Human Variability and Susceptibility to Trichloroethylene

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Although humans vary in their response to chemicals, comprehensive measures of susceptibility have generally not been incorporated into human risk assessment. The U.S. EPA dose–response-based risk assessments for cancer and the RfD/RfC (reference dose–reference concentration) approach for noncancer risk assessments are assumed to protect vulnerable human subgroups. However, these approaches generally rely on default assumptions and do not consider the specific biological basis for potential susceptibility to a given toxicant. In an effort to focus more explicitly on this issue, this article addresses biological factors that may affect human variability and susceptibility to trichloroethylene (TCE), a widely used halogenated industrial solvent. In response to Executive Order 13045, which requires federal agencies to make protection of children a high priority in implementing their policies and to take special risks to children into account when developing standards, this article examines factors that may affect risk of exposure to TCE in children. The influence of genetics, sex, altered health state, coexposure to alcohol, and enzyme induction on TCE toxicity are also examined. *Key words*: children, gender differences, human variability, susceptibility, trichloroethylene. — *Environ Health Perspect* 108(suppl 2):201–214 (2000). *http://ehpnet1.niehs.nih.gov/docs/2000/suppl-2/201-214pastino/abstract.html*

Throughout human history it has been apparent that only some individuals in a given population become sick or die after exposure to a common environmental hazard. To what extent can we make predictions concerning the effects of environmental agents on individuals or groups? Determining the risk of an individual from a specific agent requires studying the net influence of a large set of variables on the known effects of that agent. Some of these variables may enhance susceptibility, while others may diminish it.

Risk assessment has been an important environmental regulatory decision-making tool in the United States and Canada since the 1980s. It is defined as a formalized process for estimating the magnitude, likelihood, and uncertainty of environmentally induced health effects (*1–3*). While the U.S. Congress has tried to protect susceptible groups and workers since the 1970s through legislation [e.g., Clean Air Act (*4*)], both research and risk assessment on potential toxins have lagged (*5*). Historically, U.S. government agencies have focused on the maximally exposed individual in risk assessment. For example, the health of individual workers in an occupational setting is protected by setting standards that restrict exposure beyond a permissible limit of a substance (*6*).

Although humans vary in their response to a given toxicant, measures of susceptibility have not been incorporated into human risk assessment methods (*5*). Since limited baseline information is available on exposure and health outcomes in humans, most human health risk assessment is based on experimental toxicity studies of homogenous animal populations; however, these usually do not evaluate differences in sensitivity, age, or gender, particularly in human populations. While the U.S. Environmental Protection Agency (U.S. EPA) dose-response–based risk estimates are assumed to protect vulnerable human subgroups (*7*), the biological basis of susceptibility remains poorly understood. In addition, broadly based exposure baselines and data on potentially susceptible groups, such as children or the elderly, are lacking. The U.S. EPA Guidelines for Risk Characterization (*8*) have emphasized the need to identify, characterize, and include susceptible populations in risk estimation and risk management processes (*9*).

This article focuses on human variability and susceptibility in response to trichloroethylene (TCE) exposure. TCE and other halogenated hydrocarbons are widely used industrial solvents, and production of TCE increased from approximately 260,000 pounds in 1982 to 320 million pounds in 1991. Heavy use of TCE has resulted in widespread soil and groundwater contaminants in the United States; TCE is present in as many as 60% of the hazardous waste sites on the U.S. EPA National Priority List. The major environmental releases come from the air emissions of metal degreasing plants. Thus, TCE has been the subject of much toxicity testing and research, and an extensive database has been compiled (*10–13*).

In an effort to address factors that affect human variability and susceptibility, this article examines the risks of TCE exposure to children. Executive Order 13045 requires federal agencies to make protection of children a high priority in implementing their policies and to take special risks to children into account when developing standards. The order follows the recommendations of the 1993 National Research Council (NRC) report *Pesticides in the Diets of Infants and Children* (*14*), which suggested children are at disproportionate risk from environmental health threats because they receive greater exposures per unit of body weight, and their developing systems are immature.

The influence of genetics, altered state of health, and the effect of enzyme induction on TCE toxicity are also examined in this article. Ethanol and TCE interactions are examined in detail because of the availability of data. However, the studies on ethanol exposure and TCE interaction serve as an example of the potential toxic consequences of enzyme induction and exposure to mixtures.

Health Effects of TCE Exposure in Humans

Metabolism of TCE

The metabolism of TCE is described extensively in another article in this issue (*15*). Briefly, TCE is rapidly absorbed following acute exposure and distributes throughout the body, preferentially to the fat. TCE undergoes metabolic activation primarily in the liver but also in the kidneys and lungs (Figure 1). The rate-limiting step in the metabolism of TCE is the P450-mediated oxidation to chloral hydrate (CH). CH is rapidly hydrolyzed to trichloracetic acid (TCA) and free trichloroethanol (TCOH) via aldehyde and alcohol dehydrogenase (ADH), respectively. Free TCOH undergoes glucuronidation and is excreted in the urine or is converted back to TCA through CH. TCOH also undergoes enterohepatic recirculation. That is, following excretion of TCOH from the liver into the bile and then the small intestines, it is reabsorbed into the intestinal

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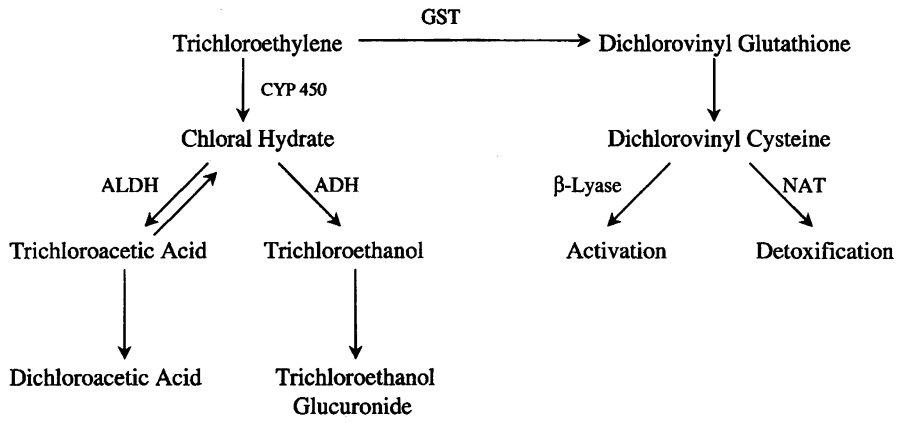

Figure 1. Oxidative and conjugative pathways for the biotransformation of TCE.

circulation and passes back through the liver. TCA is metabolized to dichloracetic acid (DCA) or is excreted in the urine. TCA, TCOH, and DCA are thought to contribute to the toxicity of TCE.

TCE also undergoes metabolism through glutathione conjugation in the liver to form dichlorovinyl glutathione (DCVG) (*15*). DCVG in turn is metabolized to the cysteine conjugate in the kidney (DCVC), which is metabolically activated to a thioacetylating agent by β-lyase or detoxified by *N*-acetyltransferase (NAT), which is excreted in the urine.

Acute Effects

Acute exposure to TCE results primarily in central nervous system (CNS) effects. Neurotoxic effects, such as dizziness, headache, sleepiness, nausea, confusion, blurred vision, and weakness occur at concentrations of approximately 100 ppm; anesthesia occurs at 2,000 ppm (*10,12*). Death has occurred at very high concentrations (10,000 ppm) and was associated with cardiac arrhythmia and massive liver damage (*12*).

Reproductive/Developmental Effects

Studies on the various reproductive and developmental effects of TCE have yielded conflicting results. One study found an increase in miscarriage among nurses exposed to TCE and other chemicals in the workplace, although no specific association with TCE was found. Another study found no increase in malformations in the children of 2,000 fathers and mothers exposed to TCE via inhalation (*12*). An association, but no direct cause-and-effect relationship, was found between elevated levels of chlorinated hydrocarbons, including TCE, and chromium in drinking water and congenital heart disease in children whose parents were exposed to this contaminated drinking water (*16,17*); follow-up studies in rats showed that TCE was a cardiac teratogen, but not a general teratogen during fetal organ development (*18*). An association between TCE exposure and cardiac anomalies and eye malformations has been found (*18–20*). Other studies, however, found no adverse reproductive effects in humans exposed to TCE in drinking water (*12,21*). An increase in abnormal sperm morphology in mice exposed to TCE by inhalation has also been found (*12,21*).

Cancer Risk

Long-term exposure to TCE can result in hepatotoxicity, nephrotoxicity, and neurotoxicity, as reviewed by Bull (*22*), Lash (*23*), and Barton (*20*), respectively. Several studies in humans have investigated the relationship between TCE exposure and cancer. The U.S. EPA has for a number of years regulated TCE on the basis of carcinogenicity, although its classification has not been resolved between group B2 (sufficient evidence in animals) and C (limited evidence) (*24–26*). Two large bodies in the past few years have reviewed the epidemiologic evidence on TCE. The American Council of Governmental Industrial Hygienists (ACGIH) (*27*), looking only at the occupational studies, classified TCE as a compound not suspected as a human carcinogen, primarily on the basis of observations from large studies of degreasers at Hill Air Force Base in Utah (*28,29*). Several years later, the International Agency for Research on Cancer (IARC) (*30*) reviewed studies from both occupational and drinking water exposures and noted consistent findings of liver/biliary tract cancer and non-Hodgkin's lymphoma in the most informative studies. Additionally, they noted elevated leukemia risks in the drinking water studies. However, these populations were exposed to other solvents in addition to TCE. These findings led IARC to classify TCE as a probable carcinogen in humans, or Group 2A, based on limited human and sufficient animal data (*30*). It should be noted that the ACGIH and IARC used different methods to evaluate the epidemiologic database.

