	NTP, 1986
Dog	9900		Narcosis	Lamson et al., 1929

TABLE A-4. ACUTE INTRAVENOUS (IV) AND INTRAPERITONEAL (IP) TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Dose	Route of administration[a]	Effect	Reference
Dog	13 mg/kg (mean dose)	IV (animals were anesthetized with pentobarbital)	Depression of myocardium; increased vulnerability of ventricles to epinephrine-induced arrhythmias; ventricular arrhythmias	Kobayashi et al., 1982
Rabbit	10 mg/kg (mean dose)	IV (animals were anesthetized with urethane)	Increased vulnerability of myocardium to tachycardia	Kobayashi et al., 1982
Dog	20 to 40 mg/kg	IV	Significant depression rate of rise of left intraventricular pressure	Kobayashi et al., 1982
Rat	1.3 mL/kg	IP	Increase in BDPF[b]; increase in Cl-, K+, decrease in protein in BDPF	Hamada and Peterson, 1977
Rat	0.3 to 2.0 mL/kg	IP	20 to 440% Increase, SGOT[c]	Cornish et al., 1973
Dog	1200 mg/kg	IP	Increased SGPT[d], 50% of animals	Klaassen and Plaa, 1967
Dog	2300mg/kg	IP	Increased retention PSP[e], 50% of animals	Klaassen and Plaa, 1967
Mouse	3900 mg/kg	IP	ED_{50}; elevation of SGPT	Gehring, 1968

TABLE A-4. (Continued)

Species	Dose (mg/kg)	Route of administration	Effect	Reference
Mouse	2.5 mL/kg	IP	Necrosis and swelling proximal convoluted tubule	Plaa and Larson, 1965
Mouse	4700 mg/kg	IP	Enlargement of hepatocytes; cellular infiltration and vacuolation; slight hepatic necrosis; slight necrosis convoluted tubule	Klaassen and Plaa, 1966

[a]IV - Intravenous injection; IP - intraperitoneal injection.
[b]BDPF: Bile duct-pancreatic flow.
[c]SGOT: Serum glutamic oxalacetic transaminase.
[d]SGPT: Serum glutamic pyruvate transaminase.
[e]PSP: Phenosulfonephthalein.

TABLE A-5. SUBCHRONIC ORAL TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Dose (mg/kg)	Dose regime	Effect	Reference
Rat	14	Daily for 90 d	No observed adverse effects	Hayes et al., 1986
Mouse	100,250,1000, 1500 or 2000	5 d/wk for 6 wk	Significant increase absolute liver weight[a]; significant increase liver triglycerides[a]	Buben and O'Flaherty, 1985
Mouse	100,250,500 or 1000	Daily for 11 d	Significant elevation in absolute liver weights[a]; hepatocellular swelling[a]; significant decrease of hepatic DNA content per gram of liver[a]; dose-related increase of hepatic DNA synthesis (1000 mg/kg)	Schumann et al., 1980
Rat	100,250,500 or 1000	Daily for 11 d	Significant elevation of liver weight (1000 mg/kg group only); minimal hepatic changes (1000 mg/kg group only)	Schumann et al., 1980
Mouse	100,200,500,1000, 1500 or 2000	5 d/wk for 6 wk	Dose-dependent increase in liver degeneration and karyorrhexis	Buben and O'Flaherty, 1985
Dog	300		Degenerative changes of liver; extensive atrophy of liver	Hall and Schillinger, 1925

TABLE A-5. (Continued)

Species	Dose (mg/kg)	Dose regime	Effect	Reference
Rat	400	Daily for 90 d	Significant decrease in body weight (females); significant increase in 5'-nucleotidase (males)	Hayes et al., 1986
Mouse	500,1000,1500, or 2000	5 d/wk for 6 wk	Significant decrease in G6P[a-b]; significant increase in SGPT[a-c]	Buben and O'Flaherty, 1985
Mouse	1000	5 d/wk for 6 wk	Significant decrease in hepatic DNA content (indicative of hypertrophy)	Buben and O'Flaherty, 1985
Rat	1400	Daily for 90 d	Significant decrease in body weight (both sexes); significant increase in 5'-nucleotidase (both sexes)	Hayes et al., 1986

[a]All dose levels.
[b]G6P: Glucose 6 phosphatase.
[c]SGPT: Serum glutamic pyruvate transaminase.

TABLE A-6. SUBCHRONIC INHALATION TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
Guinea pig (females)	100	13 exposures in 17 d	7 h/d	No observed effects	Rowe et al., 1952
Guinea pig	100	132 exposures in 185 d	7 h/d	Significant increase in liver weight (female); fat deposition in liver	Rowe et al., 1952
Rat	200	4 exposures in 4 d	6 h/d	Decreased RNA content of brain; increased nonspecific cholinesterase activity; behavioral changes	Savolainen et al., 1977
Rat	230	5 d/wk 21 exposures	8 h/d	Granular swelling and congestion of kidneys	Carpenter, 1937
Mouse	200 to 1600	Daily for 3 d	8 h/d	Inhibition of growth; increase in mortality	Schumacher et al., 1962
Rat	400	130 exposures in 183 d	7 h/d	No observed effects in mortality	Rowe et al., 1952
Rat	800	Daily for 1 month	12 h/d	Significant decrease in ACh[a]	Honma et al., 1980
Rat	1600	8 exposures in 10 d	7 h/d	Slight degeneration of germinal epithelium of testes; increase in liver weights; moderate fatty degeneration of liver	Rowe et al., 1952

