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List of abbreviations and acronyms:

AUC	area-under-the-curve.
DCA	dichloroacetic acid
DCVC	dichlorovinyl cysteine
GSH	glutathione
GSTT1	glutathione-S-transferase theta-1
IRIS	Integrated Risk Information System
NHL	non-Hodgkin lymphoma
PBPK	physiologically based pharmacokinetic
PPAR	peroxisome proliferator activated receptor
TCA	trichloroacetic acid
TCE	trichloroethylene

Abstract

Background: In support of the Integrated Risk Information System (IRIS), the Environmental Protection Agency (EPA) completed a Toxicological Review of Trichloroethylene (TCE) in September, 2011, the result of an effort spanning over twenty years.

Objectives: This article reviews key findings and scientific issues regarding the human health effects of TCE in EPA's Toxicological Review.

Methods: This assessment synthesized and characterized thousands of epidemiologic, experimental animal, and mechanistic studies, and addressed several key scientific issues through modeling of TCE toxicokinetics, meta-analyses of epidemiologic studies, and analyses of mechanistic data.

Discussion: Toxicokinetic modeling aided in characterizing the toxicological role of the complex metabolism and multiple metabolites of TCE. Meta-analyses of the epidemiologic data informed the conclusion that TCE causes kidney cancer in humans and provided strong evidence that TCE may also cause liver cancer and non-Hodgkin lymphoma. Mechanistic analyses support a key role for mutagenicity in TCE-induced kidney carcinogenicity. Recent evidence from both human and experimental animal studies indicates the involvement of TCE exposure in autoimmune disease and hypersensitivity. Recent avian and *in vitro* mechanistic studies provide biological plausibility that TCE plays a role in developmental cardiac toxicity, the subject of substantial debate due to mixed results from epidemiologic and rodent studies.

Conclusions: TCE is carcinogenic to humans by all routes of exposure and poses a potential human health hazard for non-cancer toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing embryo/fetus.

Introduction

Trichloroethylene (TCE) is a chlorinated solvent once widely used as a metal degreaser, chemical intermediate and extractant, and component of some consumer products. Total releases to the environment reported to U.S. EPA's Toxics Release Inventory have declined from over 57 million pounds in 1988 to about 2.4 million pounds in 2010 (U.S. EPA 2012b). Because it has a relatively short half-life, TCE is not commonly detected in biomonitoring surveys, and the percent of subjects with detectable levels (>0.1 ng/ml) has declined from about 10% to 1% between samples collected in 1988-1994 and those collected in 2003-2004 (CDC 2009; Wu and Schaum 2000). From a regulatory and environmental cleanup perspective, TCE has been identified in soil or groundwater at more than 700 of approximately 1300 Superfund hazardous waste sites listed by the U.S. Environmental Protection Agency (EPA) (U.S. EPA 2011c). Additionally, EPA has identified TCE as one of the volatile organic compounds to be regulated as a group in drinking water (U.S. EPA 2010, 2011a), and as one of the priority existing chemicals under review for regulatory action under TSCA (U.S. EPA 2012a). Indeed, due to its continued occurrence in the environment, most people are likely to have some exposure to TCE, either through contaminated drinking water, ambient outdoor or indoor air, or, less commonly, through contaminated foods.

EPA's Integrated Risk Information System (IRIS) program released an updated human health risk assessment of TCE in September, 2011 (U.S. EPA 2011d). This assessment was developed over the span of more than 20 years and underwent many stages of both internal- and external peer review. Key inputs were recommendations for additional analysis and research from a National Research Council (NRC) panel report reviewing the key scientific issues pertaining to TCE hazard and dose-response assessment (NRC 2006). This report, together with

a series of issue papers developed by EPA scientists (Caldwell and Keshava 2006; Chiu et al. 2006a; Chiu et al. 2006b; Keshava and Caldwell 2006; Scott and Chiu 2006), provided the foundation for developing an objective, scientifically rigorous human health risk assessment for TCE. EPA's final assessment also incorporated input from two independent peer reviews by EPA's Science Advisory Board (SAB 2002, 2011), other federal agencies (U.S. EPA 2009b, 2011b), and the public (U.S. EPA 2009a).

This article describes key findings and scientific issues addressed in EPA's 2011 Toxicological Review of TCE, covering the following topics: (1) the role of metabolism in TCE toxicity, which was informed by the development and use of an updated physiologically based pharmacokinetic (PBPK) model; (2) the carcinogenicity of TCE, including the development of meta-analyses of epidemiological studies for informing causal inferences, as recommended by the NRC, and analyses of laboratory animal mechanistic and toxicokinetic data contributing to the evaluation of biological plausibility of the epidemiologic data; and (3) non-cancer toxicity related to the two endpoints immunotoxicity and developmental cardiac toxicity, for which substantial new data have become available. For findings and issues related to other important topics not discussed here (e.g., susceptibility, mixtures/co-exposures, and dose-response assessment), readers are referred to previous reviews and the relevant chapters and appendices of EPA's Toxicological Review of TCE (e.g., Caldwell JC et al. 2008; NRC 2006; U.S. EPA 2011d).

Role of Metabolism in TCE Toxicity

TCE has a complex database of relevant information for assessing human health effects. Previous reviews have found TCE to adversely affect the central nervous system (Bale et al.