The evidence is increasing for an association between human kidney cancer and TCE exposure; newer studies consistently report elevated risk of cancer for this site (*31–35*). Additionally, nephrotoxicity and kidney dysfunction following TCE exposure have been reported (*36,37*). Several studies show elevated risks for breast and cervical cancer among women occupationally and environmentally exposed to TCE (*33,38,39*). This evidence is weaker than that for the aforementioned sites; however, it raises a question regarding susceptibility of women.

Although strict site concordance has not been established in animals and humans for each specific tumor, increases in lung, liver, and kidney tumors in animals have been seen. Inhalation exposure results in the formation of lung, liver, and kidney tumors; oral gavage results in increases in liver and kidney tumors (*22,23*). Male rats are more susceptible to the formation of kidney tumors, whereas mice are more susceptible to the formation of liver and lung tumors. The toxicities resulting from TCE exposure are likely due to the various metabolites formed from the oxidative and conjugative pathways. Qualitatively, the pathways of biotransformation in humans and animals are identical, with most metabolites identified in experimental animals also found in humans. However, quantitative differences exist that probably account for the observed species differences in the formation of TCE-induced tumors. Mice and rats metabolize TCE more efficiently than humans. For example, the maximum rate of the *in vitro* metabolism of TCE in humans is one-third that in the rat and one-fourth that in the mouse (*40*).

While the tissue-specific tumors identified in rodents are due to metabolites rather than TCE, the metabolite responsible for each tissue-specific response is likely different. Animal studies suggest that the formation of liver tumors is mediated through TCA and possibly DCA, and species differences are probably due to differences in the formation of TCA (*22*). Mice are more prone to the formation of liver tumors and metabolize TCE more rapidly than rats or humans.

As reviewed by Lash (*23*), the formation of kidney tumors in rats is likely mediated by the reactive thiol formed from the β-lyase metabolism of DCVC. DCVC has been shown to be highly nephrotoxic and mutagenic in the Ames test (*41*). The kidney tumors formed following TCE exposure are very rare and a single mode of action has not been identified, but studies in animals indicate that mutagenicity and cytotoxicity from DCVC are involved (*23*).

The relevance of the formation of kidney tumors in rats to humans has not been established. A recent study reported blood levels of DCVG, a precursor of DCVC, in humans exposed to occupationally relevant concentrations of TCE (*42*). Sex-dependent differences

were also found; peak blood levels in men were 2-fold higher than in women and were reached sooner than in females. Since male rats are more susceptible to the nephrotoxic and nephrocarcinogenic effects of TCE and also have a higher rate of glutathione (GSH) conjugation in the liver and kidney, these findings suggest that men may be at a greater risk than women of developing nephrotoxicity from TCE exposure.

The mechanism for the increased risk of lung tumors in mice is different from the liver or kidney and is thought to be mediated through the formation and accumulation of CH in the Clara cells (*43*). These cells lack the capacity to metabolize CH to TCOH, and the subsequent accumulation of CH leads to marked vacuolization of the cells. The relevance to humans has not clearly been established because of differences in morphology and metabolism in the lungs.

Analysis of Variability and Susceptibility

There has been much debate about whether environmentally related disease is attributable more to toxicant exposure or to inherent factors that affect a person's response to toxicants. Historically, many risk analyses have focused on variations in responses following different levels of exposure (e.g., occupational versus non-occupational exposure). However, the risk of developing an environmentally related illness may also be influenced by a person's genetic background, age, gender, nutritional status, behavior (e.g., exercise, alcohol, and smoking), past and current exposure patterns, and interactions between these factors and between genes.

It is important to note that susceptibility seldom remains constant. A person's susceptibility to environmental hazards may vary considerably during his or her lifetime as he or she grows and develops from infancy to adulthood, changes jobs, adopts habits that affect overall health, suffers from intermittent illness, and develops chronic diseases in old age. Factors include fundamental physiological variables such as the route of exposure, the portal of entry, and uptake route; for example, significant differences in physiology are found between children and adults. Fundamental variables may in turn be affected by constitutive factors, such as a person's genetic background (variations in metabolic proteins), sex (reproductive organs, endocrine systems), age (stage of life), and ethnicity (this combines both genetic and acquired factors such as diet, cultural practices). Acquired factors such as health status or diseases (e.g., diabetes, obesity), behavioral factors (e.g., diet, exercise, stress), and other exposures (e.g., alcohol consumption, exposure to other hazards) also affect fundamental physiological variables.

Fundamental Physiological Variables

Genetics of biotransformation reactions. **Overview.** Given equal exposure to a toxin, humans vary in their internal processing depending on genetic background, acquired characteristics, and other past or ongoing exposures (*44*). Inherited mutations that affect all cells in a person (germline mutations) differ from those that arise in a single cell and affect only the clonal descendants of that cell (somatic mutations) (*45*). Diseases in the first category tend to be rare and result from dominant single genes (e.g., Li-Fraumeni syndrome, familial bilateral retinoblastoma, defective DNA repair and proofreading), and disease is usually independent of environmental exposure (*44*).

On the other hand, susceptibility genes are defined as common polymorphic genes that are found in over 1% of the population. They usually but not exclusively consist of genes involved in metabolic and repair function, or regulators of such genes, that affect activation or detoxification of environmental agents (*46–49*). In contrast to the rare germline mutation diseases, specific environmental exposures are required to induce diseases associated with susceptibility genes. While susceptibility genes confer only a modest increase in risk to persons carrying them, larger numbers of people are affected, and these genes therefore pose a greater public health threat than the rare, single-gene diseases (*43*). Unlike diseases resulting from rare germline mutations, those attributed to susceptibility genes do not show strict familial aggregation. Indeed, the same mutation may be found in a variety of sporadic, nonhereditary diseases.

Historically, most observational studies of polymorphic metabolic genes have focused on cancer, particularly tobacco-linked lung cancer. Identifying specific metabolic gene polymorphisms in individuals may become a powerful tool in the future for identifying those at-risk persons and in targeting disease prevention. However, at present we are unable to predict environmental diseases based solely on single metabolic gene mutations. The role of each metabolic gene is complex, and may be modulated by gene–gene interactions, environmental exposure, health, nutritional status, and other factors. Indeed, a trait that provides protection against a disease resulting from one compound may increase the risk of disease from another (*6*).

There is considerable lack of data regarding genetic polymorphisms as they relate to TCE metabolism and toxicity. Most data on TCE toxicity have been obtained from studies using inbred animal strains. These studies are usually conducted on homogenous rodent strains maintained on identical diets and living conditions, while humans are more heterogeneous and vary widely in their behavior and diet. As a further complication, TCE is metabolized differently between different rodent species. Since rodent toxicity data are extrapolated as a basis for evaluating human toxicity and form the basis for human health risk assessment, a better understanding of the metabolic differences between species is needed. In addition, further studies need to be conducted on genetic polymorphisms in TCE metabolism in order to provide a complete health assessment of susceptible populations.

The metabolism of xenobiotics includes various classes of reactions, such as hydrolysis, reduction, and oxidation, which result in the formation of compounds that are slightly more hydrophilic. Examples of enzymes involved in these types of reactions include cytochrome P450s (CYP), ADH, and aldehyde dehydrogenase (ALDH). Additional biotransformation reactions include glucuronidation, sulfation, acetylation, methylation, and glutathione conjugation, which typically lead to a significant increase in the hydrophilicity of the compound, which provides for rapid urinary excretion. Each reaction as it pertains to TCE biotransformation will be discussed in further detail. Much TCE toxicity may result from its metabolites, and analyzing variations in TCE metabolism is important in understanding variable toxicity of this compound.

Metabolism by cytochrome P450. One of the major families of enzymes responsible for the oxidation of xenobiotics is CYP. This family of enzymes contains many genetic variants that show different levels of metabolic activity; up to a 10-fold difference in CYP activity has been seen in humans (*48,50*). Wide interindividual variation is found in the CYP1A2, CYP2A, CYP2C, and CYP3 gene subfamilies (*51*), and associations between altered genotypes in CYP1A1, CYP2D6, and CYP2E1 and tobacco smoke-induced lung cancer have been made (*50*).

In rats, several CYP450 enzymes metabolize TCE, including the CYP1A, 2B, 2C, and 2E subgroups (*52,53*); however, TCE metabolism is most strongly dependent on CYP2E1 activity. CYP2E1 is well conserved in mammals, and also metabolizes ethanol and a wide range of other volatile organic solvents. At present, it is not clear what CYP450 enzymes other than CYP2E1 are involved in TCE metabolism in humans (*15*). CYP2E1 metabolizes a wide range of substances including nitrosamines and chlorinated solvents (*54*). As reviewed by Lieber (*55*), CYP2E1 is involved in the metabolism of over 80 exogenous substances. It is primarily expressed in liver but also in brain, kidney, and lung.

