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May 14, 2013

Via Email and
Overnight Delivery

Ms. Cynthia Oshita
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Re: Request for Relevant Information On a Chemical Being Considered For Listing By the Authoritative Bodies Mechanism

Dear Ms. Oshita:

Enclosed are comments prepared by Exponent on behalf of Schlumberger Technology Corporation regarding the proposed listing of trichloroethylene ("TCE") as a reproductive and developmental toxicant under California Proposition 65 (Cal. Health & Safety Code §25249.5 *et seq.*). For the reasons explained in detail in the attached Exponent comments, TCE has not been "formally identified" as a reproductive and developmental toxicant by an authoritative body within the meaning of Title 27 Cal. Code Regs. §25306(d). Moreover, no authoritative body has made a formal determination that TCE causes reproductive and developmental toxicity after weighing the available scientific evidence as contemplated by §25306(d)(1).

As a result, before listing TCE as a reproductive and developmental toxicant, the issue should be referred to the Developmental and Reproductive Toxicant Identification Committee for specific consideration of the weight of scientific evidence regarding TCE.

Very truly yours,

Richard C Coffin

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May 14, 2013

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Subject: Request for Relevant Information on a Chemical Being Considered for Listing by the Authoritative Bodies Mechanism: Trichloroethylene (TCE)

Dear Ms. Oshita:

These comments are being submitted in response to the Office of Environmental Health Hazard Assessment's (OEHHA) March 15, 2013 "Request for Relevant Information on a Chemical Being Considered for Listing by the Authoritative Bodies Mechanism: Trichloroethylene (TCE)" on behalf of Schlumberger Technology Corporation. OEHHA has based the decision to list TCE as a reproductive and developmental toxicant on the findings and conclusions of the U.S. Environmental Protection Agency (EPA) as presented in their 2011 Integrated Risk information System (IRIS) toxicological review of TCE. Based on the available human epidemiological data and experimental and mechanistic studies, the IRIS toxicological review concluded that TCE can pose a potential human health hazard for non-cancer toxicity to the central nervous system, kidneys, liver, immune system, male reproductive system, and the developing fetus (EPA 2011)[1]. The IRIS toxicological review included new inhalation and oral toxicity factors, including a reference concentration (RfC) and reference dose (RfD) for non-cancer endpoints (2 $\mu g/m^3$ and 0.0005 mg/kg/day) and an inhalation unit risk (IUR) level (4×10^{-6} per $\mu g/m^3$) for cancer endpoints (EPA 2011). EPA developed a chronic inhalation RfC based on three non-cancer toxicological endpoints reported in rodent drinking water and gavage studies: (i) decreased thymus weights in mice (adults) (Keil et al. 2009); (ii) increased cardiac malformations in rats (fetuses) (Johnson et al. 2003); and (iii) toxic nephropathy (kidney effects) in rats (adults) (NTP 1988). EPA also developed a chronic oral RfD based on three non-cancer toxicological endpoints reported in rodent drinking water studies: (i) decreased thymus weights in mice (adults) (Keil et al. 2009); (ii) decreased plaque-forming cell (PFC) response and

[1] The references cited in this letter are included in Appendix A

increased delayed-type hypersensitivity in mice (pups) (Peden-Adams et al. 2006); and (iii) increased cardiac malformations in rats (fetuses) (Johnson et al. 2003).

In the IRIS toxicological review of TCE, EPA recommended exposure limits for noncarcinogenic effects that are, in fact, lower than any exposures that are associated with the various noncarcinogenic effects identified in the review and exposure levels not expected to be observed in humans. While EPA has a process for formally evaluating and characterizing the causal relationship between exposure to specific chemicals and cancer, there is no parallel, formal process for characterizing causality for noncarcinogenic health effects. The IRIS process for identifying doses below which adverse health effects might be expected in humans does not require or involve the formal identification of a causal link between a particular chemical and a particular health effect. It is possible, and even standard procedure, to identify and select exposure levels that are below levels where effects have been observed without first performing a critical evaluation of the weight-of-evidence to determine if a particular effect is, in fact, causally linked to a particular chemical exposure.

The approach of identifying doses below which specific effects have been observed, without going through the time-consuming and difficult process of developing consensus on the weight-of-evidence evaluations for the male reproductive toxicity and developmental effects associated with TCE exposure, appears to have been applied as part of the IRIS toxicological review for TCE. The IRIS toxicological review for TCE includes conflicting statements about the weight-of-evidence and strength of evidence for concluding that these two health effects are causally associated with TCE exposure. These conflicting statements may, in fact, reflect differences in opinion among authors of the IRIS toxicological review, but they certainly underscore the point that a consensus opinion on causality was not developed as part of the IRIS review process and that the IRIS toxicological review does not constitute a "formal identification" of TCE as either a male reproductive toxicant or a developmental toxicant.

While there is potentially suggestive evidence of a causal association between TCE and male reproductive and developmental effects, as noted in the IRIS toxicological review, the weight-of-evidence that the association between TCE exposure and these effects does not support a conclusion that the associations are causal. The evidence that TCE causes male reproductive effects and developmental effects is weak, and some of the key toxicological and epidemiological studies reporting such effects have fundamental methodological flaws or an not sufficient for confirming a causal association. In addition, as described in previously published reviews of the literature, there is substantial uncertainty, contradictory evidence, and even controversy regarding the identification of a causal association between TCE and reproductive or developmental effects (NAS 2006, Watson et al. 2006). Furthermore, other scientific and regulatory organizations (e.g., ATSDR, OSHA, ACGIH, NIOSH) that have developed health protective exposure limits for TCE have not selected reproductive or developmental health endpoints as the basis of their recommended limits, even though most of the reproductive toxicological and epidemiological studies that were evaluated in the IRIS toxicological review

of TCE were available when the reviews were performed by those other scientific and government organizations.

While formal evaluation of any potential link between TCE exposure and reproductive or developmental effects is not necessarily needed for purposes of recommending exposure limits, such an evaluation is needed before a chemical can be formally identified as causing such specific health effects as male reproductive toxicity or developmental effects. For reasons discussed in more detail below, the scientific evidence does not support a conclusion that a causal connection exists between exposure to TCE and male reproductive and/or developmental effects in humans.

Male Reproductive Toxicity

Several quotations from the EPA (2011) IRIS toxicological review are provided by OEHHA as support for the proposed listing of TCE under Proposition 65. EPA (2011) reviewed the epidemiological and toxicological data on TCE for male reproductive toxicity and summarized the effects reported in these studies. However, the IRIS toxicological review does not conclude that TCE causes reproductive toxicity. In fact, in several instances, the report states:

"A number of human and laboratory animal studies <u>suggest</u> that TCE exposure <u>has the potential</u> for male reproductive toxicity" [emphasis added] (EPA 2011, p. 4-628 and 6-8).

Suggestive evidence for potential toxicity falls short of demonstrating that TCE causes reproductive toxicity. The epidemiological data are very limited and insufficient to show that TCE causes male reproductive toxicity in humans. Although some of the toxicological studies report male reproductive effects, when other factors are considered, such as the animal model used, study design employed, and the systemic toxicity observed in these studies, the biological plausibility and relevance of the findings to humans is questionable.

Only ten epidemiological publications were identified in the IRIS toxicological review on male reproductive effects, including reproductive behavior effects, sperm quality, changes in endocrine function, and fertility. As presented in this letter, the reproductive toxicological and epidemiological studies that were reviewed by EPA all have methodological flaws that make it difficult to conclude that TCE is a reproductive toxicant.

An association of TCE exposure and reproductive behavior effects are reported in three publications, two of which are small studies on workers (Bardodej and Vyskocil 1956, El Ghawabi et al. 1973) that are limited by the small number of subjects (30 or 43 men), the lack of a definition of potency, self-reported effects on libido, and a the lack of control for confounding exposures. The third publication is a case report (Saihan et al. 1978), which is of limited value since exposure to TCE was not measured and, furthermore, case reports are generally considered not relevant in assessing causation.

Altered sperm quality was evaluated in only two studies cited in the IRIS toxicological review (Rasmussen et al. 1988, Chia et al. 1996). One study investigated sperm quality in 13 metal workers reportedly exposed to TCE for 20 hours per week, but measured TCE concentrations were not available (Rasmussen et al. 1988). No statistically significant differences in sperm count, morphology, or spermatozoa Y-chromosome disjunction were observed in these metal workers. Chia et al. (1996) investigated a group of 85 electronics workers with TCE exposure and the percent of workers with normal sperm morphology was reported to be low, but no statistically significant differences were seen based on a comparison of high to low exposure to TCE. A statistically significant increase in sperm density was seen in men with higher levels of urinary-TCA concentrations. This increase in sperm density in workers exposed to TCE would appear to be inconsistent with the allegation that TCE is causing reproductive toxicity. It is important to recognize that the investigator's definition of hyperzoospermia is within the WHO reference range for normal sperm counts (Cooper et al. 2009), therefore, any observed differences are arbitrary since all of the sperm density measures can be considered within the normal range. Furthermore, the observation of hyperzoospermia is clearly inconsistent with the animal studies where *decreased* sperm counts were associated with high, systemically toxic exposures to TCE. The observation of hyperzoospermia may simply be the result of chance or a consequence of biases and limitations in this study. The weight-of-evidence from these two studies does not support an effect of TCE on sperm quality.

Two publications on potential alterations in endocrine function were included by EPA in the IRIS toxicological review which report on the same population of 85 electronics workers discussed above regarding sperm quality (Chia et al. 1997, Goh et al. 1998). Although some statistically significant changes in hormone levels were reported in these studies, none of these workers have clinical abnormalities in their reproductive function. Therefore, it is not clear what the implications of these findings are and these studies do not provide any evidence of male reproductive effects.

Three publications were cited in the IRIS toxicological review regarding potential impacts on human male fertility (Sallmén et al. 1998, ATSDR 2001, Forkert et al. 2003). None of these studies reported an association between TCE exposure and fertility. Therefore, these studies do not provide any support for TCE-induced male fertility.

Overall, scant epidemiological data are available on the potential association between TCE exposure and male reproductive effects. Effects on reproductive behavior are suggested in two studies that are limited by self-reports of impacts on potency or libido and potential confounding exposures. Only one study population, (Chia et al. 1996), reported effects associated with TCE exposure that include hyperzoospermia and hormonal changes. However, it is not clear what clinical effect these changes may have and whether they represent male reproductive toxicity. Four other study populations did not observe effects on sperm quality or fertility. Thus, the weight-of-evidence does not support a conclusion that TCE is associated with, much less causes, male reproductive effects.

A total of 12 toxicological studies that provided information on the potential toxicity of TCE on the male reproductive system (increases in abnormal sperm, decreased sperm counts, decreased motility, decreased fertilization) were identified by EPA in the toxicological review. Several factors should be considered when evaluating these toxicological studies for understanding the biological plausibility of the reported findings for evaluating potential human health effects. First, the number of animals evaluated in some of these studies was very small (three to six animals), which limits the reliability of the results, particularly for sperm effects which have considerable normal variability. Second, the mouse may not be an appropriate model for assessing TCE toxicity in humans, given the potential differences in TCE metabolism. There are definitely metabolism differences in lung tissue between the mouse and human, however, no experimental data are available to demonstrate or refute differences in metabolism in male reproductive organs. Third, systemic toxicity has been generally observed in cases where male reproductive effects have been reported. Therefore, the reported effects on any reproductive parameters may be secondary to systemic toxicity.

Overall, the toxicity studies with optimal study designs that include sufficient numbers of animals and multiple dose groups indicate that male reproductive toxicity is secondary to systemic toxicity. This is supported by the other, more limited studies where high doses of TCE induced both systemic toxicity and male reproductive effects. Given the weight-of-evidence for systemic toxicity being associated with both sperm effects and reproductive organ toxicity in rodents, TCE cannot be shown to directly induce male reproductive toxicity.

Developmental Toxicity

OEHHA concludes that TCE meets the criterion of a chemical that causes developmental toxicity because of the conclusions about developmental toxicity manifested as increased cardiac malformations in rats and developmental immunotoxicity in mice following prenatal exposure.

OEHHA stated that the "authoritative body" (EPA) concludes the following in the IRIS toxicological review:

> "[B]ased on weakly suggestive epidemiologic data and fairly consistent laboratory animal data, it can be concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring" (page 6-10).
>
> "[B]ased on weakly suggestive, but overall consistent, epidemiologic data, in combination with evidence from experimental animal and mechanistic studies, it can be concluded that TCE exposure poses a potential hazard for congenital malformations, including cardiac defects, in offspring" (page 6-11).

OEHHA concludes, that this appears to meet the criterion in Section 25306(d)(1) that the chemical "is the subject of a report which is published by the authoritative body and which concludes that the chemical causes... reproductive toxicity".