2011), liver (Bull 2000), kidney (Lash et al. 2000b), immune system (Cooper et al. 2009), and reproductive systems and developing embryo/fetus (NRC 2006). As shown in Figure 1, TCE is metabolized in humans and experimental animal species by both oxidation and glutathione (GSH) conjugation metabolic pathways, with subsequent production of numerous toxicologically active compounds (Chiu et al. 2006b; Lash et al. 2000a). These include the oxidative metabolites chloral hydrate (CH), trichloroacetic acid (TCA), and dichloroacetic acid, and GSH conjugation metabolites dichlorovinyl glutathione and dichlorovinyl cysteine (DCVC). This complex assortment of metabolic compounds is generated from and transported across multiple tissues, making evaluation of mechanistic data especially challenging (JC Caldwell et al. 2008). TCE liver effects are thought to result from oxidative metabolites (Buben and O'Flaherty 1985; Bull 2000), whereas kidney effects are generally associated with metabolites resulting from GSH conjugation (Lash et al. 2000b). The identity of TCE metabolites involved in the induction of other TCE health effects is less clear, although similarities are observed between TCE and its oxidative metabolites in respiratory tract and developmental toxicity (e.g., Johnson et al. 1998a; Odum et al. 1992).

Tools such as PBPK models can be very useful for integrating complex toxicokinetic information on absorption, distribution, metabolism, and excretion of TCE and its metabolites. Many PBPK models for TCE have been developed to predict the relationship between external measures of exposure and internal dose measures (Bois 2000a, b; Clewell et al. 2000; Fisher 2000; Hack et al. 2006). Chiu et al. (2009) and Evans et al. (2009) updated and “harmonized” these efforts into a new model for use in the IRIS assessment.

Evans et al. (2009) and Chiu (2011) illustrate the importance of internal dose in investigating mechanisms of TCE toxicity, addressing the key question of whether the TCE

metabolite TCA can account for mouse hepatomegaly caused by TCE. In particular, they used the TCE PBPK model to compare the hepatomegaly response after administration of TCE with that after direct administration of its metabolite TCA, using the common internal dose measure of TCA liver concentration. If TCA were the only contributor to TCE-induced hepatomegaly, then this comparison would find equal changes in liver weight for equal TCA liver concentrations, regardless of whether TCA was the result of TCE metabolism or the result of direct TCA administration. However, as reported by Evans et al. (2009) and Chiu (2011), TCA appears to account for at most half of the hepatomegaly resulting from TCE exposure, implying that effects related to TCE exposure beyond those accounted for by TCA are also operative in TCE-induced hepatomegaly.

Carcinogenicity

Evaluation of cancer epidemiology for kidney cancer, liver cancer, and non-Hodgkin lymphoma

EPA conducted a systematic review of 76 human epidemiologic studies on TCE and cancer (Scott and Jinot 2011; U.S. EPA 2011d). Each study was evaluated with respect to explicitly identified characteristics of epidemiologic design and analysis to consider whether chance, bias, or confounding may be alternative explanations for the study's results. A more in-depth analysis (including meta-analysis) of the epidemiologic studies was conducted for kidney cancer, liver cancer and, non-Hodgkin lymphoma (NHL). These endpoints were of *a priori* interest based on the results of a preliminary review of the epidemiological database and the findings from rodent bioassays of TCE exposure.

Meta-analysis approach and results

Meta-analyses can be used to combine underpowered studies, to evaluate effects across the set of studies, and to examine consistency (or heterogeneity) of results. NRC (2006) identified a number of weaknesses in previous meta-analyses of TCE carcinogenicity, such as subjective assessment of quality and lack of sensitivity analyses. Thus, EPA conducted new meta-analyses to support evaluation of the epidemiological database on TCE (Scott and Jinot 2011). As recommended by NRC (2006), EPA established objective study inclusion criteria; fit the data to both fixed-effect and random-effects models; evaluated statistical heterogeneity across the studies; performed sensitivity analyses examining the influence of individual studies and of different measures of relative risk (RR) from studies presenting alternative estimates (e.g., incidence or mortality); and conducted tests for potential publication bias (which may occur if positive studies are more likely to be published) (Scott and Jinot 2011). The meta-analysis summary effect estimates (RR_m) from the random-effects models are shown in Figure 2, both for any TCE exposure (panel A) and for the highest TCE exposure groups (panel B).

Issues in the interpretation of cancer epidemiologic evidence

Two additional key issues regarding EPA's interpretation of the cancer epidemiologic evidence for kidney cancer, NHL, and liver cancer have been raised in peer review and public comments: (i) the modest magnitude of the RR_m estimates for the three cancer types; and (ii) the role of meta-analysis within a causality determination.

The RR_m estimates from the Scott and Jinot (2011) meta-analyses for the three cancer types were modest [e.g., with overall exposure (Figure 2A): 1.27 (95% CI: 1.13, 1.43) for kidney cancer, 1.23 (95% CI: 1.07, 1.42) for NHL, and 1.29 (95% CI: 1.07, 1.56) for liver cancer];

raising the possibility that observed associations could be the result of confounding. However, a detailed examination by EPA of potential confounding from lifestyle factors or other occupational exposures concluded that confounding was not supported as an alternative explanation for the observed excesses.

For example, although smoking can potentially confound kidney cancer results, a number of kidney cancer case-control studies reported associations with TCE exposure even after controlling for smoking in statistical analyses. In addition, had the cohort studies been confounded by smoking, increased lung cancer risk would be expected. However, increases in lung cancer risk in individual studies were either absent or insufficient to account for the observed excess kidney cancer risk. Overall, after combining studies, RRm estimates for lung cancer were 0.96 (95% CI: 0.76, 1.21) for overall TCE exposure and 0.96 (95% CI: 0.72, 1.27) for the highest exposure groups (not shown).

Another key issue is the role of meta-analysis in the overall evaluation of causality. Meta-analysis can provide an objective, quantitative method to increase statistical power and precision, since the resultant summary effect estimate is based on multiple studies. Strengths of the Scott and Jinot (2011) meta-analyses include study identification based on a systematic and transparent review, evaluations of potential publication bias, examinations of the sensitivity of the overall effect to different inputs, and investigations of possible factors responsible for any statistical heterogeneity observed across studies. However, EPA's characterization of the epidemiological evidence considered multiple aspects of the database as a whole, as discussed next, and did not rely solely on the meta-analysis findings.