Interindividual variability in humans. CYP2E1 activity *in vitro* has been studied in liver microsomes from several human populations. Peter et al. (*56*) found up to 10-fold range in activity in 14 subjects, while

Stephens et al. (*57*) and Yoo et al. (*58*) found a 50-fold variation among subjects. Pronounced differences have also been observed among Swedish, Japanese, and Chinese subjects (*54*). According to Lieber (*55*), a 6- to 20-fold variability of CYP2E1 protein or activity has been observed in humans and cannot be attributed to liver disease, cancer, alcohol, or smoking.

CYP2E1-mediated TCE metabolism in humans. In humans, CYP2E1 plays a crucial part in TCE metabolism and is responsible for over 60% of TCE clearance (*40*). An 8-fold variation in the *in vitro* CYP2E1-mediated metabolism of TCE was seen in 23 human liver samples (*40*). There were no significant sex-dependent differences in overall TCE metabolism or CYP2E1 activity, but the affinity (K_m) was lower in females than in males. Greater variability was seen in women and may have been due to the small sample size.

Although a better understanding of CYP2E1 variation will allow persons more susceptible to TCE toxicity to be more easily identified (*54*), CYP2E1 activity alone is not a sufficient indicator of risk. Genetic variation and diet both contribute to the wide variation in CYP2E1 activity, and the balance between activation of TCE to toxic metabolites and their subsequent detoxification determines the risk of exposed persons (*59*). In addition, acquired factors that increase CYP2E1 activity (e.g., alcohol exposure) may also increase susceptibility to TCE-induced toxicity.

Interspecies variability in TCE metabolism. Several species-dependent differences in the metabolism of TCE have been found. Lipscomb et al. (*40*) directly compared the metabolism of TCE in hepatic microsomes of mice, rats, and humans at occupationally relevant TCE concentrations. P450-dependent TCE metabolism was highest in mice, lower in rats, and substantially lower in humans. The maximal rate of TCE metabolism in humans was one-fourth and one-third of that in the mouse and rat, respectively. Clearance values (V_{max}/K_m) also exhibited species dependence; human microsomes were the least efficient at metabolizing TCE.

Species-dependent differences also exist in the metabolism of CH, which occurs in both the liver and blood. For example, metabolism of CH to TCOH was much lower in human blood than in mice or rats, while TCA formation was significantly higher in humans and mice than in rats (*60*). Human liver showed only about a 60% conversion of CH to TCA and TCOH compared to rodent samples. In all species, the K_m for TCOH formation in liver was at least 10-fold lower than TCA formation. K_m values for both TCOH and TCA were higher in humans than in rats and mice, and clearance of TCOH was higher than TCA (*60*).

Nakajima et al. (*52*) examined differences between rat and mouse liver microsomes in the metabolism of benzene, toluene, and TCE by P450 and concluded the species differences observed during P450 induction were more relevant to TCE toxicity than the differences in basal enzyme levels. That is, the effect of differences in CYP2E1 activity on the toxicity of TCE became more apparent following CYP2E1 induction. Different isozyme distributions and substrate affinities were observed, with mice showing greater TCE metabolism than rats (*52*).

Molecular markers and ethnic differences. Variability in the regulation of CYP2E1 expression may play a role in susceptibility. Restriction fragment length polymorphisms (RFLP) have been identified in the CYP2E1 gene using a variety of restriction enzymes (*58*). Variations in allelic distribution were observed when different ethnic groups, such as European Americans, African Americans, and Taiwanese were compared, and particular alleles were positively correlated to protection from lung cancer (*61*); however, this study did not control for gender, age, or smoking. A rare allelic form of CYP2E1, c_2, may be correlated to an increased risk for lung cancer (*62*). There was an association between increased incidence of a particular (*Dra*I) allelic distribution in Japanese lung patients (*62*). This allele was less prevalent in Finnish and Swedish patients, and the correlation between the *Dra*I marker and lung cancer was not found in a Swedish study (*63*). Kato et al. (*64*) also found no correlation between *Rsa*I markers and lung cancer risk in a study of largely Caucasian lung cancer patients. Mutations in regions outside of the CYP2E1 gene may also play a role. For example, a significant association was found for a *Rsa*I polymorphism in the gene promoter region of CYP2E1 in Swedish lung cancer patients (*64*); however, no significant correlation was found in Finnish and Japanese patients with lung cancer (*65,66*). In another example, a significantly higher distribution of the c_2 mutation was found upstream of CYP2E1 in controls for lung cancer; this region contains a putative binding site for transcription factor HNF-4. CYP2E1 may be less efficiently expressed among carriers of the c_2 mutation, making them less susceptible to xenobiotic bioactivation (*64*). Examination of CYP2E1 variants in nasopharyngeal cancer cases and controls showed an RFLP distribution similar to the Japanese population but higher than in the Finnish or Swedish population (*67*). A 5- to 7.7-fold increase in cancer risk was found in subjects homozygous for these CYP2E1 variants.

Although molecular markers clearly indicate differences in metabolic genes in different groups, at present none of these sites can be used as markers for predicting increased disease risk (*10*). In the absence of data directly relating polymorphisms to enzymatic activity, this information remains of limited use.

Alcohol dehydrogenase. ADH is a multienzyme family found in the cytosol, mitochondria, and endoplasmic reticulum of many tissues. Six human ADH genes have been characterized, and polymorphisms of several ADH genes have been identified (*68–73*). The enzymes have been divided into three classes according to isozyme composition and kinetic properties. Class I ADH consists of three nonallelic loci, ADH1, ADH2, and ADH3, which are involved in the synthesis of three types of subunits, α, β, γ, respectively (*72*). Genetic variants of ADH2 and ADH3 exist and encode for high-activity ADH (*68*). For example, the frequency of these variants is higher among Asians (*68,69*). Atypical ADH contains a variant β subunit (β2) instead of the usual β1, and is found more frequently among Asians than Caucasians (*70*). Class I ADH exhibits high catalytic activity toward short-chain aliphatic alcohols, including ethanol, as well as biologically active amines (*72*). Class II and Class III ADH exhibit catalytic activity toward longer chain alcohols (*71*). Class IV ADH consists of the enzyme that is primarily responsible for gastric metabolism of ethanol, σ-ADH (*74*). ADH is involved in the metabolism of CH to TCOH. Thus, ethnic variability in ADH metabolism may result in variations in the formation of TCOH, which can alter the hypnotic/CNS effects following TCE exposure.

Aldehyde dehydrogenase. ALDH is a group of enzymes that convert aldehydes to corresponding acids. ALDH is inducible by certain medications, such as barbiturates (*75*). Approximately 50% of East Asians have an inactive ALDH enzyme (mitochondrial ALDH2), but active ALDH is present in virtually all Caucasian, African-American, and North and South American populations studied (*70*). Persons lacking this enzyme develop a flushing reaction following ethanol consumption due to the buildup of acetaldehyde (*68*).

ALDH is involved in the metabolism of CH to TCA. A decrease in the formation of TCA, which could potentially occur in persons lacking this enzyme, could protect against the toxic effects of TCA. However, an increase in flux through the conjugative pathway can occur secondary to a decrease in the formation of TCA from CH. Thus, genetic variations in either enzyme will likely have an effect on the metabolism and toxicity of TCE. However, no studies have been conducted to examine this.

Glutathione conjugation. Glutathione *S*-transferase (GST) conjugates TCE with

glutathione to form DCVG; this is further metabolized in the kidney to the cysteine derivative DCVC, which is activated by β-lyase or detoxified by NAT. While conjugation products are generally less toxic than the parent compound, some TCE metabolites, such as the nephrotoxin DCVC, are more potent (*76*). While variations in conjugative reactions of TCE biotransformation in humans are not well documented, below are summarized the known variants in GST.

Defective GST genes are associated with an increased risk of lung and bladder cancer (*30*). About 50% of Caucasians are homozygous for the null allele of *GSTM1* (*51*) and may be at greater risk for certain cancers. In addition, different GST subfamilies show marked tissue specificity and developmental regulation. For example, placenta contains only GST π activity, and 50% of fetal liver GST activity is from the π form, which is not expressed in adult liver.

The GST family encodes multifunctional enzymes that catalyze several reactions between GST and electrophilic and hydrophobic compounds (*51*). The four multigene classes of GST subunits are α, μ, π, and θ. Approximately 60–70% of humans are conjugators who can conduct GSH-dependent detoxification of monohalomethanes, while nonconjugators cannot (*51*).

GSTμ. GSTμ1 detoxifies a number of reactive electrophilic substances. About 50–60% of Caucasians carry the GSTμ1 null phenotype (*51*). This is consistently associated with a higher risk for lung and bladder cancer (*77–79*), and possibly skin (*80*) and colon cancer (*81,82*). Indeed, 25% of all bladder cancers may be correlated to smokers carrying the GST null phenotype (*77*). Gene and environmental interactions may also be significant; nonsmokers carrying the GSTμ1 null phenotype do not show elevated bladder cancer risk, but risk increases 2- and 6-fold with smoking (*83*).