However, there are several limitations in the scientific database regarding the existence and strength of the evidence of association between exposure to TCE and developmental effects. In the Request for Relevant Information, OEHHA stated, "With regard to prenatal loss and effects on growth, the U.S. EPA Toxicological Review (2011) noted that some occupational and environmental epidemiological studies reported associations between parental exposure to TCE and spontaneous abortion or perinatal death, and decreased birth weight or SGA [small for gestational age], although other studies reported mixed or null findings, and that multiple well-conducted studies in rats and mice show analogous effects of TCE exposure; i.e., pre- or postimplantation losses, increased resorptions, perinatal death, and decreased birth weight. On that basis, U.S. EPA concluded that TCE exposure poses a potential hazard for prenatal losses and decreased growth or birth weight of offspring, based on weakly suggestive epidemiologic data and fairly consistent laboratory animal data."

Furthermore, the IRIS toxicological review does not conclude that TCE causes developmental toxicity and the review states:

> "In summary, an overall review of the weight of evidence in humans and experimental animals is <u>suggestive of the potential</u> for developmental toxicity with TCE exposure." [emphasis added] (EPA 2011, p. 4-556).

Just as in the case of male reproductive toxicity, suggestive evidence for potential toxicity falls short of demonstrating that TCE causes developmental toxicity.

The IRIS review (EPA 2011) included several epidemiological studies that evaluated the risk of a variety of developmental effects (spontaneous abortion, perinatal death, decreased birth weight, small for gestational age and postnatal growth, congenital malformations, developmental neurotoxicity, developmental immunotoxicity, other adverse birth or developmental outcomes) in the offspring of women exposed to TCE or related volatile organic compounds (VOCs) in the community through groundwater contamination or in the workplace. The review also included several developmental toxicity studies that evaluated the potential for effects on specific organ systems, including the developing nervous, immune, and pulmonary systems. In addition, EPA reviewed studies that have been conducted to characterize the mode of action for cardiac malformations that have been reported to be associated with TCE exposure.

Congenital Cardiac Defect Studies

With respect to Congenital Cardiac Defects (CCD), EPA concluded, "The epidemiological studies, while individually limited, as a whole show relatively consistent elevations, some of

which were statistically significant, in the incidence of cardiac effects in TCE-exposed populations compared to reference groups (ATSDR 2008, 2006; Yauck et al. 2004; ATSDR 2001; Bove 1996; Bove et al. 1995; Goldberg et al. 1990)." However, due to a variety of limitations, the available epidemiological studies are inadequate to support the hypothesis that TCE is associated with an increased risk of developmental effects. Methodological issues that limit the ability to establish any association between exposure to TCE and developmental effects include the types of study designs (e.g., ecologic), confounding exposure to several chemicals, lack of TCE exposure data, potential for confounding variables, non-statistically significant increases in developmental effects reported, and a lack of a specific type of cardiovascular developmental effects in the studies. The only two studies that were considered by EPA to report an increased risk of CCD after exposure to TCE were those conducted by Yauck et al. (2004) and ATSDR (2006, 2008), both of which have significant methodological limitations that affect the ability to draw conclusions about an association between exposure to TCE and the development of CCD. It is important to note that the study published by Yauck et al. (2004) did not find a link between CCD and presumed TCE exposure in mothers younger than 38 years, and for exposed older mothers, there were too few cases to determine the relative impact of CCD and age. The ATSDR study (2006, 2008) and subsequent follow-up study by Forand (2012) was ecologic in design and evaluated the risk of disease within a population, therefore, it was not specified whether infants who developed CCD were born to mothers who were actually exposed to TCE. The IRIS toxicological review concluded that the rest of the studies that evaluated CCD did not report any significant increases in TCE-exposed groups (Lagakos et al. 1986, Goldberg et al. 1990, Bove et al. 1995, Bove 1996). In some of the studies, the rate of CCD in the TCE-exposed population was actually below the background rate in the general population, because CCD are the most frequent form of birth defects—the current estimate of CCD is 9 in 1000 live births, less than 1 percent of newborns (American Heart Association website 2013). The epidemiological evidence is not sufficient to demonstrate a causal relationship between TCE exposure and CCD.

Several risk factors have been associated with CCD, including nutritional deficiencies such as folic acid, maternal diabetes, drug and alcohol use, certain viruses, and certain prescription medications. Many of these important confounding factors were not evaluated in these studies, limiting the ability to establish a causal association between TCE and developmental effects. For some of the available studies, the IRIS toxicological review reported that while they include both occupational and environmental exposures to TCE, the epidemiological studies are, overall, not highly informative due to the small numbers of cases and limited exposure characterization, or to the fact that exposures to mixed solvents were involved. A significant limitation of most of the available epidemiological studies is the lack of reported TCE exposure levels for the study populations.

Several toxicological studies have been conducted using various experimental animal models to investigate whether exposure to TCE can adversely impact normal heart development. The

IRIS toxicological review concluded that CCD were observed in Sprague-Dawley rat fetuses following the administration of TCE in drinking water to mothers during gestation (Dawson et al. 1993; Johnson et al. 2003) and following intrauterine administration (Dawson et al. 1990). These studies were all conducted by a group of investigators at the University of Arizona, which is the only research group that reported a positive association between TCE and CCD in experimental rodent studies. A few studies also reported a positive association between the oral gavage administration of TCE metabolites (TCA, DCA) and CCD in Long Evans rats (Smith et al. 1989; Epstein et al. 1992; Smith et al. 1992; Johnson et al.1998a, b). There have also been a few cardiac developmental studies with TCE or TCA conducted in chickens, some of which that have reported cardiac effects (Bross et al. 1983; Loeber et al. 1988; Boyer et al. 2000; Mishima et al. 2006; Drake et al. 2006a, b; Rufer et al 2008, 2010). In these studies, the chick embryos were injected with high concentrations of TCE administered directly to the chorioallantoic membrane, a route of exposure that it not at all representative of how pregnant women are likely to be exposed to these substances.

The relevance of these findings to humans is unclear; data in the chick model are not directly applicable to human risk due to significant developmental differences between chickens and humans and the absence of a maternal influence in the chick model system. It is important to note that with respect to some other mammalian toxicological studies that evaluated CCD, EPA (2011) stated, "However, it is notable that a number of other studies, several of which were well conducted, did not report induction of cardiac defects in rats or rabbits from TCE administered by inhalation (Carney et al. 2006; Healy et al. 1982; Hardin et al. 1981; Dorfmueller et al. 1979; Schwetz et al. 1975) or in rats and mice by gavage (Fisher et al. 2001; Narotsky and Kavlock 1995; Narotsky et al. 1995; Cosby and Dukelow 1992). EPA (2011) stated, "The potential importance of these effects warrants a more detailed discussion of possible explanations for the apparent inconsistencies in the laboratory animal studies" and the report included a discussion of some of the methodological differences that may impact the study findings regarding CCD.

There are several issues that need to be considered when attempting to extrapolate the results of the TCE experimental animal studies to humans. EPA acknowledged that it is generally recognized that response variability among developmental bioassays conducted with the same chemical agent may be related to factors such as study design (e.g., the species and strain of laboratory animal model used, day or time of day of dose administration in relation to critical developmental windows, route of exposure, vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphology and abnormalities; and whether the fetal evaluations conducted were consistent). Differences in study results may also be due to the method by which pathological examinations were conducted (e.g., whether or not cardiac evaluations were conducted using standardized dissection procedures and whether the examinations were conducted by technicians who were trained and familiar with fetal cardiac anatomy). All of the studies alleging that TCE plays a causal role in CCD were conducted at the

same laboratory at the University of Arizona, and no specific type of CCD was linked to TCE or its metabolites in these studies (Dawson et al. 1990, Dawson et al. 1993, Johnson et al. 2003). Possible reasons for the laboratory-specific positive link between TCE and CCD observed in the University of Arizona studies include their unique dissection technique and the use of non-standard statistical evaluations for developmental toxicity tests (Watson et al. 2006).

Several governmental agencies in addition to the EPA have recently reviewed the epidemiology and toxicology studies pertinent to an evaluation of a causal association between TCE and CCD (EPA 2011, SAB 2011, NAS 2006, NAC 2009). Reviews of the same studies included in the IRIS toxicological review were addressed in the reviews performed by a National Academy of Science (2006) committee and by the National Advisory Committee (NAC 2009) within the National Research Council. Both of these committees noted the same positive studies cited in the IRIS Toxicological Review, but noted the limitations of these studies and did not draw conclusions that TCE was causally linked to CCD. In the various reports produced by these Agencies, there are very few epidemiological and toxicological studies that are identified as supporting an association between TCE and CCD. The conclusion statements from EPA and other scientific panels highlight the fact that there are substantial uncertainties about the existence of an association between TCE and CCD in experimental animals and, more significantly, humans. As is noted in these reviews, the few positive studies have methodological or study design limitations that limit the value of the studies as a basis for concluding that TCE causes teratogenic effects; or more specifically, that it causes CCD. The IRIS toxicological review included a tempered conclusion that the available evidence raises "sufficient concern regarding the potential for developmental toxicity". However, following the review of the IRIS toxicological review document, the EPA Science Advisory Panel recommended that the cardiac malformations be selected as one of the health endpoints on which the TCE RfC was based. With respect to the potential for developmental cardiac teratogenicity from TCE, NAS (2006) noted the following limitations about the toxicological studies that have evaluated this endpoint: 1) rodent studies have had mixed results, suggesting either methodological or strain differences; and 2) the low-dose studies showing a positive correlation in TCE-induced developmental cardiac effects showed unusually flat dose-response curves, they also came from a single institution, and the results need to be replicated in another laboratory to clarify the dose-response relationship. Others in the scientific community have reported that epidemiological and toxicological studies that support an association between CCD and TCE in humans, and the strength of that association, are limited and weak (Hardin et al. 2005; Watson et al. 2006). A more detailed discussion of the scientific studies that have evaluated the potential for TCE to cause CCD and the conclusions about the association between TCE and developmental effects from the other authoritative bodies has been summarized in the attached document entitled, "TCE Interim Short-Term Removal Action Level White Paper" prepared by Exponent and Geosyntec Consultants and submitted to EPA in 2012 (Appendix B).

Developmental Immunotoxicity Studies

The IRIS toxicological review included a few toxicological and epidemiological studies that evaluated the potential for developmental immunotoxicity in mice following oral TCE exposures during critical pre- and postnatal stages of immune system development (Peden-Adams et al. 2006; Peden-Adams et al. 2008; Blossom and Doss 2007; Blossom et al. 2008). According to the IRIS toxicological review, the Peden-Adams et al. (2006) study showed evidence of immune system perturbation (suppression of PFC responses, increased T-cell subpopulations, decreased spleen cellularity, and increased hypersensitivity response) in mice offspring following *in utero* and 8 weeks of postnatal exposures to TCE. EPA developed a candidate RfC from this study and it is cited as one of the critical studies for developing the current chronic oral RfD for TCE. Another study by Peden-Adams et al. (2008) with autoimmune-prone mice exposed to TCE from conception until 12 months of age did not demonstrate an increase in the development of autoimmune disease markers but did report some immunological effects in offspring (e.g., increased splenic CD4-/CD8- in females; decreased thymic CD4+/CD8+ cells in males). In two other studies that focused on autoimmune responses following drinking water exposures of mice to TCE during *in utero* development and continuing until the time of sexual maturation, Blossom and Doss (2007) and Blossom et al. (2008) reported some peripheral blood changes that were indicative of treatment-related autoimmune responses in offspring. It is important to note that the mice were exposed postnatally in these studies, limiting the ability to determine what effects prenatal TCE exposure may have had on their immune system.

As stated in the IRIS toxicological profile, although the developmental immunotoxicity studies with TCE (Peden-Adams et al. (2006; 2008); Blossom and Doss (2007); Blossom et al. (2008)) exposed the offspring during critical periods of pre- and postnatal immune system development, they were not designed to assess issues such as post-treatment recovery, latent outcomes, or differences in severity of response that might be attributed to the early life exposures. EPA (2011) further stated that consistency in response in these animal studies was difficult to ascertain due to the variations in study design (e.g., animal strain used, duration of exposure, treatment levels evaluated, timing of assessments, and endpoints evaluated).

The IRIS toxicological review for TCE included two epidemiological studies that evaluated immunological development in children after maternal exposure to volatile organic compounds (VOCs) (Lehmann et al. 2002; Lehmann et al. 2001). Lehmann et al. (2001) examined 121 premature neonates and neonates at risk of atopy at 36 months of age with a median TCE air level of 0.42 $\mu g/m^3$ in their bedrooms. Blood samples were taken at 36-months and were used to measure the total IgE and specific IgE antibodies directed to egg white, milk, indoor and outdoor allergens. According to the IRIS toxicological review, there was no association between maternal exposure to TCE and allergic sensitization to egg white and milk, or to cytokine producing peripheral T-cells. Lehmann et al. (2002) examined 85 healthy, full-term neonates that had a median VOC air level in their bedroom of 0.6 $\mu g/m^3$ measured 3–4 weeks

after birth and a significant reduction of Th1 IL-2 producing T-cells was observed. These studies evaluated VOCs, which may include several other compounds besides TCE at various levels, making it impossible to establish a causal relationship with a single chemical.