TABLE A-6. (Continued)

Species	Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
Rabbit	2211	6 d/wk for 45 d	4 h/d	Significant decrease in rate of glomerular filtration; decrease of renal tubular capacity	Brancaccio et al., 1971
Guinea pig	2500	18 exposures in 24 d	7 h/d	High mortality; loss of equilibrium, coordination, and strength; rapid weight loss; increase in liver and kidney weights; fatty degeneration of liver; swelling of tubular epithelium	Rowe et al., 1952
Rabbit	2500	28 exposures in 39 d	7 h/d	Slight hepatic degeneration; CNS depression to point of helplessness	Rowe et al., 1952
Rat	2500	13 exposures in 18 d	7 h/d	CNS depression with loss of consciousness; 90% lethality	Rowe et al., 1952

[a]ACh: acetylcholine.

TABLE A-7. CHRONIC ORAL TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS (Noncarcinogenic effects only)

Species[a]	Dose[b] (mg/kg)	Dose regime	Exposure frequency	Effect	Reference
Mouse (female)	386[c]	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 62); toxic nephropathy (96%)	NCI, 1977
Rat (male)	471	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 72); toxic nephropathy (88%)	NCI, 1977
Rat (female)	474	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 74); toxic nephropathy (58%)	NCI, 1977
Mouse (male)	536[b]	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 78); toxic nephropathy (82%))	NCI, 1977
Mouse (female)	772[b]	5 d wk for 78 wk	Daily	Early mortality (50% mortality by wk 50); toxic nephropathy (100%)	NCI, 1977
Rat (male)	941	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 44); toxic nephropathy (94%)	NCI, 1977
Rat (female)	949	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 66); toxic nephropathy (76%)	NCI, 1977

TABLE A-7. (Continued)

Species	Dose[a] (mg/kg)	Dose regime	Exposure frequency	Effect	Reference
Mouse (male)	1072[b]	5 d/wk for 78 wk	Daily	Early mortality (50% mortality by wk 43); toxic nephropathy (94%)	NCI, 1977

[a]Each dose group initially had 50 animals.
[b]All values are time-weighted average doses.
[c]These dose levels are associated with an increased incidence of cancer in experimental animals. See Section 5 for additional information.

TABLE A-8. CHRONIC INHALATION TOXICITY OF TETRACHLOROETHYLENE TO ANIMALS

Species	Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
Rabbit	15	7 to 11 months	3 to 4 h/d	Reduction in agglutinin formation	Mazza, 1972
Rat	70	5 d/wk for 7 months	8 h/d	No observed effects	Carpenter, 1937
Mouse	100	5 d/wk for 103 wk	6 h/d	Significantly lower survival than controls (after wk 74); increased incidence of liver degeneration and liver necrosis	NTP, 1986
Mouse (females)	100	5 d/wk for 103 wk	6 h/d	Increased incidence of nephrosis	NTP, 1986
Guinea pig	200	158 exposures in 220 d	7 h/d	Significant depression of growth; increase in liver and kidney weight; slight to moderate fatty degeneration of liver	Rowe et al., 1952
Mouse	200	5 d/wk for 103 wk	6 h/d	Significantly lower survival than controls (after wk 78); increased incidence of liver degeneration, liver necrosis, and nephrosis	NTP, 1986
Rat	230	5 d/wk for 7 months (150 exposures)	8 h/d	Granular swelling of the liver; decrease in glycogen storage	Carpenter, 1937
Rat (males)	400	5 d/wk for 103 wk	6 h/d	Decreased survival (after wk 82)	NTP, 1986

TABLE A-8. (Continued)

Species	Concentration (ppm)	Dose regime	Exposure period	Effect	Reference
Mouse	400	169 exposures in 236 d	7 h/d	Increase in kidney weight; swelling of tubular epithelium	Rowe et al., 1952
Guinea pig	400	169 exposures in 236 d	7 h/d	Significant depression of growth; increase in liver and kidney weights; moderate fatty degeneration and slight cirrhosis of liver	Rowe et al., 1952
Rabbit	400	159 exposures in 220 d	7 h/d	No observed effects	Rowe et al., 1952
Monkey	400	179 exposures in 250 d	7 h/d	No observed effects	Rowe et al., 1952
Rat	470	5 d/wk for 7 months 150 exposures	8 h/d	Liver congestion and cloudy swelling; increased secretion, cloudy swelling and desquamation of kidney; congestion and increased pigmentation spleen	Carpenter, 1937
Rat	600	5 d/wk for 12 months	6 h/d	Increase in mortality	Leong et al., 1975
Rat	600	5 d/wk for 12 months	6 h/d	Increase in mortality (males only); inflammation of kidney cells; nephrosis	Rampy et al., 1978

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