GSTθ. GSTθ enzymes conjugate a number of chlorinated low-molecular-weight compounds, and may be primarily responsible for conjugation of TCE (*15*). The GSTθ gene is present in 60–75% of humans, and at least two classes of enzymes are found in liver and in erythrocytes (*84*). Distribution of the GSTθ1 polymorphism varies among ethnic groups (*84–86*), and is associated with an increase in sister chromatid exchange rates, particularly with exposure to tobacco smoke or diepoxybutane (*87,88*).

GSTμ1 and GSTθ1 polymorphisms may be important in renal cell cancer development following high occupational TCE exposure. Bruning et al. (*89*) found unequal distributions of the GSTμ1 and GSTθ1 genotypes between renal cell cancer patients and controls, suggesting that these polymorphisms indicate predisposition toward this disease. Enzyme polymorphisms were found in renal cell cancer patients who had high occupational TCE exposure, resulting in irreversible tubular damage. The authors concluded that glutathione-dependent formation of nephrotoxic metabolites is responsible for induction of renal cell carcinoma by TCE, and that this genetic polymorphism may indicate a predisposition for TCE-induced renal cell cancer. In addition, persons with polymorphisms in both the GSTμ and GSTθ genes show a synergistic risk for lung cancer (*90*).

Ethnicity. Different ethnic groups vary in their distribution of genotypes, and extrapolation of health outcomes among the groups is very difficult (*51*). For example, genetic polymorphisms in CYP450 were found in debrisoquin and (*S*)-mephenytoin hydroxylation (catalyzed by CYP2D6 and $CYP2C_{MP}$ respectively) when Japanese and Caucasian populations were compared. In a separate study, Shimada et al. (*91*) examined the distribution and activity of P450 isoforms in liver microsomes from 30 Japanese and 30 Caucasian patients. No significant gender-related differences were seen in either population, and the proportion of CYP2E1 in CYP450 was not affected by gender or age. While total CYP450 content and activity were higher in Caucasians, the relative CYP450 isoform levels were similar except for CYP2A6 and CYP2B6, which were higher in Caucasians. Differences in CYP4501A2, 2A6, 2D6, 2E1, and 3A4 activity levels were observed, but no difference in $CYP2C_{MP}$ was seen.

Biologically plausible mechanisms to link specific genotypes to specific outcomes are still lacking for most environmental diseases; for example, many RFLP polymorphisms in Class I genes are the result of mutations in introns or other silent areas of the human genome (*51*). RFLP analysis is therefore of limited use in predicting an individual's susceptibility at this time but may provide some insight into the mechanisms of susceptibility among different groups. DNA sequence analysis, either by direct sequencing or by hybridization-based analysis of polymorphic sites via DNA chips, is likely to be of considerably greater use in the near future, however.

Sex. Susceptibility differences between males and females have been studied only for a few environmental pollutants, most notably inhaled agents such as cigarette smoke and carbon monoxide (*92,93*). Several epidemiological studies of cigarette smoke have shown that, at the same exposure level, women differ in their resistance to lung damage compared to men. Xu et al. (*94*) report that adverse effects of smoking on pulmonary function are greater in women than in men, and other studies indicate sex differences in lung dysfunction, and in genetic and biochemical alterations in lung cancer (*95–97*).

In addition to differences in the internal processing of these toxins, sex affects many aspects of lung growth and development as well as structure and function. There are sex-related differences in how airborne pollutants are deposited in the lung and in the pulmonary response. This may be important because most TCE exposure comes from inhalation.

There are limited data on sex-dependent differences in the toxic response to TCE. Recent epidemiological studies show an excess risk of breast and cervical cancer among women exposed occupationally and environmentally to TCE (*33,38,39*). A recent analysis of the TCE and TCA National Exposure Registry (NER) revealed significant sex-dependent differences in reported adverse health outcomes (*39*). In this study, a comparison between data from the registry and national norms found significantly greater increases in diabetes, kidney problems, liver problems, and urinary tract problems in women. Although this registry contains self-reported adverse health outcomes and thus cannot be used to draw definitive conclusions, it does provide a basis for further studies.

Recent studies have found significant sex-dependent differences in the toxicokinetics of TCE. For example, higher blood concentrations of the glutathione conjugate DCVG were found in men compared to women following exposure to either 50 or 100 ppm TCE (*42*). Peak blood levels in men were 2-fold higher than in women at 100 ppm and were obtained sooner (2 and 4 hr in men and women, respectively). Since male rats are more susceptible to the nephrotoxic and nephrocarcinogenic effects of TCE and also have a higher rate of GSH conjugation in the liver and kidney (*98*), these findings suggest that men may be at a greater risk of developing nephrotoxicity from TCE exposure.

Additional studies have also revealed sex-dependent differences in the toxicokinetics of TCE. For example, *in vitro* studies using human tissue found that males and females differed significantly in their affinities to oxidized TCE (*40*). An uncertainty and variability analysis of data obtained from subjects exposed to 50 and 100 ppm TCE revealed sex-dependent differences in various simulated pharmacokinetic parameters (*99*). TCE also distributes preferentially in fat, and women have a greater percentage of fat. Thus, these pharmacokinetic differences have the potential to result in differential susceptibility between men and women exposed to TCE.

Susceptibility of children to TCE. The majority of research pertaining to environmental health risks in children relates to asthma, ozone, and environmental tobacco

smoke. Research pertaining to the effects of TCE in children has focused primarily on potential teratogenic effects. Limited studies suggest a link between exposure to TCE and cardiac malformations, but conflicting results have been found (*100,101*). The Agency for Toxic Substance and Disease Registry (ATSDR) reported no increase in malformations in children whose parents were exposed to TCE via inhalation (*12*). However, Swan et al. (*16*) and Goldberg et al. (*17*) found a link between the elevated levels of hydrocarbons, including TCE, in the drinking water and cardiac malformations. These results were consistent with studies in rats that found an increase in cardiac malformations (*100*). Drinking water studies also found an increase in CNS defects, neural tube defects, and oral cleft defects (*101*). Although these effects were attributed to TCE, there were other solvents present in the drinking water, making a distinct correlation with TCE difficult.

Attention is now being focused on the health effects of TCE in the child. Recently, in their comparison between self-reported health outcomes from persons enlisted in the TCE and TCA NER and national norms, Burg and Gist (*39*) found an increase in reports of hearing and speech impairment children 0–9 years of age, with girls reporting higher rates. In addition, an increase in the rate of urinary tract disorders was also reported for most age groups, including children. As previously discussed, data obtained from the registry are useful for identifying areas of further research but do not provide conclusive evidence. However, ATSDR has recently undertaken studies to examine the link between TCE exposure and hearing and speech impairment in children.

The main exposure routes to TCE are through ingestion and inhalation. In children, oral exposures are most often associated with consumption of water, while inhalation may occur during use of water for activities such as showering. Exposure can also occur transdermally. In addition to potentially higher exposure in children, developmental differences in absorption, distribution, metabolism, and excretion can alter susceptibility in children exposed to TCE. Specific factors that affect the formation and excretion of toxic metabolites deserve consideration, as they may alter toxicity. Data on the dosimetry of TCE in children are virtually nonexistent; therefore, the discussion below focuses on potential differences in response between adults, children, and the fetus. It should be noted that potential differences do not necessarily reflect an increase in susceptibility among children. The specific nature of each response must be considered and certain factors may actually provide a protective role.

Age-dependent effects on absorption. The absorption of TCE occurs primarily through inhalation and through the gastrointestinal tract but can also occur transdermally. Each of these processes is different in children versus adults and may affect the dosimetry of TCE. Oral absorption of chemicals in the neonate can be affected by several factors. For example, the gastric pH will affect the absorption of ionized drugs and chemicals. The gastric pH is neutral at birth as a result of the presence of amniotic fluid in the stomach (*102*), decreases to approximately 1–3 within hours after birth, reaches neutrality at 8 days, and slowly declines to 2–3 by 3 months (*103*). Although there are age-dependent differences in gastric pH, nonionized lipophilic compounds such as TCE readily diffuse across membranes. Thus, altered gastric pH will likely not affect the absorption of TCE. Prolonged gastric emptying, which is observed in the neonate (*104*), and increased gastric and intestinal motility, which occurs in young children (*105*), can potentially affect the oral absorption of TCE. TCOH, a metabolite of TCE, undergoes glucuronidation and enterohepatic recirculation, which is affected by a decrease in gastric acid secretion.

Transdermal absorption of chemicals is significantly higher in the child than the adult because of the greater surface area relative to body weight, which is approximately 2.7-fold greater in children (*103,105*). In addition, in the neonate and infant the epidermis and stratum corneum are thinner, facilitating much greater dermal absorption (*103,105*). Because of its lipophilicity, TCE could be transdermally absorbed to a greater extent in children than in adults.