The endpoints assessed in the few epidemiological studies that evaluated immunological outcomes following developmental exposures to TCE (and other VOCs) were dissimilar from those evaluated in the animal models, and so provided no clear cross-species correlation (EPA 2011). The IRIS toxicological review concluded that the most sensitive immune system response noted in the studies that exposed developing animals were the decreased PFC and increased hypersensitivity in mice exposed in the drinking water at a concentration of 1,400 ppb (Peden-Adams et al. 2006). The IRIS toxicological profile concluded that none of the other studies that treated mice during immune system development assessed these same endpoints; therefore, direct confirmation of these findings across studies was not possible.

There are only a few toxicological studies that have evaluated developmental immunological effects following exposure to TCE. The epidemiological studies included in the IRIS toxicological profile evaluated VOC exposure, not specifically TCE alone. Therefore, we conclude that the strength of the epidemiological and toxicological evidence does not support a causal role between TCE and developmental immunological effects that warrant listing TCE as a developmental toxicant by the State of California.

Conclusion

As a technical summary we note, that the weight-of-evidence from available toxicological and epidemiological literature for TCE does not support a causal association between exposure to TCE and reproductive and/or developmental effects in humans. As summarized in the EPA (2011) IRIS toxicological review of TCE, developmental and reproductive toxicology studies in mice, rats, and rabbits do not consistently report adverse effects of TCE on embryonic development (including CCD), besides embryo- or fetotoxicity associated with maternal toxicity. Furthermore, the fact that other scientific and regulatory organizations (e.g., NAC, NAS, NIOSH, OSHA, ACGIH) that also reviewed the TCE literature to develop health-protective exposure limits did not select developmental toxicity as the basis of their recommendations supports the conclusion that TCE either is not causally associated with teratogenic health effects or is not the most sensitive endpoint for establishing acute exposure limits.

We are pleased to submit these comments for your consideration and appreciate your willingness to consider the points that:

1) the IRIS toxicity summary does not constitute a consensus opinion within the EPA on the weight-of-evidence for a causal association between TCE and male reproductive

toxicity or developmental toxicity and, therefore, does not constitute a formal identification of TCE as a male reproductive or developmental toxicant; and

2) the weight of toxicology and epidemiology evidence available does not support a conclusion that TCE is causally associated with either of these health endpoints.

Sincerely,

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Enclosures (2)

Appendix A

References

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Appendix B

TCE Interim Short-Term Removal Action Level White Paper

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I. Executive Summary

As part of their management of the Middlefield-Ellis-Whisman (MEW) Superfund Site in Mountain View, California, staff at U.S. Environmental Protection Agency (EPA) Region 9 is considering development of a site-specific indoor air Removal Action Level (RAL) for trichloroethylene (TCE) of 15 $\mu g/m^3$ which would be used as a daily average workplace exposure limit. Region 9 staff is considering development of the TCE RAL from the reference concentration (RfC) for TCE included in EPA's 2011 risk assessment of TCE in its Integrated Risk Information System (IRIS) process, assuming continuous exposure for 10 hours per day and a hazard quotient of 3. Although the RfC of 2 $\mu g/m^3$ in the IRIS was developed for continuous exposure as a lifetime average concentration, Region 9 has been considering applying the RAL as a daily average concentration. Given the importance of the issue, implementation problems, and the inconsistency that the RAL would create (e.g., orders of magnitude difference between the RAL and many other existing TCE regulatory standards) Region 9 staff has stated that they have requested guidance from the Headquarters Office of Research and Development.

The impetus for applying the RAL as proposed by Region 9 in this manner is apparently based on the inclusion of congenital cardiac defects (CCD) as one of the three health endpoints on which the RfC is based (EPA 2011). The RAL assumes that developmental effects could be produced by a single day of exposure to TCE by a pregnant female, and thus, the RAL is applied to short-duration exposures. The underlying IRIS documentation for the RfC, however does not indicate that it should be applied to anything other than a chronic exposure period. No acute or other short-term RfC is provided in the IRIS database for TCE.

While there is potentially suggestive evidence of a causal association between TCE and developmental effects as cited in the IRIS 2011 toxicological review prepared by EPA, the evidence is weak; it includes contradictory findings, and some of the key studies have fundamental methodological flaws. Consequently, as described in previously published reviews of the literature, there is substantial uncertainty, contradictory evidence, and even controversy regarding the identification of a causal association between TCE and developmental effects.

Furthermore, other scientific and regulatory organizations (e.g., ACGIH, OSHA) that specifically set out to develop short-term exposure limits for TCE, and these agencies have not selected a developmental health endpoint as the basis of their recommended limits, even though most of the reproductive toxicological and epidemiological studies evaluated as the basis of the RfC in the 2011 EPA review of the TCE toxicity were available as part of the reviews performed by those other scientific and government organizations.

As has been noted by Region 9 personnel, proposed RAL would impose substantial practical implementation issues for monitoring and managing TCE exposures. It may also result in unwarranted alarm among potentially exposed individuals and would be expected to result in significant confusion, given the orders of magnitude difference between the proposed RAL and other regulatory standards and screening levels for TCE.

The explicit identification of TCE as a reproductive toxicant and the identification of a corresponding and appropriate exposure averaging time was not a focus or goal associated with the EPA (2011) TCE toxicological review. Because of the importance of the issue in the possible derivation and use of a RAL for risk management and risk communication at EPA, the issue of a causal link between TCE exposure and developmental effects warrants a more focused evaluation. A formal evaluation of any potential link between TCE exposure and developmental effects, based on careful consideration of the weight of scientific evidence, is necessary to responsibly inform risk management and risk communication issues. For the reasons detailed below, this White Paper concludes that the weight of scientific evidence does not support a conclusion that a causal connection exists between exposure to TCE and CCD in humans and the application of a RAL based on teratogenicity is unwarranted.

II. Introduction

The U.S. Environmental Protection Agency (EPA) Region 9 has proposed the development of a short-term non-residential, indoor air removal action level (RAL) of 15 μg/m^3 for trichloroethylene (TCE) for use at the Middlefield-Ellis-Whisman (MEW) Superfund site. RALs are typically used to define areas, contaminants, and/or conditions that may warrant an emergency or time-critical removal action at Superfund sites. Thus, as applied at the MEW Superfund site, 15 μg/m^3 of TCE in indoor air (referred to herein as the "short-term RAL") would trigger the cessation of work or modified duty (e.g., the use personal protective equipment) for commercial, industrial and construction workers. EPA Region 9's proposed use of a short-term RAL as an exposure limit for TCE (with attendant monitoring requirements) and as a basis for risk communication with people working at buildings at the MEW Superfund site appears to be inconsistent with the EPA Office of Solid Waste and Emergency Response (OSWER) guidance on the derivation and use of RALs (EPA 2008) because Region 9 is proposing to use a chronic exposure factor (i.e., the chronic RfC) as the basis of an acute (i.e., 1-day) exposure limit. Region 9's use of the short-term RAL of 15 μg/m^3 as a one-day exposure limit is apparently based on the assumption that TCE is teratogenic[1] and that a one-day exposure averaging time is applicable to teratogenic effects.

The EPA inhalation chronic reference concentration (RfC) for TCE was used as the toxicity factor for developing the short-term RAL, and one of the three critical endpoints selected as the basis for the RfC was fetal cardiac malformations (teratogenicity). As discussed below, however, the identification of TCE as a teratogen was not a focus of the IRIS evaluation of TCE. A thorough and objective weight-of-evidence analysis would likely conclude that TCE should not be identified as a teratogen. Scientists familiar with the epidemiology and toxicology studies on the topic do not agree on the significance of many of the key individual studies or

[1] A teratogen is defined as any agent or factor that induces or increases the incidence of abnormal prenatal development. The EPA IRIS definition of teratogenic is "Structural developmental defects due to exposure to a chemical agent during formation of individual organs."

that the weight of evidence from the collection of available studies shows that TCE is a teratogen.

While the IRIS evaluation selected three critical health endpoints (immune system effects, kidney effects, and fetal cardiac malfomations) as the basis of its chronic RfC for TCE, it was not necessary to resolve the debate associated with the weight of evidence for identifying TCE as a teratogen as part of the IRIS process because the selected RfC would have been the same had it been based on the other two critical endpoints individually. The issue of a causal association between TCE exposure and teratogenicity did not receive necessary critical evaluation and weight of evidence analysis; had teratogenicity alone been the basis of the RfC, such evaluations would have been performed. The TCE toxicological review was not a complete and formal review of the teratogenicity of TCE, and the IRIS process does not purport to be a complete and formal review of the issue. In addition, other governmental and non-governmental organizations that have established short-term standards for TCE did not select teratogenicity as the basis of their standards, even though the key reproductive studies cited by EPA in the IRIS evaluation were available when these other short-term standards were developed.

III. Basis of the Short-Term RAL for TCE Proposed by EPA Region 9

EPA Region 9 proposes to select the RfC for TCE from EPA's Integrated Risk Information System (IRIS 2011) database as the relevant toxicity factor for developing the proposed short-term RAL. EPA's OSWER (2008) produced a guidance document for use at Superfund sites regarding the derivation and use of RALs. In the September 21, 2008 Memorandum, "Revised Superfund Removal Action Levels", OSWER explains that, "RALs are chemical-specific concentrations for individual contaminants that may be used to support the decision for EPA to undertake a removal action." (EPA 2008). In this document, OSWER further explains that the RAL is "…not meant to define protective level…" and that RALs should not be confused with cleanup levels or cleanup standards (EPA 2008). As discussed in this OSWER document, while RALs are not means to define protective levels, they can be risk based. When based on an RfC or RfD, the OSWER policy calls for setting RALs at levels that correspond to a hazard quotient of 3 (EPA 2008). The OSWER policy on RALs grants regional Superfund managers discretion in setting RALs and notes that "…conditions at a site may warrant RALs based on shorter exposure durations and the use of toxicity criteria other than RfDs and RfCs" (EPA 2008).

This site-specific remediation goal of 5 $\mu g/m^3$ for TCE was derived by multiplying the chronic RfC of 2 $\mu g/m^3$ by 24 hr/10 hr to develop a concentration that would result in the same exposure level for a 10-hour work day as a 24-hour residential exposure. The resulting site-specific remediation goal of 4.8 $\mu g/m^3$ for workplaces in the MEW Superfund site was rounded to 5 $\mu g/m^3$. Multiplying 5 $\mu g/m^3$ by 3 produced an indoor air concentration of 15 $\mu g/m^3$ for workplaces in the MEW area, which corresponded to a chronic hazard quotient (HQ) of 3, as discussed in the OSWER policy memorandum on RALs (EPA 2008).

Based on discussions with Region 9 personnel, we understand that the intent is to apply the short-term RAL of 15 $\mu g/m^3$ as a one-day (i.e., 10-hour) exposure limit. This exposure averaging time for the RAL is much shorter than the averaging time that would be applied to a chronic RfC. As was noted in the EPA toxicological review for TCE (EPA 2011):

> "Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration."

There is no indication in the EPA toxicity review document or in the on-line IRIS file for TCE indicating the RfC is intended for anything other than chronic exposure averaging. Therefore, it is inconsistent to establish a RAL based on an acute or short-term exposure from a regulatory level established for a chronic exposure duration. Region 9 appears to have made the determination that the RfC should be applied as a one-day exposure limit because one of the health effects on which the RfC is based on is fetal heart malformations (i.e., teratogenicity). This determination is at odds with the fact that the IRIS file does not indicate that the RfC should be implemented as a one day exposure limit.

IV. Comparison of the Short-Term RAL to Current Short-term Exposure Limits for TCE

The TCE short-term RAL of 15 µg/m^3 proposed by EPA Region 9 is orders of magnitude lower than other short-term TCE exposure limits developed for the workplace and community by governmental agencies. The large variation between the RAL under consideration and established thresholds and regulatory standards underscores the very different scientific assumptions that the other regulatory agencies have relied upon and the need for a rigorous weight-of-evidence analysis. As shown in Table 1 in Section VIII, the short-term exposure limit for TCE recommended by the National Advisory Committee for Acute Exposure Guideline Levels for Hazardous Substances (NAC AEGL Committee) for the general public is 77 ppm (410,000 µg/m^3) as an 8-hour average (NAC/COT 2009). AEGLs represent threshold exposure limits and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours, based on varying degrees of severity of toxic effects of a substance. The recommended exposure levels are applicable to the general population, including infants and children, and other individuals who may be sensitive or susceptible. The AEGLs for TCE, published in 2009, are all based on preventing neurological effects or death and are all are several orders of magnitude higher than the proposed short-term RAL. Furthermore, they are not based on developmental endpoints, even though the developmental studies that were reviewed by EPA in the IRIS toxicological review were available.