Synthesis of epidemiologic evidence

The synthesis of the epidemiologic evidence was organized according to the key concepts proposed by Hill (1965) and is summarized in Table 1. For TCE and kidney cancer, there was convincing evidence of a causal association in humans. Particularly compelling was the consistency of increased kidney cancer RR estimates across the 15 independent epidemiologic studies of different designs and of populations from different countries that met the criteria for meta-analysis inclusion (Figure 2). An increased RR_m estimate was observed in a meta-analysis of kidney cancer and overall TCE exposure. This finding was robust, not being sensitive to different study or RR inputs. There was also no evidence of heterogeneity among studies or publication bias. The observations of a greater RR_m estimate with the highest exposure groups (Figure 2B) and of statistically significant trends between TCE exposure and kidney cancer in two high-quality epidemiologic studies (Charbotel et al. 2006; Moore et al. 2010) support an exposure-response gradient. Finally, potential confounding from smoking or other occupational exposures was unlikely to explain the association of TCE exposure with kidney cancer.

The evidence on carcinogenicity from epidemiologic studies of TCE exposure was strong for NHL, though less convincing than for kidney cancer. Of the 17 studies that met the criteria for meta-analysis inclusion, most observed increased RR estimates (Figure 2A). The increased RR_m estimate observed in the meta-analysis of NHL and overall TCE exposure was robust, as it was not sensitive to different study or RR inputs. However, some heterogeneity among studies was observed, although it was not statistically significant. Some evidence of potential publication bias was also seen. An exposure-response gradient is supported by observations of a greater RR_m estimate with the highest exposure groups (Figure 2B) and of a statistically

significant trend between TCE exposure and NHL in a high-quality epidemiologic study (Purdue et al. 2011).

The epidemiologic evidence was more limited for liver cancer, where only cohort studies with small numbers of cases were available. Of the 9 studies that met the criteria for meta-analysis inclusion, most observed increased RR estimates (Figure 2A). A statistically significantly increased RRm estimate was observed in a meta-analysis of liver cancer and overall TCE exposure, but the statistical significance depended on the large study by Raaschou-Nielsen et al. (2003). There was no evidence of heterogeneity or publication bias. However, the data available did not support an exposure-response gradient, as a lower RRm estimate was observed with the highest exposure groups (Figure 2B) and none of the available studies reported a statistically significant trend between TCE exposure and liver cancer.

Experimental animal studies, analysis of mode-of-action, and toxicokinetic considerations

There is clear evidence of TCE carcinogenicity in rodents. Particularly notable is the site-concordant finding of TCE-induced kidney tumors in multiple strains/sexes of rats exposed by inhalation or gavage (Maltoni et al. 1986; NTP 1988, 1990). Although the increased incidences were low, they were sometimes statistically significant and were considered biologically significant in light of the very low historical incidences of renal tumors in control rats in various laboratories. Site concordance is also found for liver tumors, which were reported in both Swiss and B6C3F1 mice (strains with lower and higher background rates of this tumor, respectively), and in both sexes in the latter strain (Maltoni et al. 1986; NCI 1976; NTP 1990). The evidence was more limited for TCE-induced lymphohematopoietic cancers in rats and mice, (Henschler et al. 1980; Maltoni et al. 1986; NTP 1988, 1990). TCE inhalation bioassays have

demonstrated a statistically significant increase in pulmonary tumors in mice (Fukuda et al., 1983; Maltoni et al., 1986, 1988) but not other species (i.e. rats and hamsters; Henschler et al., 1980; Fukuda et al., 1983; Maltoni et al., 1986, 1988). Finally, testicular (interstitial cell and Leydig cell) tumors were significantly increased in Sprague-Dawley rats exposed via inhalation (Maltoni et al., 1986, 1988) and Marshall rats exposed via gavage (NTP, 1988). In three other tested rat strains, ACI, August, and F344/N, a high (> 75%) control rate of testicular tumors limited the ability to detect a treatment effect, though in ACI rats, a positive trend was reported (NTP, 1988, 1990). Overall, the rodent cancer data add substantial biological plausibility for TCE carcinogenicity in humans, particularly when coupled with the mechanistic data findings, discussed next.

Hypothesized modes of action and mechanistic data informative to the evaluation of TCE's carcinogenic mode of action were considered for liver, kidney, and other tumors (summarized in Table 2). Mode-of-action analyses can inform judgments regarding the human relevance of animal bioassay results and aid in the identification of particularly susceptible populations or lifestages (U.S. EPA 2005). For kidney carcinogenicity, EPA concluded that a mutagenic mode of action is operative for TCE, providing further biological plausibility for the epidemiologic findings of TCE-induced kidney cancer. The identification of the mutagenic metabolites as being derived from the GSH conjugation pathway further suggests increased susceptibility in populations with greater metabolism through this pathway. Consistent with this hypothesis, Moore et al. (2010) found a statistically significant association among TCE-exposed persons with an active GSTT1 enzyme (OR, 1.88; 95% CI, 1.06–3.33), but not among those with no GSTT1 activity (OR, 0.93; 95% CI, 0.35–2.44). Although data on early-life susceptibility to TCE carcinogenicity are lacking, the analysis by Barton et al. (2005) suggests increased

susceptibility to cancer from early-life exposures, particularly for chemicals acting through a mutagenic mode of action. For other endpoints, there are inadequate data to support a particular hypothesized mode of action.

The evaluation of TCE carcinogenicity also considered toxicokinetic data on TCE and metabolites, which are consistent with qualitatively similar absorption, distribution, metabolism, and excretion across species and routes of exposure (Lash et al. 2000a). Mice, rats, and humans all metabolize TCE via the pathways illustrated in Figure 1. Thus, toxicokinetic data support the biological plausibility of TCE carcinogenicity in humans, as humans and experimental animals have similar mixtures of TCE and metabolites in target tissues.