Inhalation also differs between children and adults because of physiological and anatomical differences, as reviewed in Guzelian et al. (*106*) and Snodgrass (*107*). The newborn has approximately 10 million alveoli; adult levels of 300 million are not reached until 8 years of age. The alveolar surface area increases from 3 m^2 at birth to 75 m^2 in adulthood; as a result, the air-to-tissue gas exchange increases more than 20-fold. In addition, although the respiratory volume is the same in adults and children (10 mL/kg body weight/breath), the number of breaths per minute is increased in the infant [40 breaths/minute in the infant versus 15 breaths per minute in the adult; (*107*)]. Thus, the respiratory minute ventilation is greater in children [133 vs 2 mL/kg body weight/m^2 lung surface area/min; (*107*)]. One of the primary routes of exposure to TCE is via inhalation, and all of these factors may alter the dosimetry of TCE in the child compared to the adult. However, no studies have been conducted to evaluate these factors.

Age-dependent factors in distribution. Factors such as blood flow, tissue volume, and protein binding affect the distribution of chemicals and also vary with age. For example, protein binding is decreased in children primarily as a result of an increase in the concentration of nonesterified fatty acids (*108,109*). Plasma albumin is also decreased in children (*108,109*). These factors will likely not affect the distribution of TCE in children because TCE does not bind significantly to proteins.

However, the distribution of TCE will likely be affected by factors that affect the volume of distribution. Lipid-soluble chemicals such as TCE should have a smaller volume of distribution in infants because of children's larger percentage of total body water. Total body water constitutes as much as 85% of body weight in preterm and 78% of body weight in full-term neonates (*110,111*), decreasing to 55% by 12 years of age (*112*). Because fat content also varies throughout development, periods of fat reduction may result in increased dosage to the liver or other target organs. For example, fat content increases between 5 and 10 years of age, followed by a decrease in boys at age 17 (*111*). In girls there is a rapid increase at puberty; young females have approximately 2 times greater percentage body fat than boys, suggesting that females will accumulate TCE to a greater extent than males. The half-life of TCE in the fat of adults is 3.5 hr, whereas in rapidly and slowly perfused tissue the half-life is 2–4 min (*11*). Therefore, TCE can be released from the fat for several hours, resulting in prolonged delivery to various target organs.

TCE is a centrally acting chemical and distributes to the brain. Thus, differences in brain volume and blood flow to the brain can alter the toxicokinetics of TCE in children compared to adults. The brain weight in the newborn is approximately 33% of adult values, whereas the body weight of the newborn is 4%. After birth, the brain continues to develop and is therefore susceptible to insult. Brain growth is very rapid during the first 2 years and contains 75% of all cell types by age 2, but further growth is due to myelination of subcortical white matter, elaboration of neuronal dendrites and axons, and an increase in the number of glial cells. Thus, the newborn's brain is more lipophilic, which can affect the distribution of chemicals such as TCE.

Age-dependent effects on metabolism. In general, children are believed to metabolize and clear xenobiotics faster than adults. However, the metabolism of xenobiotics in the infant and child is dependent on the specific enzyme systems involved for each chemical. Some enzymes present at birth do not reach adult activity levels for months or years.

Others are present in the fetus and reach adult capacity at birth. It is therefore important to consider chemical-specific metabolic pathways when examining the potential risks in children, since the ontogenicity differs for each enzyme system.

CYP2E1. As with adults, one of the most important enzymes to consider in the evaluation of toxicity from exposure to environmental chemicals in children is CYP2E1. This enzyme is responsible for the metabolism of over 80 exogenous substances, including TCE (*55*). Expression of CYP2E1 in the placenta or fetus could potentially result in the formation of high local concentrations of metabolites and lead to higher exposures in fetal tissues than would be expected from maternal exposure. Depending on the physiochemical properties, these metabolites may be unable to exit the placenta and accumulate in the fetal compartment. Moreover, the possibility for transplacental induction poses additional threats.

Recent studies have been conducted to examine the expression and activity of CYP2E1 in the human fetus and placenta. Many of these studies were motivated by an attempt to elucidate a mechanism for the effects of ethanol on the fetus. In one study, CYP2E1 was detected in human fetal liver during the second trimester, at about 16 weeks of gestation (*113*). The molecular weight was slightly lower than in adults, but the enzyme was able to metabolize ethanol at 12 and 27% of adult capacity. However, at 10 weeks of gestation, CYP2E1 mRNA levels were undetectable in embryo liver samples. CYP2E1 was inducible in human fetal hepatocytes treated with ethanol and clofibrate, and a 4-fold induction in CYP3A1 was also found following treatment with rifampicin. CYP2E1 has also been detected in extrahepatic tissues. For example, the expression of CYP2E1 was detected in human placenta (*114–116*). CYP2E1 was also identified in prenatal human cephalic tissues (*117*). Northern blot analyses of mRNA levels from tissues obtained between days 54 and 78 of gestation showed that CYP2E1 levels increased as a function of age but were much lower than hepatic levels.

Despite these findings, conflicting reports exist regarding the presence of CYP2E1 in fetal liver. Vieira et al. (*118*) found that CYP2E1 was absent from fetal liver but rose immediately after birth regardless of the gestational age at birth, which ranged between 25 and 40 weeks. This suggested CYP2E1 regulation was directly related to parturition events rather than temporal maturation itself. The level of the protein and its catalytic activity steadily increased during the first year to reach adult values in children age 1–10 years. In addition, the kinetic properties of CYP2E1 were investigated using cloroxazone as a substrate in both neonatal and adult microsomal preparation. The affinity was greater in newborns compared to adults; K_m values obtained were 15.8 and 28.8 μM, respectively. However, the capacity was similar, with a V_{max} of 0.96 nmol/mg protein/min in the neonate and 1.0 nmol/mg protein/min in the adult. These findings are consistent with previous studies examining the expression of CYP2E1 in fetal tissues (*119*). In this study, CYP2E1 expression was absent in the placenta either at 10 weeks or 18 weeks of gestation. In addition, CYP2E1 expression was not detected in fetal liver, kidney, lung, and placenta at 18 weeks or in the liver at 6 weeks of gestation.

Differences between studies detecting the presence of CYP2E1 and those that do not are due, at least in part, to timing of the studies. The placenta and fetus are continually growing and thus expression of CYP2E1 is likely dependent upon the developmental stage. While some human studies have shown the presence of CYP2E1 in fetal hepatic and extrahepatic tissues of different gestational ages, in animals CYP2E1 is present only after birth. The rat CYP2E1 gene appears to be transcriptionally activated at birth, only after which is it detected in rat hepatocytes (*120*). The expression of CYP2E1 in rabbits did not begin until 2 weeks of age and reached twice the adult level between weeks 3–5, the time of weaning (*121*). In kidney, CYP2E1 was expressed at 1 week of age.

Clearly, the data indicate that developmental expression and activity of CYP2E1 in humans must be explored more fully. This is particularly important because this enzyme is responsible for the biotransformation of many xenobiotics, including metabolically activated compounds such as TCE. A complete assessment of the health effects of TCE in the fetus and child requires knowledge of the metabolism, particularly by CYP2E1, in this population.

Additional metabolizing enzymes. As with the CYP450 enzymes, the expression and activity of several other enzyme systems in humans vary depending on the developmental stage. For example, glucuronidation in the child reaches adult values at 3–6 months (*122*). Thus, the fetus and neonate probably lack adult capacity to form the glucuronide of TCOH, which can potentially lead to an increase in the formation of TCA and DCA. Since these metabolites are toxic, the fetus and neonate could potentially be more susceptible to TCE-related toxicity.

Of additional concern to children is the decrease in ADH activity. ADH activity is present in fetal liver at the second month of gestation, but this activity is only 3% of adult levels (*123*). The diminished ADH activity appears to have an effect on the clearance of several drugs and chemicals, including CH, a metabolite of TCE. For example, the elimination half-life of CH was much greater in the preterm fetus (39.8 hr) than in the neonate (27.8) or child (9.7, age 1–13 years) (*124*).

Metabolism in Rodents. Studies in pregnant and immature rodents can lend insight into mechanisms of effects of exposure to environmental chemicals and potential toxicity to humans, particularly with regard to the contribution of metabolic differences. Several studies have been conducted examining the expression of metabolic enzymes in the developing rodent. Hepatic microsomal monooxygenase activity is very low during fetal development in most mammals but increases rapidly after birth. Sex-specific regulation of certain P450 isozymes occurs during development. For example, CYP2C11 was found at significant levels in immature (4-week-old) male rats, and liver levels increased 30-fold in male but not female rats at puberty (*125,126*). In addition, microsomal protein levels were higher in immature male rats versus females; protein levels increased during development, but at puberty no sex differences were found. No difference in net P450 content was seen between immature male and female rats, but marked differences were seen at puberty. Pregnancy decreased the P450 content but not the level of microsomal protein.

Nakajima et al. (*52*) examined the effects of sex, age, and pregnancy on toluene and TCE metabolism in rat liver. CYP2E1 levels were higher in immature than in mature rats, especially at low substrate levels. At puberty, CYP2E1 levels were higher in females than in males. In general, TCE metabolism was higher in immature than in mature rats, especially at low substrate levels. No sex differences in metabolism were seen with age or with varied TCE concentration. However, pregnancy decreased the metabolism of both toluene and TCE.

Age-dependent effects on excretion. Several processes involved in the excretion of chemicals may be affected by age. Polar compounds can be excreted unchanged, whereas most lipophilic compounds are readily excreted following metabolism to more polar forms. Lipophilic chemicals are also eliminated through exhalation. Pulmonary excretion occurs primarily with gases and vapors, such as TCE. As described above, lung development is different in children and may affect how TCE is excreted in breath.