The Agency for Toxic Substances and Disease Registry (ATSDR) has developed an acute-duration inhalation minimal risk level (MRL) of 2 ppm (11,000 µg/m^3) and an intermediate inhalation MRL of 0.1 ppm (540 µg/m^3) based on neurological effects, values that are several orders of magnitude higher than the proposed short-term RAL (ATSDR 1997). An acute MRL for inhalation exposure is an estimate of daily human exposure to an air concentration of a chemical that is likely to be without an appreciable risk of adverse non-carcinogenic effects over 14 days or less of exposure. An intermediate MRL for inhalation exposure is an estimate of daily human exposure to an air concentration of a chemical that is likely to be without an appreciable risk of adverse non-carcinogenic effects over 15–364 days of exposure. The OSHA permissible exposure limit (PEL) is an 8-hour time-weighted average (TWA) of 100 ppm

(537,000 μg/m^3), with 300 ppm (1,612,000 μg/m^3) as a 5-minute maximum short-term exposure limit (STEL) allowable in any 2-hour period in the workplace. The American Conference of Governmental Industrial Hygienists (ACGIH) recommends an 8-hour TWA of 10 ppm (54,000 μg/m^3) and a STEL of 25 ppm (134,000 μg/m^3) based on central nervous system impairment, cognitive decrements, and renal toxicity. The National Institute for Occupational Safety and Health (NIOSH) recommends an exposure limit of 25 ppm (134,000 μg/m^3) as a 10-hour TWA.

The fact that the short-term RAL under consideration by EPA Region 9 is many orders of magnitude lower than most of the short-term TCE exposure limits developed for the workplace and community is noteworthy because these expert regulatory and environmental health organizations go through the same process of identifying the lowest dose needed to protect exposed (including sensitive) populations. The community and workplace exposure limits developed by these other organizations are based on neurological endpoints, not reproductive or developmental endpoints as in the case of the proposed short-term RAL, even though many of the same reproductive and developmental studies cited in the EPA toxicity review were available when the other organizations developed their recommendations. Some of the key reproductive studies cited in the EPA toxicity review were specifically cited and not selected as the basis of the AEGLs, for example. The fact that these other organizations, which deliberately set out to establish short-term exposure limits, did not select reproductive or developmental endpoints as the most sensitive endpoints for developing their limits is at odds with the application of the short-term RAL as a single-day exposure limit for developmental effects.

V. EPA IRIS Chronic Reference Concentration for TCE

Summary of the Derivation of the Inhalation RfC for TCE

Over the last several decades a substantial amount of research has been conducted on the dose-response relationships for cancer and non-cancer effects associated with TCE exposures. Several publications have reviewed the available toxicological and epidemiological studies on TCE, including an issue of *Environmental Health Perspectives* published in 2000 that was dedicated to the "state of the science" of TCE (Scott and Cogliano 2000), a TCE-dedicated mini-monograph (Chiu et al. 2006), a review of the critical TCE issues by the NAS (NRC 2006), as well as other published studies, reviews, and meta-analyses. Scott and Cogliano (2000) described a series of 16 papers that were sponsored by the EPA, the U.S. Air Force, the U.S. Department of Energy, the National Institute of Environmental Health Sciences, and the Halogenated Solvents Industry Alliance. These studies had been used previously to generate a draft risk assessment text for TCE that emphasized mode of action and pharmacokinetic data to understand and characterize potential non-cancer and cancer health risks (EPA 2001).

As mentioned previously, EPA published an IRIS toxicological review of TCE on September 28, 2011, which included new inhalation and oral toxicity factors, including an RfC and RfD for non-cancer endpoints and an inhalation unit risk (IUR) level for cancer endpoints (EPA 2011). Based on the available human epidemiological data and experimental and mechanistic studies, the IRIS toxicological review concluded that TCE can pose a potential human health hazard for non-cancer toxicity to the central nervous system, kidney, liver, immune system, male reproductive system, and the developing fetus (EPA 2011).

The current final RfC of 2 $\mu g/m^3$ for chronic inhalation exposure to TCE was developed by EPA (2011) following a review of the available toxicological and epidemiological studies. The derivation of the RfC is based on three non-cancer toxicological endpoints reported in rodent drinking water and gavage studies: (i) decreased thymus weights in mice (adults) (Keil et al. 2009), (ii) increased cardiac malformations in rats (fetuses) (Johnson et al. 2003), and (iii) toxic nephropathy (kidney effects) in rats (adults) (NTP 1988). In a previous EPA draft TCE health

risk assessment, the RfC of 40 μg/m^3 was based on critical effects in the central nervous system, liver, and endocrine system and not on developmental effects (EPA 2001).

To develop an RfC, EPA identifies suitable point-of-departure (POD) values from the toxicity database and applies uncertainty factors (UFs) to reflect limitations in the data. The POD value for the Keil et al. (2003) study was a lowest-observed-adverse-effect-level (LOAEL) whereas the POD values for the Johnson et al (2003) and NTP (1988) studies were Benchmark Dose Levels (BMDLs). Although the LOAEL and BMDL differ in meaning, both represent points that correlate dose with an observed response and both are suitable POD values for developing toxicity values. In developing the current RfC, EPA (2011) derived POD values for thymus weight change in female mice and heart malformations in fetal rats reported in two separate studies (Keil et al. 2009; Johnson et al. 2003). EPA also derived a POD for kidney effects in female rats as a supporting study for developing the RfC (NTP 1988).

EPA applied a physiologically based pharmacokinetic (PBPK) model to the POD values to derive Human Equivalency Concentration (HEC) values. The HEC values were then adjusted to reflect: (i) uncertainty in extrapolating from a LOAEL instead of a no-observed-adverse-effect-level (NOAEL), (UF=10); (ii) the possibility that humans may be more sensitive to TCE than rats due to toxicodynamic differences (UF=3)[2]; and/or (iii) the possibility that some humans may be more sensitive to TCE due to toxicodynamic differences among humans (UF=3). Table 2 presents a summary of the two critical studies and the supporting study selected by EPA to develop the current TCE RfC, including the HEC values, UFs, and candidate RfCs. From these RfC estimates, EPA developed a final RfC of 0.0004 ppm (2 μg/m^3) and concluded that the RfC reflects the midpoint between the estimates for the two critical endpoint RfCs for thymus weight and fetal heart malformations, and is similar to the supporting RfC for toxic nephropathy.

[2] Note that UF values of "3" actually represent $10^{0.5}$, and when two such values are multiplied together, the result is 10 rather than 9.

Review of Candidate Studies for Developing the RfC for TCE

A key step in identifying the critical effect for a dose-response assessment and the development of toxicity criteria (e.g., RfC) is evaluating the quality of the scientific data from epidemiological and animal studies and other supporting information. Specifically, the evidence must support a causal relationship between exposure and outcome to establish a dose-response relationship. In epidemiology, the following criteria, known as Hill's postulates (1965), are typically used as guidelines for assessing causality: (i) temporal sequence (exposure before outcome); (ii) strength (statistical significance) of association; (iii) consistency of association across time and place; (iv) dose-response relationship; (v) biological plausibility; and (vi) experimental evidence. These same criteria are applicable to evaluating the weight of evidence for determining confidence in the toxicological database, individual studies, and the RfC itself.

For toxicological databases, higher confidence is given to those that include epidemiological studies; experimental studies of several animal species, routes, and durations of exposure, and that evaluate a variety of health end points. A robust database is critical for characterizing the chemical's spectrum of potential human toxicity and identifying target organs and the dose ranges associated with adverse effects. Consistency of exposure and effect between studies also tends to increase confidence in the database. Because numerous studies were available for potential candidate critical effects, the IRIS evaluation characterized the overall confidence in the TCE database as high. Note, however, that high confidence in the database is not synonymous with high confidence in each individual study or for each health endpoint.

For individual studies, confidence is related to the study design, study execution, and reporting as well as the relevance of the study (route, dose) to potential human exposures. Often, the inclination is to select the studies that report toxicity at the lowest exposure levels for developing PODs and, ultimately, RfCs. However, critical studies should be identified based on a weight-of-evidence approach that considers all aspects of the study (e.g., study design, methodology, statistical analysis), not just the results. In the case of TCE, there may be reduced confidence due to the ways in which TCE was administered during the studies; TCE was

administered orally in the two critical studies and via oral gavage[3] in the supporting study that were used to derive the inhalation RfC. Higher confidence would be given to RfCs developed from inhalation studies, rather than extrapolating data from oral studies. Notably, there are TCE inhalation studies that evaluated CCD and candidate RfCs could possibly be derived from these studies that may be more appropriate and scientifically robust (e.g., Carney et al. 2006). A weight-of-evidence evaluation of POD values from inhalation studies for the cardiac developmental endpoint, in comparison to the level of confidence in the POD values from the oral studies with TCE, would suggest that the candidate RfCs from inhalation studies would be more scientifically robust than the candidate RfC determined from the Johnson et al. (2003) study.

As stated previously, the final chronic RfC for TCE was based on a range of RfCs developed for three different toxicological endpoints reported to be associated with exposure to TCE. This approach was apparently taken because it is consistent with recommendations from the report entitled, "A Review of the Reference Dose and Reference Concentration Process," which proposes that reference values be based on consideration of all relevant and appropriate endpoints carried through to the derivation of sample reference values, with the selection of the limiting value protective of all endpoints (EPA 2002). Although more studies resulting in similar RfCs will provide, to some extent, more confidence in that range of RfCs, the confidence associated with the individual RfCs in that range should be considered carefully using a weight-of-evidence approach. The IRIS evaluation characterized the confidence in the specific studies used to develop an RfC for TCE as medium-to-high for the decreased thymus endpoint (Kiel et al. 2009), medium for fetal heart malformations (Johnson et al. 2003), and low to medium for kidney effects (NTP 1988); these confidence levels reflect the confidence in the evidence of the effect as well as uncertainties associated with the dose-response assessment (e.g., PBPK modeling). Overall, the IRIS evaluation concluded that confidence in the final RfC for TCE is characterized as high because the multiple candidate RfCs fall within a narrow range, providing support for the final value (EPA 2011).

[3] Gavage is the administration through the use of a tube inserted through the esophagus into the stomach to directly orally administer a test substance.

With respect to CCD, however, the TCE toxicological review concluded that the critical study by Johnson et al. (2003) has important limitations (EPA 2011). As discussed below, in addition to the uncertainties associated with the Johnson et al. (2003) study, there is very limited support for an association between TCE and CCD from all of the available epidemiological and toxicological studies with TCE.

VI. Cardiac Developmental Studies for TCE

The association between TCE exposure and congenital cardiac defects (CCD) is important, because the concern for this potential teratogenic effect appears to be the basis for the proposed application of the short-term RAL as a single-day exposure limit, and because of the substantial uncertainty surrounding the question of whether the association is causal. The available epidemiological and toxicological studies that have been cited in discussions of the association between TCE exposure and CCD are summarized below, and some of the key concerns associated with these studies are identified.

Epidemiological Studies that have Evaluated Congenital Cardiac Defects in TCE Exposed Populations

Summaries of Epidemiological Studies

There have been several epidemiological studies conducted that evaluated the risk of a variety of developmental effects, including CCD, in the offspring of women exposed to TCE or related volatile organic compounds in the community through groundwater contamination or in the workplace (Tola et al. 1980; Lagakos et al. 1986; Flood and Chapin 1988; Swan et al. 1989; Deane et al. 1989; Wrensch et al. 1990; Goldberg et al. 1990; Shaw et al. 1990; Hertz-Picciotto et al. 1992; Bove et al. 1995; Bove 1996; ATSDR 1998; Lorente et al. 2000; Yauck et al. 2004; ATSDR 2006; ATSDR 2008 Forand et al. 2012). A few of the community-based studies specifically examined the potential for CCD associated with exposure to TCE in groundwater or well water (Lagakos et al. 1986; Goldberg et al. 1990; Bove et al. 1995; ATSDR 1998) or in the air as a result of vapor intrusion (Yauck et al. 2004; ATSDR 2006; ATSDR 2008; Forand et al. 2012). Two of the studies reported results regarding the risk of CCD in populations exposed to water containing trichloroethane (Swan et al. 1989; Shaw et al. 1990). The remaining studies listed above that evaluated congenital malformations in women exposed to TCE or related substances did not report increased levels of CCD in the offspring; however, it is not clear whether they were designed to evaluate CCD in the study cohorts. The studies that evaluated CCD in TCE-exposed cohorts are summarized in Table 3 and are described briefly below.

Lagakos et al. (1986) conducted a telephone survey of residents of Woburn, Massachusetts, to collect data on residential history and information on a variety of adverse health outcomes. Completed surveys were obtained from approximately 57% of the town residences, which included 4,978 children born since 1960. Two of the wells providing the town's water supply from 1964 to 1979 had been found to be contaminated with several volatile organic compounds (e.g., TCE, tetrachloroethylene, chloroform). Lagakos et al. (1986) used information from a study by the Massachusetts Department of Environmental Quality and Engineering to estimate the contribution of water from the two contaminated wells to the residence of each participant, based on zones within the town that received different mixtures of water from various wells, for the period in which the contaminated wells were operating (EPA 2011). This exposure information was used to estimate a cumulative exposure to the solvents based on each child's length of residence in Woburn. Only five cases of cardiovascular abnormalities were reported among exposed subjects, which corresponds to approximately 0.1% of births, and the investigators concluded that there was no significant association with TCE. This level is well below the background rate in the general population, because CCD are the most frequent form of birth defects—the current estimate of CCD is 9 in 1000 live births, or not quite 1% of newborns (American Heart Association website 2012).