Another issue informed by toxicokinetic data is whether TCE carcinogenicity depends on route of exposure, given that the vast majority of the available epidemiologic data are from inhalation exposures to TCE. Because TCE is systemically distributed and undergoes systemic metabolism from all routes of exposure, there is no reason to expect that cancers such as kidney cancer, NHL, or liver cancer, which originate in separate tissues, would be dependent on route of exposure. Also, TCE-induced tumors have been reported in rodents by both the oral and inhalation routes. Therefore, conclusions regarding TCE carcinogenicity would apply equally to any exposure route.

Conclusions as to Carcinogenic Hazard

Supported by the analyses described above, and following EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA 2005), TCE is characterized as "carcinogenic to humans" by all routes of exposure (U.S. EPA 2011d). This conclusion was based primarily on convincing evidence of a causal association between TCE exposure and kidney cancer in humans. The

epidemiologic evidence is strong for NHL, although less convincing than for kidney cancer. Issues increasing the uncertainty in the NHL association include study heterogeneity, potential publication bias, and less evidence for an exposure-response gradient. The epidemiologic evidence was more limited for liver cancer, where only cohort studies with small numbers of cases were available. Finally, animal bioassay, mechanistic, and toxicokinetic data provide further corroboration and biological plausibility to the epidemiologic findings, thus supporting a causal link between TCE exposure and cancer (Table 1).

Non-cancer toxicity

As part of its evaluation of TCE non-cancer toxicity, EPA analyzed the available experimental animal, human epidemiologic, and mechanistic studies of TCE. A table of the relevant studies for each endpoint is provided in Supplemental Material, Table S1. The discussions below focus on the data pertaining to immunotoxicity and developmental cardiac toxicity, for which there are substantial new experimental and epidemiologic studies (U.S. EPA 2011d), and about which scientific issues have been raised by reviewers or comments. This is followed by an overall summary of the hazard conclusions for non-cancer toxicity.

Immunotoxicity

As recently reviewed by Cooper et al. (2009) and documented in the TCE assessment (U.S. EPA 2011d), the human and laboratory animal studies of TCE and immune-related effects provide strong evidence that TCE exposure increases the risk of autoimmune disease and a specific type of generalized hypersensitivity syndrome. In addition to the epidemiologic studies of specific diseases (e.g., systemic sclerosis), changes in cytokine levels reflecting an

inflammatory immune response have been reported in relation to TCE exposure in occupational (Iavicoli et al. 2005) and residential (i.e., infants exposed to TCE in indoor air) (Lehmann et al. 2001; Lehmann et al. 2002) settings. Also, a large number of case reports have associated a severe hypersensitivity skin disorder, distinct from contact dermatitis and often accompanied by hepatitis, with occupational TCE exposure, with prevalences as high as 13% of workers in the same location (Kamijima et al. 2007; Kamijima et al. 2008).

Human evidence for autoimmune-related effects is supported by experimental animal studies. Numerous studies have demonstrated TCE-induced progressive, accelerated autoimmune responses in autoimmune-prone mice [also reviewed in (Cooper et al. 2009)]. After shorter exposure periods, changes in cytokine levels appear similar to those reported in human studies. Longer exposure periods led to more severe effects, including autoimmune hepatitis, inflammatory skin lesions, and alopecia, that differ from the “normal” expression of autoimmune effects in these mice. TCE-induced autoimmune effects have also been reported in B6C3F1 mice, which are not known to have any particular immune-related susceptibility (Gilkeson et al. 2004; Peden-Adams et al. 2006). Treatment-related increase in delayed hypersensitivity response accompanied by hepatic damage has been observed in guinea pigs following intradermal TCE injection (Tang et al. 2002; Tang et al. 2008), and increased hypersensitivity response was also seen in mice exposed via drinking water pre- and postnatally (gestation day 0 through to 8 weeks of age) (Peden-Adams et al. 2006).

There is less evidence regarding a possible role of TCE exposure in immunosuppression. Immunosuppressive effects have been reported in a number of experimental studies in mice and rats (see Supplemental Material, Table S1). Reported effects include reduced responses to bacterial challenge in mice (Aranyi et al. 1986; Selgrade and Gilmour 2010) and decreased

numbers of antibody-forming cells in rats and developmentally exposed mice (Peden-Adams et al. 2006; Woolhiser et al. 2006).

Overall, the concordance of human and laboratory animal studies and the spectrum of effects (from biomarkers to frank expressions of disease) strongly support the conclusion that TCE causes immunotoxicity, particularly in the form of autoimmune disease and a specific type of severe hypersensitivity skin disorder, with more limited evidence for immunosuppression. Moreover, these findings lend additional biological plausibility to the association between TCE and NHL, as alterations in immune status are associated with increased risk of NHL (Grulich et al., 2007)

Developmental Cardiac Toxicity

The TCE database includes a number of epidemiological and animal toxicity studies that indicate TCE-induced developmental toxicity. Congenital malformations, particularly cardiac defects, have been associated with exposures to TCE and/or its metabolites in both humans and experimental animals. Other TCE-related developmental outcomes observed in both humans and experimental animals include embryonic or fetal mortality, prenatal growth inhibition, and neurological and immunological functional deficits.