Biliary and fecal excretion are additional pathways. Many metabolites are excreted from the liver into the bile by specialized transport systems similar to those in the renal tubule, and competition for transport can occur between ions of like charges. These chemicals are either excreted in the

feces or reabsorbed into the blood and excreted into the urine, a process known as enterohepatic recirculation (EHR). EHR occurs primarily with organic ions, such as glucuronides or cations. In the child, biliary acid secretion is decreased, which will alter EHR (*127*). The glucuronide conjugate, trichloroethanol glucuronide (TCOG), is excreted in the bile, undergoes EHR, and does not get excreted into the feces (*15*). Thus, decreased biliary acid secretion in the child will probably alter EHR of TCOG and may alter the toxicity of TCE.

Elimination of chemicals can also be affected by factors such as the glomerular filtration rate (GFR). The GFR in newborns is low but reaches adult levels by 3 months of age (*128*). Since many chemicals are eliminated in the urine, including several TCE metabolites, developmental differences in the GFR may affect overall elimination.

A potential excretory pathway for TCE in adult women that may be of particular concern for infants is lactation. TCE is lipophilic and is excreted in the breast milk. However, no studies have been conducted on the dosimetry of TCE in breast-fed infants. Recently, a physiologically based pharmacokinetic (PBPK) model was developed to describe the transfer of volatile chemicals in breast milk, including TCE (*129*). An intermittent acute exposure to 50 ppm of TCE was simulated using previously published physiological and metabolic values for a lactating woman (2–3 months postpartum). The simulated nursing schedule consisted of eight 12-min nursing bouts over a 24-hr period. The predicted amount of TCE ingested by the infant over the 24-hr period was 0.496 mg compared to the U.S. EPA Health Advisory Intake Limits of 0.6 mg/day for an adult consuming 2 L of water per day.

While this estimate would suggest that breast-fed infants are not at increased risk from exposure to TCE in breast milk, several factors need to be considered. The output, that is, the amount of TCE ingested by the lactating infant, is compared to drinking water limits for an adult and may or may not be appropriate for the child. Moreover, the model simulated the 2- to 3-month-old infant not the newborn or older infants. The model also simulates only parent compound and does not consider the transfer of metabolites. Studies in rats showed that TCA reaches the suckling pup at 30 and 50% of maternal levels following exposure of the mother to TCE in the drinking water and through inhalation, respectively (*130*). Thus, given these factors and the small difference between PBPK model predictions and the U.S. EPA advisory, conclusions regarding the safety of exposure to TCE in breast milk need to be reevaluated.

Clearly there are many factors that have the potential to alter the dosimetry and toxicity of TCE in the child. Any one of these factors alone may play a role in susceptibility. However, in order to provide a scientifically justifiable assessment of risk to children, these factors need to be considered simultaneously. The most useful tool for accomplishing this task would be a PBPK model for TCE in the growing child. Despite potential limitations, the development of the PBPK model described above is a useful step in this process.

Acquired Factors That Affect Physiological Variables

A number of acquired factors affect metabolic gene expression and function, and include health status, prior and concurrent exposure to other substances, and behavioral patterns. Susceptibility to TCE can be altered by these factors because many of them have the potential to alter toxicity secondary to CYP2E1 induction. Factors that lead to an induction of CYP2E1 include, but are not limited to, uncontrolled diabetes, obesity, and prior exposure to certain solvents (*131*). CYP2E1 is also induced by certain medications, such as barbiturates, and by excessive alcohol consumption (*55,132*). The chronic use of barbiturates, as often occurs in epileptics, increases protein and lipid content of hepatic smooth endoplasmic reticulum, and also increases the activity of glucuronyl transferase and various CYPP450s, including CYP2E1 (*133*). Comprehensive reviews on the mechanism for CYP2E1 induction in various populations, and on the physiological and pathological role of CYP2E1, are provided by Raucy et al. (*131*) and Lieber (*55*), respectively.

It is important to consider these populations in evaluating susceptibility, given the number of persons who fall into one or more of these categories. For example, 14 million people in the United States meet the diagnostic criteria for alcohol abuse or alcohol dependence (*68*). The prevalence of insulin-dependent diabetes in the United States is 300,000–500,000, with 30,000 new cases diagnosed each year (*134*). The incidence of diabetes is affected by sex, race, and age (*134,135*).

Very few studies have been conducted on toxicokinetic interactions between TCE and these risk factors, with the exception of ethanol. Although the discussion below will focus primarily on interactions between ethanol and TCE, this serves as an example of potential consequences of TCE exposure in persons with induced CYP2E1, as well as the influence of exposure to mixtures.

Ethanol Interactions. Concurrent exposure to TCE and ethanol is likely to occur in a large number of people, and ethanol may increase susceptibility to the adverse health effects of TCE. Alternatively, it can be viewed that exposure to TCE can aggravate the effects of ethanol. This interaction may be an important health problem because, as discussed above, both alcohol and TCE exposure are widespread in the United States. Ethanol and TCE are centrally acting chemicals and exposure to high levels can cause profound CNS depression (*75,136*). Many enzymes responsible for TCE metabolism are also involved in the metabolism of ethanol.

Approximately 75–90% of ethanol is metabolized through oxidation in the liver, primarily by ADH, although metabolism can also occur in other tissues, such as the kidneys, gastric mucosa, and brain (Figure 2) (*137–139*). Ethanol is also metabolized by CYP2E1 (*140*). The contribution of CYP2E1 to the metabolism of ethanol *in vivo* is unclear but is thought to contribute at higher doses and following chronic exposure to ethanol (*55,132,138*). Chronic ethanol consumption can result in up to a 10-fold induction in CYP2E1 (*55*). The catalase system also contributes to the metabolism of ethanol and requires hydrogen peroxide. Thus, its relative role in the metabolism of ethanol *in vivo* is thought to be minimal (*141*).

Several studies have been conducted on the toxicokinetic interactions between ethanol and TCE in male rats. Exposure to ethanol in the diet produced a 6-fold increase in the *in vitro* metabolism of TCE and an increase in cytochrome P450 content and activity (*142*). *In vivo* elimination was increased following inhalation exposure to concentrations of TCE ranging from 500 to 8,000 ppm. Blood TCE concentrations were decreased and the urinary excretion of TCA and TCOH was enhanced at all exposure concentrations (*142*). These toxicokinetic interactions between chronic ethanol exposure and TCE *in vivo* are dependent upon TCE exposure concentration. For

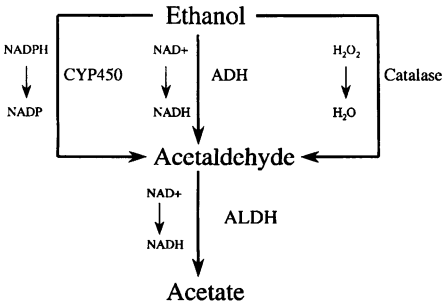

Figure 2. Biotransformation of ethanol. Metabolism occurs primarily by hepatic ADH at low ethanol concentrations and following acute exposure. At higher concentrations and following chronic exposure to ethanol cytochromes, P450 (CYP450), particularly CYP2E1, contributes to the metabolism. Catalase plays a minor role in ethanol metabolism. Each pathway results in the formation of acetaldehyde, which is metabolized to acetate via ALDH.

example, an increase in the *in vivo* elimination of TCE following chronic ethanol exposure in male rats was found at TCE concentrations greater than 100 ppm; exposure to either 50 or 100 ppm produced no difference in blood TCE concentration or in the amounts of TCA and TCOH in the urine (*143*). The duration of exposure (*142,144*) and the composition of the ethanol-containing diet (*55,145*) can also influence the metabolic interactions and toxicity.

Data from human males also suggest potential influences of chronic ethanol consumption on the toxicity of TCE. A study of 188 factory workers exposed to TCE revealed increased hepatotoxicity in subjects who consumed ethanol (*146*). On average, workers were exposed for 7 hr/day for 7 years to TCE concentrations ranging from 50 to 150 ppm. Heavy drinkers consumed 1.5 L of wine per day for at least 5 years. Of the 51 workers identified as heavy drinkers, 41 showed clinical signs of liver impairment. This prevalence was statistically significantly increased compared to the prevalence among workers who were not heavy drinkers. However, because chronic ethanol consumption causes liver impairment, these observed effects may be due to the presence of ethanol rather than TCE. That is, TCE may be enhancing the toxicity of ethanol.

Acute ethanol exposure also affects the toxicokinetics of TCE. Concurrent exposure to ethanol and TCE in human males inhibited the metabolism of TCE at an exposure concentration of 50 ppm for 6 hr on 5 consecutive days (*147*). Plasma TCA and TCOH concentrations were decreased by one-half and one-third, respectively, compared to controls. The total amount of TCA and TCOH excreted in the urine also decreased 43 and 20%, respectively. Similarly, ethanol almost completely inhibited the metabolism of a single exposure to 100 ppm TCE for 6 hr (*147*). Plasma TCA levels were essentially unchanged, blood TCE concentrations increased 2-fold, and breath TCE concentrations increased 3-fold when ethanol was consumed prior to and during the TCE exposure.