A birth-registry-based observational study was conducted by Goldberg et al. (1990) to evaluate the incidence of CCD among residents from Tucson Valley, Arizona. Interviews were conducted with parents of 707 children with a CCD born between 1969 and 1987 that were identified from birth registries. Of the 707 case families included, 246 (35%) were exposed to wells providing drinking water found to be contaminated with TCE (range = 6–239 ppb), among other substances (e.g., dichloroethylene, chromium) during their first trimester, while 461 controls had no exposure to contaminated water during pregnancy. The investigators reported that 6.8 in 1000 live births of mothers exposed to contaminated water had a CCD, compared to 2.6 in 1000 live births of mothers residing in non-contaminated areas. Goldberg et al. (1990) noted that the odds ratio (OR) for CCD in offspring declined from three-fold higher in exposed populations to no difference as compared to controls after TCE-contaminated drinking water wells were closed, which suggested a causal relationship. The prevalence of any particular type of CCD was not statistically significantly different in exposed versus non-exposed mothers of

afflicted infants, indicating that TCE did not induce a specific effect on the heart. In addition, these levels are below the background rate of 1% for CCD in the general population. EPA (2011) concluded that this study reported no significant differences in cardiac lesions between exposed and non-exposed groups.

A cross-sectional study was conducted to evaluate the incidence of congenital abnormalities in infants from75 towns in New Jersey that reported several contaminants, including TCE (average of 55 ppb TCE), in the water supply between 1985 and 1988 (Bove et al. 1995). Birth records of 80,938 live births and 594 fetal deaths in the towns during this time period were reviewed. From this population, 346 infants (including live births and stillborns) had CCD and were considered cases, and 52,334 infants had no birth defects and were considered to be controls. The amount of maternal TCE exposure was estimated based on tap water data for the area. The author reported weak associations between TCE exposure and CCDs in women exposed to water levels exceeding 10 ppb TCE and an increased risk of ventricle septal defects in women exposed to levels of TCE exceeding 5 ppb. The incidence levels of CCD were not statistically significant, therefore they do not provide support for an association between TCE and CCD. In addition, in the study population, only 0.4% (346 in 80,938) CCD were reported, which is lower than the U.S. background incidence of approximately 1%.

ATSDR examined pregnancy outcomes among women living at the U.S. Marine Corps Base in Camp Lejeune, North Carolina during the years 1968 to 1985 in a retrospective cohort study (ATSDR 1998). In early 1982, TCE was found in tap water samples from one water distribution system on Camp Lejeune at concentrations as high as 1,400 ppb and by July, the concentration in that distribution system had dropped to a maximum level of 20 ppb. However, in 1985, the TCE concentration in another water distribution system at the base was 1,148 ppb. The retrospective cohort study was conducted to determine whether a link existed between TCE exposure and adverse birth outcomes in infants born between January 1, 1968, and December 31, 1985, based on birth and infant death certificates. The study population included 141 infants born to women with short-term exposure to TCE and a second cohort of 31 infants born to women with long-term exposure to TCE. The investigators controlled for sex of the infant, maternal and paternal ages, parity, maternal race, maternal and paternal education, military pay

grade, adequacy of maternal care, marital status, and year of birth. No association between TCE exposure and CCD was observed.

A case-control study was conducted of 4,025 infants born in 1997–1999 in Milwaukee, Wisconsin, to evaluate the association between human maternal TCE exposure and CCDs (Yauck et al. 2004). Cases included 245 infants with a CCD and controls included 3,870 infants without a CCD, based on diagnostic information obtained from hospital records. Information about potential confounders and the location of the mother's residence for both cases and controls was obtained from birth records. TCE emissions data were ascertained from state and EPA databases, and distance between maternal residence and the emission source was determined by geographic information system software. Exposure was defined as those residing within 1.32 miles of at least one site, but no TCE exposure measurements were reported in the study (761 exposed and 3,264 unexposed mothers). Of 245 CCD cases, 8 (3.3%) were born to mothers ≥38 years old. Of the 3,780 controls, only 19 (0.5%) were born to older exposed mothers. An increased risk of CCD was reported in the offspring of mothers ≥38 years old with presumed TCE exposure (OR = 6.2, CI = 2.6–14.5) and for offspring of unexposed mothers ≥38 years old (OR = 1.9, CI = 1.1-3.5). No increased risk of CCD was reported for offspring of exposed or unexposed mothers <38 years old. It is important to note that there were statistically significant increased risks for CCD associated with preexisting diabetes, chronic hypertension, or alcohol use during pregnancy—potential confounding variables for CCD. Several limitations need to be considered when interpreting the results from this study, including the lack of TCE exposure data, lack of information about potential confounding variables (e.g., diet, vitamin intake), and lack of information about pregnancy termination rates. Also, maternal residence at the time of delivery was assumed to be the residence during pregnancy, and the sample size for older exposed mothers was very small (n = 27). Although the authors claim that advanced maternal age (defined as ≥38 years of age) can make women more susceptible to adverse effects of TCE on the developing heart compared to younger women, advanced maternal age is associated with an increased risk of CCD (Watson et al. 2006). Taking into consideration the potentially confounding effect of advanced maternal age in conjunction with the small number of cases, it is difficult to establish the relative roles that TCE exposure and maternal age might play in the increased risk of CCD. In addition, the authors failed to evaluate gradients of risk

associated with either increasing distance from the facilities or with increasing maternal age (Scialli and Gibb 2004).

ATSDR conducted a study to evaluate the risk of birth defects among residents of Endicott, New York, who may have been exposed to volatile organic compounds (VOCs) via soil vapor intrusion as a result of groundwater contamination (ATSDR 2006; 2008; Forand et al. 2012). The study was conducted to determine whether the prevalence of birth defects between 1983 and 2000, and the rate of other adverse birth outcomes between 1978 and 2002 among Endicott area residents living in the area where VOCs had been found in soil vapor, were similar to those of New York State, excluding New York City. A total of 1,440 births occurred among residents in the two study areas between 1978 and 2002. Between 1983 and 2000, there were 61 congenital defects, compared to 59 expected, resulting in no elevation of risk; however, both total cardiac defects (n = 15; OR = 1.94, 95% CI = 1.21–3.12) and major cardiac defects (n = 6; OR: 2.52, 95% CI: 1.2–5.29) were statistically increased in the study population. A follow-up study by ATSDR (2008) reported that conotruncal heart malformations were particularly elevated (n = 4; rate ratio = 4.83, 95% CI = 1.81–12.89), and the results remained significantly elevated when infants with Down syndrome were excluded from the analysis. However, these results were based on a very small number of cases. The ATSDR study was ecologic in design and evaluated the risk of disease within a population, therefore, it was not specified whether individuals who developed adverse health outcomes (e.g., CCD) were those who were actually exposed to VOCs. In addition, there were no measures of individual exposures and there was limited information about the levels of VOCs in indoor air, and no information regarding duration of exposure. Individual exposure to VOCs would vary with the length of time the person lived in the study area before diagnosis, levels of VOCs in their house, and amount of time they spent in the home each day. In addition, personal information such as medical history, dietary and lifestyle choices such as smoking and drinking, and occupational exposures to chemicals were not examined. These limitations make it very difficult to draw conclusions regarding the existence of an association between TCE and CCD.

Discussion of the Epidemiological Studies that EPA (2011) Concluded Were Positive for Congenital Cardiac Defects

The IRIS toxicological review of TCE concluded that, although the epidemiological studies have individual limitations, the studies as a whole show relatively consistent elevation in the incidence of congenital cardiac defects in TCE-exposed populations compared to reference groups (EPA 2011). However, the only two studies that were considered to report an increased risk of CCD after exposure to TCE were those conducted by Yauck et al. (2004) and ATSDR (2006, 2008), both of which have significant methodological limitations that affect the ability to draw conclusions about an association between exposure to TCE and the development of CCD. It is important to note that the study published by Yauck et al. (2004) did not find a link between CCD and presumed TCE exposure in mothers younger than 38 years, and for exposed older mothers, there were too few cases to determine the relative impact of CCD and age. The ATSDR (2006, 2008) study was ecologic in design and evaluated the risk of disease within a population; therefore, it was not specified whether infants who developed CCD were born to mothers who were actually exposed to TCE. The toxicological review (EPA 2011) concluded that the rest of the studies that evaluated CCD did not report any significant increases in TCE-exposed groups (Lagakos et al. 1986; Goldberg et al. 1990; Bove et al. 1995; Bove 1996).

Overall Conclusions and Limitations of the Cardiac Developmental Epidemiological Studies with TCE

Due to a variety of limitations, the available epidemiological studies are inadequate to support the hypothesis that TCE is associated with an increased risk of CCD. Methodological issues that limit the ability to establish any association between exposure to TCE and CCD include the types of study designs (e.g., ecologic), exposure to several chemicals, lack of TCE exposure data, potential for confounding variables, non-statistically significant increases in CCDs reported, and a lack of a specific type of cardiovascular developmental effect in the studies. In addition, several risk factors have been associated with CCD, including Down syndrome, nutritional deficiencies such as folic acid, maternal diabetes, drug and alcohol use, certain viruses, and certain prescription medications and many of these important confounding factors were not evaluated in the studies, limiting the ability to establish a causal association between TCE and CCD. For some of the available studies, the toxicological review (EPA 2011) reported

that while they include both occupational and environmental exposures to TCE, the epidemiological studies are, overall, not highly informative due to their small numbers of cases and limited exposure characterization, or to the fact that exposures to mixed solvents were involved. A significant limitation of most of the available epidemiological studies is the lack of TCE exposure levels. When attempting to establish a causal association between CCD and exposure to a chemical such as TCE, it is important to quantify exposure for pregnant women during the first trimester when organogenesis is underway and the developing heart is most susceptible to environmental insult (Watson et al. 2006). The concentration of TCE in the drinking water and the amount of residential water ingested by the pregnant subjects is necessary to quantify exposure, information that is lacking from the epidemiological studies that evaluated CCD. Estimates of TCE inhalation exposure are not available, because the studies did not report TCE exposure levels for the subjects or their residences. Furthermore, most of the epidemiological studies examined solvents in general, and the proportion of TCE present in the mixtures of organic solvents was not known, making it impossible to quantify TCE exposure. Conclusions about TCE based on studies of organic solvents in general would not be directly relevant to the evaluation of TCE toxicity, because it is not possible to determine which solvent may be associated with the observed adverse effect. In addition, it is important to note that some investigators concluded that there was a non-statistically significant increase in CCD among exposed populations (Goldberg et al. 1990; Bove et al. 1995), although the prevalence of CCD in these groups was well within the expected range of CCD in the general population (Watson et al. 2006). Furthermore, the epidemiological and toxicological studies have not identified an increase in any one particular type of CCD, making it difficult to evaluate biological plausibility and the mechanism of any association between TCE and CCD.

The epidemiological studies have additional limitations regarding the manner in which the data were collected. For example, the validity of the data relies on the quality of the parental interview or on the rigor with which CCDs were detected and reported in birth defects registries (Watson et al. 2006). An intrinsic problem with studies that use questionnaires or interviews to obtain health effects information is that the validity of the findings is limited by the recall of the subjects. It is probable that the parents of children with a CCD would be more eager to participate in a study evaluating possible reasons for their child's condition and/or may have

already given considerable thought to how maternal exposure might have influenced their child's condition (Watson et al. 2006). Overall, the relatively large number of available epidemiological studies does not provide convincing evidence that TCE exposure during pregnancy is associated with the development of CCD in offspring.

Toxicological Studies That Evaluated Cardiac Developmental Endpoints

Several toxicological studies have been conducted using various experimental animal models to investigate whether exposure to TCE can adversely impact normal heart development. However, there are several issues that need to be considered when attempting to extrapolate the results of the TCE experimental animal studies to humans. For example, there are notable differences in how rodents and humans metabolize TCE. Specifically, mice and rats metabolize TCE more efficiently than humans; the maximum rate of TCE metabolism in humans is one-third that of the rat and one-fourth that of the mouse (Pastino et al. 2000). In rodents, a greater proportion of TCE is metabolized to dichloroacetic acid (DCA) mercapturic acid and a reactive thiol, whereas humans metabolize a greater proportion of TCE to trichloroacetic acid (TCA). Furthermore, when considering the relevance of animal data to human health, it must be determined whether the experimental exposure concentration and route of exposure are relevant to humans. Many of the TCE developmental studies have been performed at doses far exceeding what would be expected from environmental exposure, and it may not be possible to reasonably extrapolate data at these high doses to human health risk.