As noted by NRC (2006), the cardiac teratogenicity of TCE has been the focus of considerable study and analysis (Bove et al. 2002; Hardin et al. 2005; Johnson et al. 1998b; Watson et al. 2006). Only geographic-based epidemiology studies have evaluated whether there is an association between maternal TCE exposure and cardiac defects in offspring (see Supplemental Material, Table S1), with some reporting statistically significant elevations in a variety of cardiac defects (ATSDR 2006, 2008; Yauck et al. 2004), and others reporting non-

statistically significant elevations in risk (Bove et al. 1995; Bove 1996; Goldberg et al. 1990). Interpretation of these data has been controversial, as many of the studies are limited by small numbers of cases, insufficient exposure characterization, chemical co-exposures, and other methodological deficiencies. In addition, these studies aggregate a broad array of TCE-associated cardiac malformations and have inadequate statistical power to identify any particular kind(s) of defect that may be more susceptible to induction by TCE. The NRC noted that the epidemiological studies, although individually limited, as a whole show relatively consistent elevations for cardiac malformations, with similar relative effect sizes of 2- to 3-fold, some of which were statistically significant, associated with TCE exposure across multiple studies (NRC 2006).

The outcomes of studies in rodents exposed to TCE during gestation show an inconsistent pattern. Some studies identified significant treatment-related increases in the overall incidence of cardiac anomalies at environmentally relevant exposure levels (e.g., Johnson et al. 2003; Johnson et al. 2005), while others reported no excess cardiac abnormalities at much higher dose levels (e.g., Carney et al. 2006; Fisher et al. 2001). A number of methodological factors may contribute to differences across study outcomes, such as the route of administration, test substance purity, test species or strain, timing of dosing or fetal evaluation, procedures used in dissecting and examining fetal hearts, statistical approaches applied to data evaluation, and generally uncharacterized inter-laboratory variation.

Other available data providing evidence of TCE cardiac teratogenicity come from avian and *in vitro* mechanistic studies (NRC 2006). For instance, studies in chick embryos reported consistent effects on cardiogenesis (many demonstrating septal and valvular alterations) when TCE was administered during critical stages of heart development (Drake et al. 2006a; Drake et

al. 2006b; Loeber et al. 1988; Rufer et al. 2010); these findings are similar to some of the cardiac defects observed in rodent studies following in utero TCE exposures. The events of cardiac morphogenesis in birds and mammals are similar; both involving mesenchymal cells that form endocardial cushion tissue with subsequent differentiation into septa and valvular structures in the adult heart (NRC 2006). Thus, cultured embryonic chick atrioventricular canal cushion cells have been used to examine chemically induced disruptions in cardiac morphogenesis. In this model, TCE has inhibited endothelial separations and mesenchymal cell formation (Boyer et al. 2000; Mishima et al. 2006) or adhesive properties of endocardial cells (Hoffman et al. 2004), either of which could potentially result in septal or valvular malformations. Other TCE-induced effects that may have morphologic consequences in the developing heart include disruption of endothelial oxide synthetase, which has a role in endothelial cell proliferation (Ou et al. 2003), and interference with proteins involved in intercellular Ca^{2+} regulation, which may result in altered blood flow (PT Caldwell et al. 2008; Caldwell et al. 2010; Collier et al. 2003; Selmin et al. 2008).

Overall, the avian and *in vitro* data substantially increase the biological plausibility for TCE-induced cardiac teratogenesis, and thus strongly support the more limited epidemiologic and *in vivo* rodent data suggesting that TCE induces cardiac teratogenicity. Moreover, the mechanistic data support the possibility that multiple modes of action with different targets within the developing heart may be operant in eliciting cardiac malformations, consistent with the reported association between TCE and overall cardiac malformations in the absence of a strong association with any particular type of defect.

Conclusions as to non-cancer hazard

Table 3 summarizes the evidence for TCE non-cancer toxicity across target organs/systems (see U.S. EPA 2011d for additional details). In addition to the immunotoxicity and developmental cardiac toxicity discussed above, there is strong evidence for TCE-induced neurotoxicity, kidney toxicity, liver toxicity, male reproductive toxicity, and several developmental effects in addition to cardiac toxicity. More limited evidence exists for TCE respiratory tract and female reproductive system toxicity.

Summary

TCE is carcinogenic to humans by all routes of exposure and poses a potential human health hazard for non-cancer toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing embryo/fetus. These conclusions are based on analyses of a broad spectrum of information from thousands of scientific studies and input from numerous scientific reviews. In the last decade, substantial new scientific data on the human health effects of TCE have become available. Moreover, methodological advancements—such as modeling TCE toxicokinetics, meta-analyses of epidemiologic studies, and analyses of mechanistic and non-cancer hazard information—have improved the scientific rigor and transparency of data interpretation. EPA’s analysis approaches and conclusions are consistent with the recommendations of the NRC (2006) and were affirmed by independent peer review through EPA’s Science Advisory Board (SAB 2011). Additionally, the International Agency for Research on Cancer (IARC) recently upgraded its carcinogenicity classification of TCE to “carcinogenic to humans” (Guha et al., 2012). Finally, research on TCE health effects continue

to report similar findings as those described in EPA's assessment, such as kidney carcinogenicity and toxicity (Karami et al., 2012; Vermeulen et al., 2012), immunotoxicity (Hosgood et al., 2011), and developmental cardiac toxicity (Forand et al. 2012).

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Table 1. Primary components for a causality determination based on the epidemiological database for TCE.