Studies in rodents confirm the acute effects of ethanol in adult men. Ethanol added directly to microsomal incubations obtained from rats inhibited the *in vitro* metabolism of TCE (*148*). An acute ethanol administration also inhibited the *in vivo* elimination of TCE when ethanol was still present in the livers of male rats (*149*). Steady-state blood TCE concentrations were elevated in female rats exposed to 50 or 100 ppm TCE and 0.8 mL/kg ethanol (*149*). These toxicokinetic interactions are likely affected by the balance of cofactors necessary for TCE and ethanol oxidation, and inhibition of TCE following acute exposure to ethanol is likely due to a shift in the NADH:NAD^+ ratio (*150–152*).

Acutely, ethanol may also interact with the metabolites of TCE, specifically CH and TCOH, since ethanol and CH are both metabolized by ADH and have similar pharmacological properties (*75*). For example, male subjects who were administered ethanol following the administration of CH had significantly higher plasma TCOH levels compared to CH alone, and levels were prolonged in the ethanol-exposed subjects (*153*). A decrease in plasma TCA was also found in the ethanol-exposed subjects, as well as an increase in urinary TCA and a decrease in urinary TCOH production. The authors suggest the ethanol-induced increase in plasma TCOH concentration is due to an increase in the metabolism of CH to TCOH and a decrease in the glucuronidation of TCOH.

Although experimental studies in humans exposed to both TCE and ethanol are limited, pharmacokinetic modeling has provided insight regarding the effects of potential interactions. Simulation studies using a PBPK model for TCE and ethanol were conducted to examine the interactions between acute and chronic ethanol and TCE (*154*). Based on previous studies conducted in male rats, the metabolism of TCE following acute exposure to ethanol was assumed to be competitively inhibited and was described using the Michaelis-Menten expression for competitive inhibition. CYP2E1 induction following chronic ethanol exposure was simulated by an increase in the V_{max} for the metabolism of TCE.

Moderate doses of ethanol consumed 15 min prior to a 6-hr exposure to 50 ppm TCE resulted in increases in blood TCE concentrations and corresponding decreases in the rate of excretion of urinary metabolites (*152*). At the highest ethanol dose (20 mmol/kg), blood TCE concentrations increased 70% and the urinary excretion rate of total urinary metabolites decreased 40%. This dose of ethanol corresponds to approximately 6–9 standard drinks in a 70-kg man and 5–8 drinks in a 60-kg female.

The induction of CYP2E1, simulated by a 5-fold increase in the V_{max}, had a slight effect on TCE metabolism (*154*). Blood TCE concentrations were decreased 10% and the excretions of urinary TCA and TCOH was increased by less than 10%. Simulations at TCE concentrations higher than 500 ppm showed a much greater effect on the decrease in blood TCE and the increase in urinary TCA and TCOH formation.

The minimal effect of CYP2E1 induction on the metabolism of low concentrations of TCE is due, at least in part, to the intrinsic clearance of TCE. When concentrations of TCE are low, the metabolism is blood-flow limited and the effect of CYP2E1 induction is minimal. At higher concentrations when metabolism is saturated, CYP2E1 induction results in an increase in the capacity to metabolize TCE, which increases the *in vivo* elimination and formation of toxic metabolites.

Predictions based on *in vitro* studies of TCE metabolism (*40*) and PBPK model simulations in human males (*155*) suggest the metabolism of TCE in humans at occupationally relevant concentrations (50 ppm) is not saturated. Accordingly, a significant increase in the formation of toxic metabolites formed from the oxidative pathway would not be expected. However, a 10% increase in the formation of toxic metabolites, as predicted by the PBPK model, might be enough to enhance the toxicity of TCE, particularly over long-term TCE exposure. The simulations accounted for a 5-fold induction in CYP2E1, and chronic ethanol consumption can result in up to a 10-fold induction of CYP2E1 (*132*). Moreover, up to a 20-fold variation in CYP2E1 activity can occur (*138*).

The extent of TCE metabolism in humans was found to be correlated with cytochrome 450 activity and content (*40*). Although Lipscomb et al. found that CYP2E1 activity varied less than 10-fold, these studies were conducted on a limited number of subjects and cannot necessarily be generalized to all populations. For example, subjects who consumed alcohol were not included in this study. Furthermore, the short-term exposure limit for TCE is 200 ppm and was set to protect against the anesthetic effects of TCE (*29*). The minimal risk level (MRL), which is the estimated daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of time and is set by ATSDR, is well below 200 ppm for TCE (*12*). The MRLs for acute and intermediate inhalation exposure to TCE are 2 and 0.1 ppm, respectively. Thus, when CYP2E1 is induced, an increase in the formation of toxic metabolites will likely occur at TCE concentrations at or near 200 ppm. The anesthetic effects of acute ethanol and TCE will be enhanced when exposure to both occurs at this concentration.

Another possible interaction that has not been studied is the effect of ethanol-induced glutathione depletion on the metabolism of TCE to DCVG. Ethanol decreases hepatic mitochondrial glutathione levels (*156*), which in turn impairs mitochondrial function (*157*). The formation of DCVG from glutathione conjugation has been implicated as a possible mechanism for the nephrotoxicity and nephrocarcinogencity of TCE (*15*). Thus, ethanol exposure has the potential to affect susceptibility to TCE via interaction with this pathway.

Most studies on the interaction between TCE and ethanol were conducted in male rats, as well as a limited number of studies in human males. Caution should be used when extrapolating these findings to females, particularly since significant sex-dependent differences in the affinity to metabolize TCE were observed in humans (*40*). As discussed above, sex-dependent difference in the toxicity of TCE and TCA also exists. In addition, there are sex-dependent differences in the pharmacokinetics and toxicity of ethanol; women are more susceptible to the adverse effects of ethanol (*158–163*).

While studies have not been conducted on the interactions between ethanol and TCE in pregnancy, studies in rats showed an elevation in cardiac malformation in rat pups exposed to DCA, TCA, or TCE *in utero* (*18,100,164*). An increase in cardiac anomalies was found in children born to mothers exposed to drinking water containing, among other contaminants, TCE and DCA (*17,18*). Exposure to ethanol *in utero* also results in cardiac malformations in humans (*165*).

The induction of CYP2E1 by ethanol in the fetus and developing infant may also increase the likelihood of developmental effects of TCE and/or ethanol. Recent studies found elevated CYP2E1 levels and an increase in activity in rat pups exposed to ethanol *in utero* (*166*) as well as pups exposed translactationally (*167*). Ethanol has been measured in the breast milk of lactating women (*168*). Recent studies in women with a history of heavy drinking found an increase in the expression of CYP2E1 in third-term placentas (*169*). A 2-fold induction in CYP2E1 levels was found in fetal hepatocyte cultures treated with ethanol (*170*). The estimated incidence of fetal alcohol syndrome (FAS), a syndrome of anomalies resulting from exposure to ethanol *in utero*, is 9.7 per 10,000 live births; among heavy drinkers, 4.3% of children are born with FAS (*171*). Therefore, the possibility of induction of CYP2E1 in the placenta and/or human fetus exists and this may lead to a subsequent increase in susceptibility to the developmental effects of TCE.

The data presented thus far illustrate that interactions between ethanol and TCE occur in both rodents and humans following acute and chronic ethanol exposure. There are limited data in rodents and humans, suggesting that chronic ethanol consumption leads to greater hepatotoxicity of TCE and that acute ethanol consumption increases the CNS effects of TCE. Alcoholics are often warned about a potential increase in the adverse health effects of many drugs. For example, the U.S. Food and Drug Administration will be requiring an alcohol warning on all over-the-counter medications containing acetaminophen because induction of CYP2E1 causes an increase in the hepatotoxicity of acetaminophen (*172*). Given that there are over 80 exogenous chemicals metabolized by CYP2E1, physicians should probably warn alcoholics of the potential increase in adverse health effects of environmental contaminants.

Prior and concurrent exposures. Susceptibility may be altered simply by increasing the body burden of a given environmental agent or its metabolites. In some cases a threshold may be reached. Previous exposures may enhance susceptibility by sensitizing the individual to the compound, either directly or by cross-sensitization. Prior exposure to various chemicals may also interfere with metabolism through inhibition or induction, which in turn can alter susceptibility.

For example, Lipscomb et al. (*40*) studied the effect of TCE on specific cytochrome P450 isozyme activities using mouse, rat, and human hepatic microsomes. TCE was a competitive inhibitor of CYP2E1 activity, and a noncompetitive inhibitor of CYP3A and CYP2B. Addition of TCE at 1,000 ppm inhibited CYP2E1 activity in all three species, and CYP3A activity in mice and rats. Prior exposure to TCE had no effect on CYP2A activity but did increase CYP1A1/1A2 activity. In addition, animal studies show DCA inhibits its own metabolism (*173*). Thus, DCA levels may accumulate with prior or prolonged exposure.