The IRIS toxicological review of TCE concluded that CCD were not observed in several studies in which TCE was administered during the period of fetal cardiac development, including inhalation studies in rats (Schwetz et al. 1975; Dorfmueller et al. 1979; Hardin et al. 1981; Healy et al. 1982; Carney et al. 2006) and rabbits (Hardin et al. 1981), and gavage studies in rats (Narotsky and Kavlock 1995; Narotsky et al. 1995; Fisher et al. 2001) and mice (Cosby and Dukelow 1992). The IRIS review of TCE also concluded that CCD were observed in Sprague-Dawley rat fetuses following the administration of TCE in drinking water to mothers during gestation (Dawson et al. 1993; Johnson et al. 2003) and following intrauterine administration (Dawson et al. 1990). These studies were all conducted by a group of investigators at the

University of Arizona, which is the only research group that reported a positive association between TCE and CCD in experimental rodent studies. A few studies also reported a positive association between the oral gavage administration of TCE metabolites (TCA, DCA) and CCD in Long Evans rats (Smith et al. 1989; Epstein et al. 1992; Smith et al. 1992; Johnson et al.1998a,b).

In contrast to the few studies reporting positive CCD findings from the University of Arizona, statistical analysis of the data from the inhalation studies reporting negative findings were always performed on a per-litter basis rather than a per-fetus basis. Counting each neonate as a separate observation may lead to incorrect conclusions, and it is generally recommended that the number of observations for each outcome be based on the number of treated females or whole litters (Festing 2006; DeSesso and Willhite 2009). Because the maternal animal, and not the conceptus, is the individual treated during gestation, data generally are calculated as incidence per litter or as number and percent of litters with particular endpoints (EPA 1991).

There have also been a few cardiac developmental studies with TCE or TCA conducted in chickens, some of which that have reported cardiac effects (Bross et al. 1983; Loeber et al. 1988; Boyer et al. 2000; Mishima et al. 2006; Drake et al. 2006a,b; Rufer et al 2008; 2010). In the studies, the chick embryos were injected with high concentrations of TCE administered directly to the chorioallantoic membrane, a route of exposure that it not at all representative of how pregnant women are likely to be exposed to these substances. The relevance of these findings to humans is unclear; data in the chick model are not directly applicable to human risk due to significant developmental differences between chickens and humans and the absence of a maternal influence in the chick model system. Because of the uncertainties regarding extrapolating results from avian studies to humans, those studies are not summarized in this review.

The following is a discussion of the available toxicological studies that evaluated developmental cardiac toxicity in experimental animals following exposure to TCE. These studies are summarized in Table 4.

Inhalation Studies with TCE

Schwetz et al. (1975) exposed Sprague–Dawley rats and Swiss Webster mice to 300 ppm TCE vapors for 7 h daily throughout GD 6–15 and no significant maternal, embryonal or fetal toxicity was reported at this concentration.

Dorfmueller et al. (1979) exposed Long Evans rats to higher concentrations of TCE vapors (1800±200 ppm), and examined the effects of exposure to TCE for 2 weeks before mating and/or during pregnancy. Groups of rats were exposed before mating only, during pregnancy only, and throughout pre-mating, mating, and pregnancy. No treatment-related CCDs, or any other developmental effects were reported.

Hardin et al. (1981) conducted a study to evaluate the effects of inhalation exposure of 500 ppm TCE in rats and rabbits on GD 1–19 and 1–24, respectively, and did not observe evidence of CCD.

Healy et al. (1982) exposed pregnant Wistar rats to 100 ppm TCE for 4 h daily from GD 8 to 21. On GD 21, fetuses were examined for developmental abnormalities, including CCDs. There were no significant increases in CCD following exposure to TCE.

A TCE inhalation developmental study with Sprague-Dawley rats was conducted by Carney et al. (2006). This study was compliant with EPA Office of Pesticides and Toxic Substances Guideline 870.3700 for prenatal and developmental toxicity studies, as well as the Organization for Economic Co-operation and Development Guideline No. 414 for developmental toxicity studies. Pregnant Sprague–Dawley rats were exposed to 50, 150, or 600 ppm TCE vapors for 6 hours a day during gestational day (GD) 6–20. At least half of all fetuses in each litter were chosen randomly for complete visceral examinations, including a thorough dissection of the heart and great vessels. Dams treated with 600 ppm TCE exhibited a significant decrease in body weight gain; however, there were no indications of developmental toxicity, including CCD observed at any dose level, and the no-observed-effect-concentration (NOEC) was 600 ppm.

Oral Studies with TCE

National Toxicology Program (NTP) TCE developmental studies were conducted with Swiss CD-1 mice and Fischer 344 rats treated by oral gavage (NTP 1985; NTP 1986). Mice were administered 100, 300, or 700 mg/kg/day throughout pregnancy, and rats were administered 76, 156, or 289 mg/kg/day. There was no correlation between TCE and CCDs identified in the offspring of any treatment group.

In a study performed by Fisher et al. (2001), 20 presumed-pregnant rats per group were administered a daily oral gavage dose of 500 mg/kg TCE, 300 mg/kg TCA, or 300 mg/kg DCA from GD 6 to 15. Negative controls were administered and soybean oil or water, and 12 pregnant dams were administered a daily dose of retinoic acid, a known cardiac teratogen, as a positive control. Fetal hearts were dissected according to the fresh dissection method previously described to have been used by the University of Arizona investigators, and the team of observers included members of the University of Arizona laboratory. All observers were blinded to treatment. Although gestational treatment with TCA and DCA led to a statistically significant decrease in fetal body weight, neither the percentage of fetuses with cardiac anomalies nor the percentage of litters with a CCD was higher in the TCE, TCA or DCA groups compared to water or soybean oil controls. As expected, retinoic acid administration to dams led to a statistically significant increase in CCD compared to both control groups.

Dawson et al. (1993) conducted a drinking-water study with Sprague-Dawley rats administered 1.5 ppm TCE, 1100 ppm TCE, 0.15 ppm DCE, or 110 ppm DCE prior to mating only, prior to mating and during pregnancy, and during pregnancy only. For this study, and for all subsequent studies performed in this laboratory that evaluated the effects of TCE on the rodent heart, the Dawson dissection technique, which differs from methods typically employed for examining the heart, was employed. Using this method, the investigators reported a significant increase (on a per-fetus basis) in the incidence of CCDs in the following treatment groups: 1100 ppm TCE during pregnancy (10.4%), 1100 ppm TCE before and during pregnancy (9.2%), 1.5 ppm TCE before and during pregnancy (8.2%), 0.15 ppm DCE before and during pregnancy (11.6%), and 110 ppm DCE before and during pregnancy.

A study was conducted by Johnson et al. (2003) to add additional TCE dose levels to those that were evaluated in the Dawson et al. (1993) study. Johnson et al. (2003) also summarized the Dawson et al. (1990, 1993) studies in an attempt to identify a threshold concentration of TCE at which an increased risk to the developing heart would be expected. In the Johnson et al. (2003) study, Sprague-Dawley rats were randomly placed in test groups and exposed to various concentrations of TCE (0, 2.5 ppb, 250 ppb, 1.5 ppm, 1,100 ppm) in drinking water throughout pregnancy. When the data from the studies were pooled, Johnson et al. (2003) reported that the percentages of abnormal hearts were 2.2%, 0%, 4.5%, 1.5%, and 10.5% at concentrations of 0 ppb, 2.5 ppb, 250 ppb, 1.5 ppm, and 1100 ppm TCE, respectively. Johnson et al. (2003) reported that when analyzed on a per-fetus and per-litter basis, the 2.5-ppb and 1100-ppm concentrations led to a statistically significant increase in the number of abnormal hearts, although the marked absence of a dose–response relationship should be noted. For each treatment group, there were 9–13 litters, and the control group (consisting of animals used in 1993 and 2003) contained 55 litters. To calculate the per-litter statistics, the authors appear to have divided the number of litters with at least one CCD by the total number of litters in the group. In contrast, the correct way to conduct per-litter statistics is by examining the proportion of pups per litter (DeSesso and Willhite 2009; Watson et al. 2006). Per-litter analysis is the accepted method of analysis for developmental effects related to chemical exposure during pregnancy, as recommended by the EPA Office of Research and Development (EPA 1991). Furthermore, pooling of controls is not an appropriate statistical practice and is likely to have exaggerated the alleged statistical significance (Watson et al. 2006).

Intrauterine Administration of TCE

In the study by Dawson et al. (1990), 15 ppm TCE, 1500 ppm TCE, 1.5 ppm dichloroethylene (DCE), and 150 ppm DCE in saline were pumped into the uterine lumen using osmotic pumps inserted into the uterine horn of pregnant Sprague-Dawley rats GD 7-22. Heart defects (primarily atrial septal defects) were observed in 3% of control animals, 9% of animals exposed to 1.5 ppm TCE, and 14% of animals exposed to 1500 ppm TCE, 12% of animals exposed to 0.15 ppm DCE, and 21% of animals exposed to 150 ppm DCE. The increase in the percentage of CCD in the TCE-treated animals was statistically significant on a per-fetus basis. There were no specific CCD observed.

TCE Metabolites Studies

Trichloroacetic acid (TCA) administered to Long Evans rats by oral gavage during GD 6–15 (includes the sensitive period of organogenesis) at doses of 330, 800, 1200, or 1800 mg/kg/day was associated with a significant increase in the CCDs observed in offspring (Smith et al. 1989). The most common findings after treatment with TCA were levocardia (at 330 mg/kg/day and greater) and interventricular septal defect (800 mg/kg/day and greater). Smith et al. (1992) reported statistically significant increases in CCD in Long Evans rats administered oral gavage doses of DCA ranging from 140 to 2400 mg/kg/day administered during GD 6–15. With DCA, the most common cardiac malformations were a defect between the ascending aorta and right ventricle (at 140 mg/kg/day and greater), levocardia (at 900 mg/kg/day and greater), and intraventricular septal defect (at 1,400 mg/kg/day and greater). Epstein et al. (1992) reported a positive association between DCA treatment and the prevalence of CCDs in the pups of Long Evans rat dams treated with 1900 mg/kg DCA by gavage on GD 9–11 or 12–15. The heart defects found were predominantly high interventricular septal defects and, less commonly, interventricular septal defects. Johnson et al. (1998a,b) administered pregnant Sprague-Dawley rats drinking water with various metabolites of TCE or DCE at doses equivalent to that expected if all of the high dose of TCE (1100 ppm, which is above the limit of solubility at 20ºC), was to completely break down to the metabolites. Of the metabolites evaluated, TCA (2730 ppm) was the only treatment that resulted in a statistically significant increase in a variety of cardiac malformations (10.53% versus 2.15% in the control group). According to NAS, limitations associated with the Johnson et al. (1998b) study include discrepancies in the number of affected hearts and fetuses reported in the study and failure to disclose that the control group was not concurrent.

Evaluation of the Johnson et al. 2003 Study

As discussed previously, EPA identified the Johnson et al. (2003) study of fetal heart malformations in rats as a critical study for developing a candidate RfC for TCE, and the RfC was developed to be protective of CCD in humans. The Johnson et al. (2003) study was conducted to re-evaluate the data reported by Dawson et al. (1993) by including information on two lower test concentrations of TCE (0.0025 and 0.25 ppm). Johnson et al. (2003) concluded

that their analysis identified 0.25 ppm as a threshold above which rats exposed to increasing levels of TCE during pregnancy have increasing incidences of developmental cardiac effects in their fetuses. Concerns about the studies from the Johnson et al (2003) research group regarding the methodology, reported findings, and the scientific credibility of the study have been expressed by other researchers (Hardin et al. 2004; Watson et al. 2005). Several other laboratories have not observed CCD in the same species at higher exposure levels. In addition, the original study (Dawson et al. 1993) was statistically significant for CCD only after a re-evaluation of the statistics using a different control group from a later study (Johnson et al. 2003). It is important to note that the data were accumulated over ten years; deficiencies in study design and reporting make the interpretation of data tentative at best; and the major effect was increased incidence of atrial septal defects, which may actually have been related to the cardiac examination procedure or possible delays in development, rather than actual heart defects. These methodological deficiencies and concerns about the results of the Johnson et al. (2003) study should be considered carefully and evaluated when conducting a weight-of-evidence analysis of the causal association between TCE and CCD. Furthermore, a critical analysis and a weight-of-evidence analysis should be conducted prior to selecting an individual study, such as the Johnson study for deriving regulatory levels for TCE.

Although EPA (2011) selected this study as one of the critical studies for developing a RfC for TCE, concerns about the Johnson et al. (2003) study have been expressed by EPA and by the scientific community, including NAS (2006). This study has several methodological issues that warrant examination and careful consideration, particularly when relying on the reported data for developing regulatory levels for TCE. Furthermore, it is important to note that the only positive animal studies reporting a causal association between TCE and developmental heart effects are reported by a single laboratory group (Dawson et al. 1990; Dawson et al. 1993; Johnson et al. 2003).