Consideration	Summary of Weight of Evidence
Consistency of observed association	<ul style="list-style-type: none"> • Strong evidence of consistency for kidney cancer – consistently elevated RRs. Meta-analysis yielded robust, statistically significant summary RR, with no evidence of heterogeneity or potential publication bias (Scott and Jinot 2011). • Moderate evidence of consistency for NHL – consistently elevated RRs; RR estimates more variable, compared with kidney cancer. Meta-analysis yielded robust, statistically significant summary RR, with some heterogeneity (not statistically significant) and some evidence for potential publication bias. • Limited evidence of consistency for liver cancer – fewer studies overall, more variable results. Meta-analysis showed no evidence of heterogeneity or potential publication bias, but the statistical significance of the summary estimate depends on the large study by Raaschou-Nielsen et al. (2003).
Strength of observed association	<ul style="list-style-type: none"> • Strength of association is modest. Other known or suspected risk factors (smoking, body mass index, hypertension, co-exposure to other occupational agents such as cutting or petroleum oils) cannot fully explain the observed elevations in kidney cancer RRs. The alternative explanation of smoking was ruled out by the finding of no increased risk of lung cancer. Indirect examination of some specific risk factors for liver cancer or NHL did not suggest confounding as an alternative explanation.
Specificity	<ul style="list-style-type: none"> • Limited evidence suggesting particular von Hippel-Lindau mutations in kidney tumors may be caused by TCE (Brauch et al. 1999; Brauch et al. 2004; Brüning et al. 1997; Nickerson et al. 2008; Schraml et al. 1999); additional research addressing this issue warranted.
Biological gradient (exposure-response relationship)	<ul style="list-style-type: none"> • Only a few epidemiologic studies examined exposure-response relationships. Studies with well-designed exposure assessments reported a statistically significant trend of increasing risk of kidney cancer (Charbotel et al. 2006; Moore et al. 2010; Zhao et al. 2005) or NHL (Purdue et al. 2011) with increasing TCE exposure. Further support was provided by the meta-analyses; higher summary RR estimates for kidney cancer and NHL were observed for the highest exposure groups than for overall TCE exposure, taking possible reporting bias into account. Liver cancer studies generally had few cases, limiting ability to assess exposure-response relationships. The meta-analysis for liver cancer did not provide support for a biological gradient (lower summary RR estimate for highest exposure groups than for overall TCE exposure, taking possible reporting bias into account).

Table 1 (continued).

Consideration	Summary of Weight of Evidence
Biological plausibility and coherence	<ul style="list-style-type: none"> • TCE metabolism results in reactive, genotoxic, and/or toxicologically active metabolites at target sites in humans and in rodent test species. • Active GSTT1 enzyme in humans associated with increased kidney cancer risk, while lack of active enzyme associated with no increased risk (Moore et al., 2010). • TCE is carcinogenic in rodents; cancer types with increased incidences include kidney, liver, and lymphohematopoietic cancers. • A mutagenic mode of action is considered operative for TCE-induced kidney tumors, based on mutagenicity of GSH-conjugation metabolites and the toxicokinetic availability of these metabolites to the target tissue. • Modes of action are not established for other rodent cancer findings; human relevance is not precluded by any hypothesized modes of action due to inadequate support.

Table 2. Selected key mode-of-action hypotheses and support.

Endpoint	Hypothesized mode of action	Summary of weight of evidence
Kidney tumors	<p>Mutagenicity:</p> <ul style="list-style-type: none"> • GSH conjugation-derived metabolites are produced in the kidney. • Metabolites directly induce mutations in kidney cells, advancing acquisition of critical traits contributing to carcinogenesis. 	<p>Data sufficient to conclude a mutagenic mode of action is operative:</p> <ul style="list-style-type: none"> • Studies demonstrate TCE metabolism via GSH conjugation pathway; availability of metabolites to the kidney in laboratory animals and humans. • Predominance of positive genotoxicity data for GSH pathway metabolites in experimental systems.
	<p>Cytotoxicity and regenerative proliferation:</p> <ul style="list-style-type: none"> • GSH conjugation-derived metabolites are produced in kidney. • Metabolites directly induce death in kidney cells (cytotoxicity) • Compensatory cell proliferation occurs to repair damage. • Clonal expansion of initiated cells occurs, leading to cancer. 	<p>Data consistent with cytotoxicity contributing to carcinogenesis in rodents, but the evidence is not as strong as that for a mutagenic mode of action:</p> <ul style="list-style-type: none"> • Studies demonstrate TCE metabolism via GSH conjugation pathway; availability of metabolites to the kidney in humans and laboratory animals. • Studies demonstrating TCE-induced rare form of nephrotoxicity in laboratory animals; similarity of renal tubular effects induced by TCE and its GSH metabolites. However, cytopathology involves changes in cell and nuclear sizes. • Data linking TCE-induction of proliferation and clonal expansion are lacking

Table 2 (continued).

Endpoint	Hypothesized mode of action	Summary of weight of evidence
Liver tumors	<p>Mutagenicity:</p> <ul style="list-style-type: none"> • Oxidation–pathway-derived metabolites are produced in and/or distributed to the liver. • Metabolites directly induce mutations in liver, advancing acquisition of critical traits contributing to carcinogenesis. 	<p>Data are inadequate to support a mutagenic mode of action:</p> <ul style="list-style-type: none"> • Studies demonstrate TCE metabolism via oxidative pathway: availability of numerous metabolites to the liver. • Strong data for mutagenic potential is CH, but difficult to assess the contributions from CH along with genotoxic and non-genotoxic effects of other oxidative metabolites.
	<p>Peroxisome proliferation activated receptor alpha (PPARα) activation:</p> <ul style="list-style-type: none"> • Oxidation–pathway-derived PPAR agonist metabolites (TCA and/or DCA) are produced in and/or distributed to the liver. • Metabolites activate PPARα in the liver. • Alteration of cell proliferation and apoptosis occurs. • Clonal expansion of initiated cells occurs, leading to cancer. 	<p>Data are inadequate to support a PPARα activation mode of action:</p> <ul style="list-style-type: none"> • Studies demonstrate TCE metabolism via oxidative pathway: availability of some metabolites that are PPAR agonists to the liver. • Studies demonstrating activation of hepatic PPARα in rodents exposed to TCE and TCA. • However, inadequate evidence that PPARα is necessary for liver tumors induced by TCE or that hypothesized key events are collectively sufficient for carcinogenesis.
Other endpoints and/or modes of action	<p>Inadequate data to support one or more of the following:</p> <ul style="list-style-type: none"> • An identified sequence of key events. • TCE or metabolites induce key events. • Key events are individually necessary for inducing the endpoint. • Key events are collectively sufficient for inducing the endpoint. 	