Nakajima et al. (*52*) compared the relative contribution of the P450 isozymes in the metabolism of TCE in rats. Hepatic microsomes were obtained from control rats, and rats previously exposed to phenobarbital, ethanol, or 3-methylcholanthrene were used. CYP2E1, CYP2E11/6, CYPIIB1/2, and CYPIA1/2 were involved in conversion of TCE to CH, with some variation in isozyme levels at different TCE concentrations. At low TCE levels, adding anti-CYP2E1 antibodies inhibited TCE metabolism in ethanol-treated rats more greatly than in controls. In contrast, CH formation was inhibited by anti-CYP2C11/6 in control and PB-treated rats at high but not low TCE concentrations, with a lower net inhibition than from using anti-CYP4502E1. Anti-CYP3B1/2 and anti-CYPA1/2 inhibited CH formation from phenobarbital and 3-methylcholantherene-treated rats, particularly at high TCE concentrations. CYP2B1 contributed more to TCE oxidation than CYPIC1/6, but the reverse was seen for toluene metabolism. In conclusion, CYP2E1, 1A1/2, 2C11/6, and 2B1/2 are all involved in metabolism of benzene, toluene, and TCE, indicating broad and overlapping substrate specificity of these volatile compounds.

Prior exposure to solvents such as toluene can induce CYP450s, which in turn can affect TCE metabolism (*174*). Both sex and age influence CYP450 induction after toluene exposure; in general, induction is greater in younger animals and higher in males (*55*). Neonatal exposure of rats to toluene greatly affected liver microsomal CYP450 activity at birth, whereas minimal effects were seen in rats exposed at 3 weeks of age (*174*).

Altered health state. An additional population that deserves consideration in the evaluation of susceptibility to environmental toxins is persons whose health state is altered. This is important because of the effects of certain diseases on the ability of the body to process chemicals. For example, the ability to renally excrete chemicals can be diminished in people with kidney impairment, leading to an increase in toxicity. The phenomenon is readily apparent with antibiotics. The majority of antibiotics and their metabolites, such as vancomycin and aminoglycosides, are eliminated by the kidneys. In patients with renal insufficiency, dosages of these drugs are adjusted accordingly to avoid overt excessive toxicity (*175*). Liver disease also affects the ability to metabolize and eliminate chemicals. For example, rifampin and isoniazid have prolonged half-lives in people with cirrhosis (*175*). These same principles apply following exposure to environmental chemicals and may contribute to differences in susceptibility.

As previously discussed, uncontrolled diabetes results in an induction of CYP2E1, which can lead to an increase in the formation of toxic metabolites. Diabetics are also at greater risk for liver cancer. Recent studies in the Swedish population show a 4-fold increase in the risk for development of primary liver cancer in patients with diabetes mellitus versus the general population (*176*). Similar results were reported in studies from Italy, Denmark, and Los Angeles (*177–179*). Diabetics may be a population susceptible to the potential hepatotoxic effects of TCE.

The effect of DCA on blood glucose and serum insulin levels is of additional concern for diabetics. An increase in serum insulin levels in mice exposed to ≤ 0.5 mg/L DCA in the drinking water was found (*22*). This has not been found in humans. But DCA reduces hyperglycemia in insulin and non-insulin–dependent diabetics by stimulating blood glucose oxidation in peripheral tissues, stimulating glycogensis from glucose in adipocytes, and depleting hepatic and muscle glycogen levels (*180*). These effects have been observed in both animals and humans. DCA also decreases hyperglycemia through stimulation of pyruvate dehydrogenase (PDH) (*181*). DCA inhibits pyruvate dehydrogenase kinase, which maintains PDH in its unphosphorylated, catalytically active state (*181*). PDH controls the rate of glucose and pyruvate oxidation, and the net effect is a decrease in blood glucose levels, as well as a dramatic

and prolonged decrease in lactate and aniline levels (*181*).

Several studies have been conducted in humans to evaluate the potential use of DCA in the treatment of hyperglycemia in diabetics, as well as in the treatment of lactic acidosis, hyperlipidemia, ischemic heart disease, and heart failure. The treatment regimen used in lactic acidosis consists of a 30-min intravenous infusion of 50 mg/kg followed by a second infusion of the same dose 2 hr after initiation of the first dose (*182*). This dosing regimen, which results in a rapid decrease in plasma lactate and glucose concentrations, produced maximum plasma DCA concentrations of 144 ± 92 mg/L and 180 ± 92 mg/L following the first and second infusion, respectively, in adult patients with lactic acidosis (*182*). These levels are much greater than those measured in healthy human subjects exposed to occupationally relevant concentrations of TCE (*183*). In this study, subjects were exposed to 100 ppm TCE for 4 hr, and TCE and metabolite levels were measured in blood and urine. DCA was detected intermittently, with as high as 0.014 mg/L measured in one subject. It is unlikely that exposure to occupationally relevant TCE concentrations will produce blood DCA levels that would result in a decrease in hyperglycemia in diabetics.

However, DCA administration in humans has been limited to 2 weeks and chronic administration has not been examined. Because DCA inhibits its own metabolism, prolonged and/or repeated administration may lead to sustained concentrations that may result in an irreversible inactivation of the PDH (*180*). Thus, an increase in susceptibility of diabetics to the effects of the TCE metabolite DCA on blood glucose levels cannot be ruled out until further studies are conducted.

Discussion

As discussed earlier, for most environmental chemicals data on susceptible subgroups are rarely available. The use of linear low-dose extrapolation in cancer assessments has been assumed to be conservative enough not to underestimate risk for susceptible members of the population (*9*). Noncancer assessments acknowledge response variability within the population by an uncertainty factor (current practice uses a default value of 10)(*183*). However, the scientific basis for default assumptions remains questionable, particularly in light of the myriad factors that can potentially alter toxicity. A person's response to environmental hazards is affected by physiological variables including exposure route (respiratory rates, dermal penetration/irritation, etc.), pharmacokinetics (distribution, absorption, metabolism, excretion), and pharmacodynamics (receptor density, organ specificity). These variables are in turn affected by constitutive factors (genetics, gender, age, ethnicity) and acquired factors (disease state, diet, exercise, stress, effects of previous or concurrent exposures).

While a small number of human carcinogens show variable effects in outcome following exposure, our understanding of the inherent variability in subpopulations is limited, and epidemiological and laboratory studies on this subject are in their infancy (*184*). While heredity clearly has an impact on exposure-driven cancer, not enough is known about the correlation to harness it in improving public health (*184*).

Ethnic groups vary in their distributions of genotypes, and extrapolation of health outcomes between the different groups is very difficult (*51*). In the United States, overall disease rates are higher in African-Americans than in Caucasians. Molecular epidemiology suggests different patterns of exposure and/or internal handling of exogenous or endogenous carcinogens along racial or ethnic lines, and different patterns of susceptibility markers are seen (*184*).

Despite the observational correlations, biologically plausible mechanisms to link specific genotypes to specific outcomes are still lacking for most diseases; for example, many RFLP polymorphisms in Class I genes are the result of mutations in introns or other silent areas of the human genome (*51*), and molecular methods are still of limited utility in predicting susceptibility at this time. Furthermore, it is difficult to disentangle outcomes that are the result of genetic traits, particularly when associated with ethnicity, from socioeconomic and behavioral factors that also may affect environmental exposure or susceptibility.

In some cases, risk reduction can be achieved by going beyond risk regulation and taking a public health approach promoting healthy activities in conjunction with an increased understanding of the biological mechanisms of toxicity, which may allow development of more effective interventions. Examples of successful interventions include controlling smoking and radon exposure, smoking and asbestos exposure, and CYP450 induction and alcohol/smoking.

While environmental prevention strategies focus on reducing exposure in the workplace and environment, intervention strategies are geared toward the population as a whole and do not inherently address genetic differences among individuals (*185*). Some contend that current environmental risk assessment methods do not provide sufficient protection, as they assume similar biological responses from all individuals for a specified dose of a toxic agent (*185*). This results in regulatory and health policies aimed at protecting the "average American," ignoring a sizable, more vulnerable fraction of the population (*51,184*). Conversely, others contend that the default safety factors used in human risk assessment are overprotective of the population, resulting in high regulatory costs at the expense of industry.

The new U.S. EPA cancer guidelines proposed in 1996 discuss the importance of incorporating information on susceptibility. The NRC and the Presidential Commission on Risk Assessment and Risk Management have recommended that the U.S. EPA develop explicit methods for assessing susceptibility, using molecular epidemiology and other population-based information to determine the magnitude of variability (*5*).

In order to better protect vulnerable groups and the population as a whole, accurate data on susceptible groups are needed and should be directly incorporated into risk assessment processes to allow development of better health-based policies (*3,5*). More mechanistic information is needed to allow better understanding of how different factors influence toxicity, and how these vary according to genetic profile, behavior, and age. For example, molecular biomarkers of exposure and effect are increasingly being used in epidemiological analyses to improve the resolution of risk factors and to better understand the basic underlying mechanisms responsible for disease (*185*). Biomarkers can serve as an early indicator of exposure or susceptibility before clinical signs manifest, allowing early interventions. The distribution of biomarkers in a population can potentially be used to improve the quantitative estimates of risk from a given exposure, and to identify susceptible groups or even individuals who may be at risk (*51,185*).

A more comprehensive documentation and understanding of sex- and species-dependent differences in metabolism is essential for extrapolating animal data to humans, for refining risk assessment models and methods, and for better determining safe exposure levels for humans.

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