Overall Conclusions and Limitations of the Cardiac Developmental Toxicological Studies with TCE

With respect to the variable results reported in various oral and inhalation toxicological studies that evaluated CCD, EPA acknowledged that it is generally recognized that response variability

among developmental bioassays conducted with the same chemical agent may be related to factors such as study design (e.g., the species and strain of laboratory animal model used, day or time of day of dose administration in relation to critical developmental windows, route of exposure, vehicle used, the day of study termination), or the study methodologies (e.g., how fetuses were processed, fixed, and examined; what standard procedures were used in the evaluation of morphology and abnormalities; and whether the fetal evaluations conducted were consistent). Differences in study results may also be due to the method by which pathological examinations were conducted (e.g. whether or not cardiac evaluations were conducted using standardized dissection procedures and whether the examinations were conducted by technicians who were trained and familiar with fetal cardiac anatomy). The IRIS evaluation concluded that many of the developmental studies used a traditional free-hand section technique on fixed fetal specimens, whereas a fresh dissection technique that can enhance the detection of anomalies was used in the positive studies by Dawson et al. (1990, 1993) and Johnson et al. (2003). In addition, interpretation of the findings may be influenced by the quantitative approaches applied to the data, as well as historical incidence data for the species and strain of interest as reviewed by Watson et al. (2006) and Hardin et al. (2005).

Most of the available studies, including those that reported an association between TCE and CCD, were performed at concentrations several orders of magnitude greater than the highest concentration of TCE ever detected in drinking water (~1400 ppb) (Watson et al. 2006). For example, a concentration of 1100 ppm (~129 mg/kg/day) TCE was administered to rats in drinking water throughout pregnancy (Dawson et al. 1993), gavage doses of 500 mg/kg/day were administered to rats from GD 6 to 15 in a study by Fisher et al. (2001), and 1500 ppm TCE was injected directly into the pumps inserted into rodent uterine horns (Dawson et al. 1990). In comparison, the solubility limits of TCE in water are 1070 ppm at 20ºC and 1366 ppm at 25ºC and the odor threshold is approximately 28 ppm. Therefore, the toxicological studies that reported a positive association between TCE and CCD were performed at concentrations that are much higher than concentrations that should be used to estimate human risk from environmental exposure to TCE in water or air.

All of the studies alleging that TCE plays a causal role in CCD were conducted at the same laboratory at the University of Arizona, and no specific type of CCD was linked to TCE or its metabolites in these studies (Dawson et al. 1990; Dawson et al. 1993, Johnson et al. 2003). The positive CCD findings from these studies cannot be explained by the high exposure level, because Fisher et al. (2001) also administered a high dose of TCE (500 mg/kg/day) during GD 6–15 and observed no CCD (Watson et al. 2006). The mode of exposure at the University of Arizona laboratory (via drinking water throughout pregnancy), rather than limiting exposure to GD 6–15 (the sensitive period of organogenesis), also cannot explain the differences between the positive and negative findings (Watson et al. 2006). The heart is formed during the period of organogenesis; therefore, exposure to TCE prior to or after this period would not increase the likelihood of a CCD. Dorfmueller et al. (1979) and Hardin et al. (1981) exposed animals to high concentrations of TCE for all or most of pregnancy and also reported negative results. Possible reasons for the laboratory-specific positive link between TCE and CCD observed in the University of Arizona studies include their unique dissection technique and the use of non-standard statistical evaluations for developmental toxicity tests (Watson et al. 2006).

VII. Conclusions from Various Governmental Agencies Regarding the Teratogenicity of TCE

As previously mentioned, governmental agencies in addition to the USEPA have recently reviewed the epidemiology and toxicology studies on pertinent to an evaluation of a causal association between TCE and CCD. (EPA 2011, SAB 2011, NAS 2006, NAC/COT 2009). In the various reports produced by these Agencies, there are very few epidemiological and toxicological studies that are identified as supporting an association between TCE and CCD. As is noted in these reviews the few positive studies have methodological or study design limitations that limit the value of the studies as a basis for concluding that TCE causes teratogenic effects; or more specifically, that is causes CCD. As noted below, the 2011 toxicological review that was developed to support to the RfC presented in the EPA's IRIS data base included a tempered conclusion that the available evidence raises "sufficient concern regarding the potential for developmental toxicity" (EPA 2011). However, following the review of this toxicological review document, the EPA Science Advisory Panel recommended that the cardiac malformations be selected as one of the health endpoints on which the TCE RfC was based. The conclusion about CCD presented in the IRIS file itself was restated in a stronger form than was expressed in the underlying 2011 toxicological review, but the reason for this difference is not discussed in the IRIS file. Reviews of the same studies included in the EPA 2011 Toxicological Review were also addressed in the reviews performed by a National Academy of Science (2006) committee and by the National Advisory Committee (NAC/COT 2009) within the National Research Council. Both of these committees noted the same positive studies cited in the EPA (2011) Toxicological Review, but noted the limitations of these studies and did not draw conclusions that TCE was causally linked to CCD. To illustrate the inconclusiveness of the existence of an association between TCE and CCD, the conclusions that have been developed by EPA, NAS, and NAC/COT are presented below.

EPA (2011) Toxicological Review

The EPA toxicological review concluded the following regarding the association between TCE and CCD in the section entitled, "Summary of the Weight of Evidence on Cardiac Malformations" (EPA 2011, p. 4-565):

> "The evidence for an association between TCE exposures in the human population and the occurrence of congenital cardiac defects is not particularly strong. Many of the epidemiological study designs were not sufficiently robust to detect exposure-related birth defects with a high degree of confidence. However, two well-conducted studies by ATSDR (2006, 2008) clearly demonstrated an elevation in cardiac defects. It could be surmised that the identified cardiac defects were detected because they were severe, and that additional cases with less severe cardiac anomalies may have gone undetected.
>
> The animal data provide strong, but not unequivocal, evidence of the potential for TCE-induced cardiac malformations following oral exposures during gestation. Strengths of the evidence are the duplication of the adverse response in several studies from the same laboratory group, detection of treatment-related cardiac defects in both mammalian and avian species (i.e., rat and chicken), general cross-study consistency in the positive association of increased cardiac malformations with test species (i.e., rat), route of administration (i.e., oral), and the methodologies used in cardiac morphological evaluation (i.e., fresh dissection of fetal hearts). Furthermore, when differences in response are observed across studies, they can generally be attributed to obvious methodological differences, and a number of *in ovo* and *in vitro* studies demonstrate a consistent and biologically plausible mode of action for one type of malformation observed. Weaknesses in the evidence include lack of a clear dose-related response in the incidence of cardiac defects, and the broad variety of cardiac defects observed, such that they cannot all be grouped easily by type or etiology.
>
> Taken together, the epidemiological and animal study evidence raise sufficient concern regarding the potential for developmental toxicity (increased incidence of cardiac defects) with in utero TCE exposures."

By noting the updated evaluation of the Endicott study in the summary evaluation, it appears that EPA is giving this study substantial weight, even though the study has the limit of being an ecological study. The statement that one could "surmise" the existence of additional, undetected effects is speculation that undermines the credibility and apparent objectivity of the statement regarding the epidemiology data. The characterization of the Endicott study appears to be contradictory to the more measure final conclusion, although the final conclusion is vague.

EPA IRIS File (2011)

The IRIS 2011 file concluded the following about TCE and developmental cardiac effects (IRIS 2011):

> "For developmental cardiac effects, although the available study (Johnson et al., 2003) has important limitations, the overall weight of evidence supports an effect of TCE on cardiac development."

EPA Science Advisory Panel (2011)

The SAB (2011) reviewed the draft EPA toxicological review document before it was finalized, and concluded the following about CCD:

> "The Panel recommended that the two endpoints for immune effects from Keil et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered the principal studies supporting the RfC. The Panel also recommended that the endpoints for immune effects from Keil et al. (2009) and Peden-Adams et al. (2009) and the cardiac malformations from Johnson et al. (2003) be considered as the principal studies supporting the RfD."

> "Thus, the Panel agreed that kidney toxicity was indisputably a key effect of TCE from a hazard identification perspective. However, as discussed above, the Panel concluded that the three p-cRfCs for renal endpoints were based on an uncertain dose metric, especially in regard to the relative rate of formation of the toxic metabolite in humans and animals.

Although there was somewhat less confidence in the immune and cardiac malformation endpoints from a hazard identification perspective, for reasons discussed extensively in other sections of this response, there was sufficient confidence in them to consider them critical endpoints to support the RfC. While the confidence in these three endpoints was less than for the kidney endpoints as far as hazard identification, the three p-cRfCs for these endpoints were based on relatively certain dose metrics."

National Academy of Sciences, National Research Council (NAS 2006)

With respect to cardiac teratogenesis, NAS (2006) concluded the following:

"The committee is aware that considerable controversy has existed regarding cardiac teratogenesis, with some reviewers on both sides of the argument (Kaneko et al. 1997; Johnson et al. 1998b; Bove et al. 2002; Hardin et al. 2005). Multiple studies in several animal models, including mammalian (Smith et al. 1989, 1992; Epstein et al. 1992; Dawson et al. 1993; Drake et al. 2006) and avian (Bross et al. 1983; Loeber et al. 1988), suggest that trichloroethylene, or one or more of its metabolites (trichloroacetic acid and dichloroacetic acid), can cause cardiac teratogenesis. Of the studies performed, the avian studies are the most convincing, and mechanistic studies in birds have been performed. Although some rodent studies have shown effects (Smith et al. 1989, 1992; Dawson et al. 1993; Epstein et al. 1992), other studies have not (NTP 1985, 1986b; Fisher et al. 2001), suggesting either methodological or strain differences. The committee noted that the rodent studies showing trichloroethylene induced cardiac teratogenesis at low doses were performed by investigators from a single institution. Also noted were the unusually flat dose-response curves in the low-dose studies from these investigators. For example, the incidences of heart malformations at trichloroethylene concentrations of 1.5 and 1,100 ppm (almost three orders of magnitude greater) were 8.2% to 9.2% (prepregnancy and during pregnancy) to 10.4% (during pregnancy only) (Dawson et al. 1993). The same pattern occurred with dichloroethylene. Thus, the animal data are inconsistent, and the apparent species differences have not been addressed.

> Of the human epidemiologic studies, the Bove et al. (2002) reanalysis of the widely criticized, but positive, study by Goldberg et al. (1990) also found a positive association. Methodological problems limited the committee's consideration of the Santa Clara County data for congenital heart disease. The recent report of an increased incidence among residents of the Endicott, New York, area was also consistent with the Goldberg study. Of note, the effect size of a 2- to 3-fold increase in risk is similar across multiple studies. Plausibility for trichloroethylene-induced cardiac teratogenesis is increased by the fact that the most frequently observed cardiac defects in the human studies, those of the interventricular septae and the valves, are consistent with the most common defects seen in the animal studies. In addition, these specific defects are consistent with mechanistic studies demonstrating altered increased proliferation in the endocardial cushions at low dose (Drake et al. 2006) or alterations in endothelial cell activation and decreased expression of the transcription factor Mox-1 and extracellular matrix protein fibrillin 2, two markers of epithelial mesenchymal cell transformation, a key process in valve and septum formation (Boyer et al. 2000). Evidence that trichloroacetic acid and dichloroacetic acid are as potent as the parent compound suggests that CYP2E1 metabolic activation, as well as the fractional formation of trichloroacetic acid from chloral, is important in trichloroethylene cardiac teratogenesis."

With respect to the ATSDR Endicott study that was ongoing at the time of publication, NAS (2006) concluded the following:

> "The evaluation of health effects at Endicott is an ongoing study and additional analyses and data refinements are planned. The current study is limited by the lack of individual exposure information, including concentration and duration of exposure. Birth defect cases were not validated by record review. Insufficient power was available to evaluate most birth defects.

NAC/COT (2009) Conclusions

The Interim Acute Exposure Guideline Levels document did not include conclusions regarding TCE and CCD (NAC/COT Subcommittee 2009). The AEGLs that were developed in this

report were all based on neurological endpoints, not developmental endpoints. With respect to the teratogenic potential of TCE, NAC/COT concluded the following, based on a single study that reported an association between TCE and fluid in the skull (hydrocephalus) in rabbit fetuses by Beliles at al. (1980)[4]:

> "Limited developmental studies in rats suggest that trichloroethylene when inhaled throughout pregnancy may delay development. The result of one rabbit study suggests teratogenic potential but the evidence is not conclusive." (p. 36)

With respect to cardiac effects, NAC/COT (2009) stated the following:

> "Another oral developmental rat study indicates that via this exposure route trichloroethylene may induce fetal heart defects. This study was prompted by the observation of an increased risk for these effects in an epidemiological community survey. After exposure of rats via drinking-water before and during pregnancy, increased rates of fetal heart defects were seen at both of the widely spaced dose levels (0.18 and 132 mg/kg bw/day). This increase did not show a clear dose response relation (incidences 8.2 and 9.2% versus 3% in controls) (Dawson et al. 1993)." (p. 30)

Summary of Conclusions from Various Governmental Agencies

The conclusion statements from EPA and other scientific panels highlight the fact that there are substantial uncertainties about the existence of an association between TCE and CCD in experimental animals and, more significantly, humans. The primary toxicological studies that are cited by these groups as providing support for an association are the studies by Dawson et al. (1993) and Johnson et al. (2003). These studies were conducted by a group of investigators at the University of Arizona, which is the only research group that that reported a positive association between TCE and CCD in experimental rodent studies. Potential reasons for the laboratory-specific positive link between TCE and CCD observed in the University of Arizona studies include their unique dissection technique and the use of non-standard statistical

[4] According to NAC/COT, the Beliles et al. (1980) stated that the evidence for a teratogenic effect was not conclusive.

evaluations for developmental toxicity tests. Several studies from a variety of laboratories reviewed in this White Paper have not reported CCD in experimental animals treated with TCE.