Table 3. Key conclusions for TCE non-cancer toxicity.

Tissue or organ system	Key conclusions as to human health hazard
Central Nervous System	<p>Strong evidence, based on multiple human and experimental animal studies, that TCE causes:</p> <ul style="list-style-type: none"> • Changes in trigeminal nerve function or morphology. • Impairment of vestibular function. <p>Limited evidence, primarily from experimental animal studies, with fewer/more limited human studies, that TCE causes:</p> <ul style="list-style-type: none"> • Delayed motor function, including during neurodevelopment. • Changes in auditory, visual, and cognitive function or performance.
Kidney	<p>Strong evidence, based on experimental animal studies, a few human studies, and mechanistic studies, that TCE causes nephrotoxicity, particularly in the form of tubular toxicity. Nephrotoxicity is likely mediated primarily through the TCE GSH conjugation metabolite DCVC.</p>
Liver	<p>Limited evidence in humans and strong evidence from experimental animal studies that TCE causes hepatotoxicity, but not necrosis. Mice appear to be more sensitive than other experimental species, and hepatotoxicity is likely mediated through oxidative metabolites, including, but not exclusively, TCA.</p>
Immune System	<p>Strong evidence, based on multiple human and experimental animal studies, that TCE exposure causes:</p> <ul style="list-style-type: none"> • Autoimmune disease, including scleroderma. • A specific type of generalized hypersensitivity disorder. <p>Limited evidence, primarily from experimental animal studies, with fewer/more limited human studies, that TCE causes immunosuppression.</p>
Respiratory tract	<p>Suggestive evidence, primarily from short-term experimental animal studies, that TCE causes respiratory tract toxicity, primarily in Clara cells.</p>
Reproductive system	<p>Strong evidence, based on multiple human and experimental animal studies, that TCE causes male reproductive toxicity, primarily through effects on the testes, epididymides, sperm, or hormone levels.</p> <p>Suggestive evidence, based on few/limited human and experimental animal studies, that TCE causes female reproductive toxicity.</p>

Table 3 (continued).**Tissue or organ system Key conclusions as to human health hazard**

Development

Strong evidence, based on weakly suggestive epidemiologic studies, limited experimental animal studies, and multiple mechanistic studies, that TCE causes fetal cardiac malformations. Limited experimental evidence that oxidative metabolites, such as TCA and/or DCA, cause similar effects.

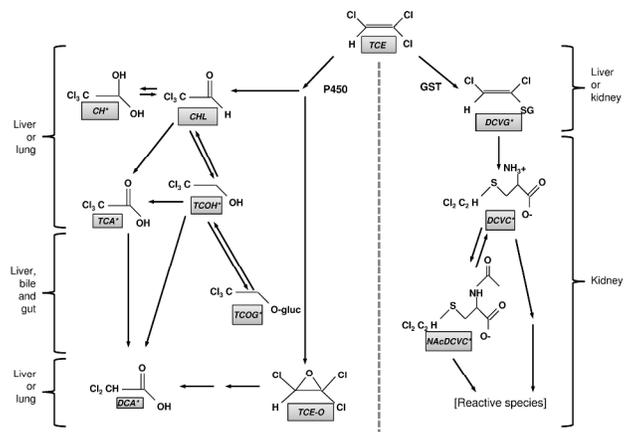
Limited evidence, primarily from experimental animal studies, with weakly suggestive epidemiologic studies, that TCE causes fetal malformations (in addition to cardiac), prenatal losses, decreased growth or birth weight of offspring, and alterations in immune system function.

Figure Legends

Figure 1. Simplified trichloroethylene (TCE) metabolism scheme. Metabolism of TCE occurs through two main irreversible pathways: oxidation via the microsomal mixed-function oxidase system (i.e., cytochrome P450s) and conjugation with glutathione (GSH) by glutathione *S*-transferases (GSTs). Oxidation occurs predominantly in the liver, and to a lesser extent in the lung; the first metabolic products are TCE-oxide (TCE-O), chloral (CHL), and chloral hydrate (CH), with the latter two quickly transformed to trichloroethanol (TCOH) (a reversible reaction) and trichloroacetic acid (TCA). TCOH is glucuronidated to form TCOH-glucuronide (TCOG), which undergoes enterohepatic recirculation (excretion in bile with regeneration and reabsorption of TCOH from the gut). TCA and TCOG are excreted in urine. Further metabolism of TCA and TCOH has not been well characterized, but may include dichloroacetic acid (DCA) (Lash et al. 2000a). TCE-O may also form DCA, among other species (Cai and Guengerich 1999). TCE conjugation with GSH in the liver or kidney form dichlorovinyl glutathione (DCVG), which is further processed in the kidney, forming the cysteine conjugate *S*-dichlorovinyl-L-cysteine (DCVC). DCVC may be bioactivated by beta-lyase or flavin-containing monooxygenases to reactive species (Anders et al. 1988; Krause et al. 2003; Lash et al. 2003), or (reversibly) undergo *N*-acetylation to the mercapturate *N*-acetyl dichlorovinyl cysteine (NAcDCVC), which is then excreted in urine or sulfoxidated by CYP3A to reactive species (Bernauer et al. 1996; Birner et al. 1993; Werner et al. 1995a; Werner et al. 1995b).

Figure 2. Forest plots from random-effects models of overall (i.e., “ever” or “any”) TCE exposure (A) and highest TCE exposure groups (B), adapted from Scott and Jinot (2011). Individual study relative risks (RR) and summary RR estimates from the meta-analysis (RR_m)

are plotted with 95% confidence intervals for each cancer type (blue: kidney cancer; orange: non-Hodgkin lymphoma; green: liver cancer). Symbol sizes reflect relative weight of the studies.



* Metabolites identified in blood or urine following in vivo TCE exposure (rodent or human).

Figure 1
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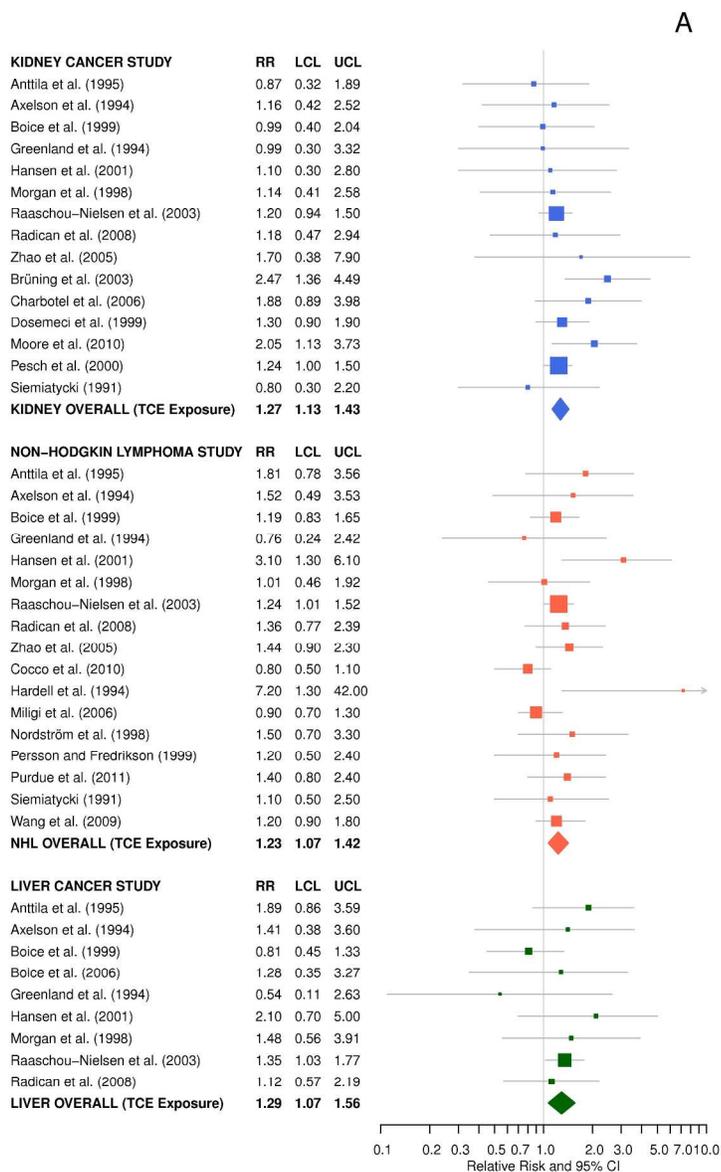


Figure 2A
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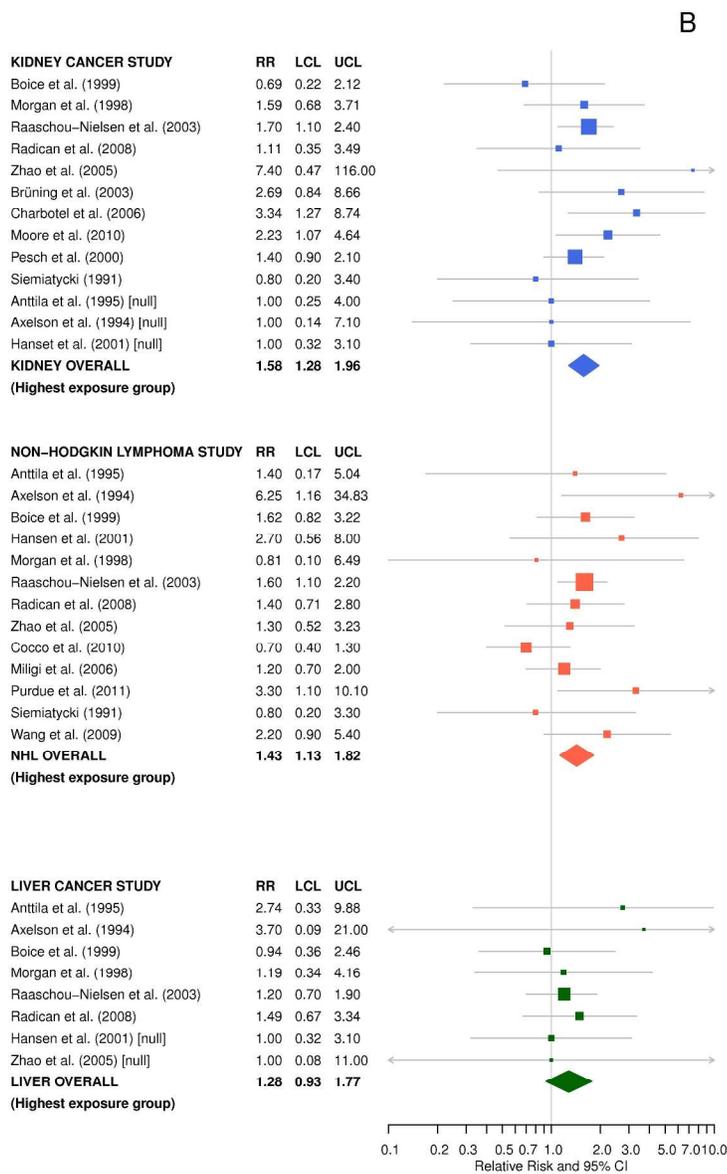


Figure 2B
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