The only epidemiological study that is cited by EPA in the toxicological review's Weight-of-the-Evidence Summary as "clearly demonstrating and elevation in cardiac defects (ATSDR 2006)" is an ecologic study of a population in Endicott, NY that has significant methodological limitations, including, no control for confounding variables, multiple volatile organic chemicals, no measures of individual exposure, and no information about exposure duration. Although the ATSDR study of the population in Endicott New York was noted in the reviews by the NAS and NAC/COT committees, an updated evaluation of results was available for the 2011 EPA Toxicological Review. While the fundamental limitations of ecological studies, such as the Endicott study, remain after the re-evaluation of results, this study appears to have had a significant influence on the EPA assessment of the issue. Such uncertainties warrant a thorough weight-of-evidence analysis of the developmental studies to determine if there is an association between TCE and CCD before regulatory values are developed based on teratogenicity as an endpoint.

VIII. Conclusions Regarding Strength of the Evidence for an Association Between TCE and Congenital Cardiac Defects

As summarized in the EPA (2011) toxicological review of TCE, developmental and reproductive toxicology studies in mice, rats, and rabbits do not consistently report adverse effects of TCE on embryonic development (including CCD), besides embryo- or fetotoxicity associated with maternal toxicity. The investigators, Johnson and Dawson, along with their collaborators, appear to be the only researchers to consistently report that TCE is causally associated with CCD in rodent studies (Dawson et al. 1990, 1993; Johnson et al. 1998a,b; Johnson 2003). Others in the scientific community have reported that epidemiological and toxicological studies that support an association between CCD and TCE in humans, and the strength of that association, are limited and weak (Hardin et al. 2005; NAS 2006; Watson et al. 2006). With respect to the potential for developmental cardiac teratogenicity from TCE, NAS (2006) noted the following limitations about the toxicological studies that have evaluated this endpoint: 1) rodent studies have had mixed results, suggesting either methodological or strain differences; and 2) the low-dose studies showing a positive correlation in TCE-induced developmental cardiac effects showed unusually flat dose-response curves, they also came from a single institution, and the results need to be replicated in another laboratory to clarify the dose-response relationship. Specifically, NAS (2006) pointed out that there was no dose response in the Johnson et al. (2003) reanalysis of the Dawson et al. (1993) data, whereby the authors concluded that their reanalysis identified a "threshold level of less than 0.25 ppm TCE, above which rats exposed to increasing levels of TCE during pregnancy have increasing incidences of cardiac malformations in their fetuses." However, as pointed out by NAS (2006), in the Dawson et al. (1993) study, the incidences of congenital cardiac defects at TCE concentrations of 1.5 and 1,100 ppm were 8.2% to 9.2% (pre-pregnancy and during pregnancy) to 10.4% (during pregnancy only), bringing into question the existence of an increasing risk of CCD with increasing exposure levels of TCE. NAS (2006) suggested that additional studies evaluating a LOAEL and mode of action for TCE-induced developmental effects are needed to determine the most appropriate species for human modeling. NAS also noted that epidemiologic

investigations of communities exposed to TCE have reported mixed results regarding CCD and suggested that data from previous epidemiological studies could be reanalyzed.

As stated previously, the RfC for TCE in the 2011 IRIS process was developed on the basis of three sensitive endpoints, one of which was increased fetal cardiac malformations. More typically, RfCs are based on a single health endpoint, and a high standard of critical evaluation is applied to the basis for selecting the critical endpoint(s) and studies for developing the RfC. In developing the RfC from multiple endpoints, the normal standard for adequacy of data does not appear to have been applied by EPA in identifying an association between fetal cardiac malformations and exposure to TCE. The scientific data regarding the existence of a causal link between TCE exposure and CCD are uncertain and there are significant questions about the study (Johnson et al. 2003) that was used as the basis for the candidate RfC value. Furthermore, if a causal association between TCE and CCD is assumed, there are significant questions about the dose response and identification of a NOAEL for developmental effects, as well as the appropriate averaging time to be applied to the NOAEL.

In conclusion, the weight-of-evidence from available toxicological and epidemiological studies does not support the conclusion that there is a causal association between exposure to TCE and CCD in humans. The fact that other scientific and regulatory organizations (e.g., NAC, ACGIH, OSHA) that also reviewed the TCE literature to develop health-protective exposure limits did not select developmental toxicity as the basis of their recommendations supports the conclusion that TCE either is not causally associated with teratogenic health effects or is not the most sensitive endpoint for establishing acute exposure limits.

IX. Tables 1-4

Table 1 - Summary of Interim AEGL Values for TCE

Table 1 Summary of Interim AEGL Values for TCE in ppm [µg/m^3]						
Classification	**10-min**	**30-min**	**1-hr**	**4-hr**	**8-hr**	**Endpoint (Reference)**
AEGL-1	260 [1,400,000]	180 [970,000]	130 [700,000]	84 [450,000]	77 [410,000]	Marginal CNS-effects in 1 out of 8 volunteers exposed to 300 ppm for 2 hrs (Vernon and Ferguson 1969).
AEGL-2 (Disabling)	960 [5,200,000]	620 [3,300,000]	450 [2,400,000]	270 [1,400,000]	240 [1,300,000]	Light-headedness, dizziness, or lethargy in combination with reduced performance in neurobehavioral test at 1000 ppm for 2 hrs (Vernon and Ferguson 1969)
AEGL-3 (Lethal)	6100 [33,000,000]	6100 [33,000,000]	3800 [20,000,000]	1500 [8,100,000]	970 [5,200,000]	NOEL for mortality in mice: 4600 ppm for 4 hrs (Friberg et al. 1953)
AEGL – Acute Exposure Guideline Level Source: NAC/COT Subcommittee for AEGLS. Trichloroethylene Interim Acute Exposure Guideline Levels (AEGLs), 2009						

Table 2 - Summary of Interim AEGL Values for TCE

Table 2 Candidate RfC Values Developed by EPA (2011)								
Study	Species	Endpoint	POD	HEC (ppm)	LOAEL to NOAEL UF	Intra-species UF	Inter-species UF	Candidate RfC (ppm)
Keil et al. 2009	Female Mice	Thymus Weight Change	LOAEL	0.033	10	3	3	0.00033
Johnson et al. 2003	Rat Fetuses	Fetal Heart Malformation	BMDL	0.0037	1	3	3	0.00037
NTP 1988	Female Rats	Kidney Effects	BMDL	0.0056	1	3	3	0.00056

NOAEL – no observed adverse effect level
LOAEL – lowest observed adverse effect level
BMDL – benchmark dose level
HEC – human equivalency concentration
UF – uncertainty factor

Table 3 - Epidemiological Studies Evaluating TCE and Congenital Cardiac Defects (CCD)

Reference, Location, Date, Type of study	Route of Exposure	Concentration of TCE	Study Subjects	Findings	Comments
Lagakos et al. 1986 Woburn, MA 1960-1982 Observational Study – Telephone Survey	Water	267 ppb TCE 21 ppb tetrachloroethylene 12 ppb chloroform	Survey of parents of live infants born between 1970-1982 (4,396 pregnancies) 5 infants with CCD	No association reported	No association between TCE exposure and CCD
Goldberg et al. 1990 Tucson Valley, AZ 1969-1987 Observational Study – Birth Registry	Water	6-239 ppb TCE	Parents of 707 children with a CCD Cases: 246 CCD infants born in TCE contaminated area Controls: 461 CCD infants born outside TCE contaminated area	Incidence of CCDs in TCE contaminated area was 6.8/1000, and the incidence in non-TCE area was 2.6/1000	No statistically significant increase in CCD Lower incidence than the U.S. background rate of CCD in exposed and control groups
Bove et al. 1995 75 towns in Northern New Jersey 1985-1988 Cross Sectional Study	Water	55 ppb TCE	Birth records between 1985 and 1988. 80,938 live births; 594 fetal deaths Cases: 346 infants with CCD Controls: 52,334 live births with no birth defects	Drinking water exposure associated with a slight increase in major CCDs at >10 ppb TCE; OR= 1.24; 50% CI = 0.75–1.94 Increase in ventricular septal defects at >5 ppb TCE; OR= 1.3, 50% CI = 0.88–1.87 The incidence of CCDs was 346/80,938 (4/1000)	No statistically significant increase in CCD Incidence of CCD below background levels Exposure not quantified Water contained multiple chemicals, so not possible to attribute reported effects to TCE

Reference, Location, Date, Type of study	Route of Exposure	Concentration of TCE	Study Subjects	Findings	Comments
ATSDR 1998 Camp Lejeune, North Carolina 1968-1985 Retrospective Cohort	Water	20 – 1400 ppb TCE	Birth certificates of infants born between 1968 and 1985 172 infants born to women exposed to TCE	No association reported	No association between TCE exposure and CCD
Yauck et al. 2004 Milwaukee, WI 1997–1999 Case control study	Air	Maternal residence within 1.32 miles from at least one TCE emissions source No exposure levels reported	4,025 infants born with congenital cardiac defects	Increase in CCD for mothers ≥38 yrs Exposed: OR: 6.2, 95% CI: 2.6–14.5 Unexposed: OR: 1.9, 95% CI: 1.1–3.5 No increase in CCD for exposed mothers <38 yrs old: OR: 0.9, 95% CI: 0.6–1.2	TCE exposure not quantified Effect reported in exposed and unexposed mothers ≥35 yrs Small number of births in older mothers making it difficult to attribute effect to TCE or age
ATSDR 2006, 2008, Forand et al. 2012 Endicott, NY 1978–2000 Ecologic Study	Air	Indoor air from soil vapor: 0.18 - 140 mg/m^3 in the "Eastern Study Area"	1,440 pregnancies among residents during this time period	Increase in total cardiac defects: RR: 1.94, 95% CI: 1.21–3.12 Increase in major cardiac defects: RR: 2.52, 95% CI: 1.2–5.29 Increase in conotruncal heart defects: RR: 4.83, 95% CI: 1.81–12.89	Ecologic study No control for confounding variables Multiple VOCs No measures of individual exposure No information about exposure duration

Table 4 - Toxicological Studies Evaluating TCE and Congenital Cardiac Defects (CCD)

Reference	Route	Number Animals	Dose and Duration	NOAEL or LOAEL	Cardiac Effect(s) Reported and Comments
Inhalation Studies with TCE					
Schwetz et al. 1975	Inhalation	Sprague-Dawley rats 20-35/group Swiss Webster mice 30-40/group	0 or 300 ppm TCE 7 hr/day GD 6-15	Developmental NOAEL: 300 ppm	No CCD observed
Dorfmueller et al. 1979	Inhalation	Long-Evans rats 30/group	0 or 1800 ± 200 ppm TCE 6 hr/day, 5 d/wk for 2 weeks and/or on GD 0-20	Developmental NOAEL: 1,800 ± 200 ppm	No CCD observed
Hardin et al. 1981	Inhalation	Sprague-Dawley rats 20-35/group New Zealand rabbits 15-20/group	Rats: 0 or 500 ppm TCE 6-7 hr/day, GD 1-19 Rabbits: 0 or 500 ppm TCE 6-7 hr/day, GD 1-24	Developmental NOAEL: 500 ppm	No CCD observed
Healy et al. 1982	Inhalation	Wistar rats 31-32/group	0 or 100 ppm TCE 7 hr/day, GD 8-21	Developmental NOAEL: 100 ppm	No CCD observed
Carney et al. 2006	Inhalation	Sprague-Dawley rats	0, 50, 150, 600 ppm TCE 600 ppm = 3.2 mg/L 6 hr/day, GD 6-20	Developmental NOAEL: 600 ppm	No CCD observed
Intrauterine Administration of TCE					
Dawson et al. 1990	Intraperitoneal osmotic pump inserted into uterus	Sprague-Dawley rats	15 ppm or 1500 ppm TCE 1.5 ppm DCE or 150 ppm DCE Pump inserted into uterus on GD 7 through GD 22	TCE: 15 ppm LOAEL PCE: 1.5 ppm LOAEL	CCD observed in 3% controls, 9% 15ppm TCE, 14% 1,500 ppm TCE, 12% in 0.15 ppm DCE, and 21% in 150 ppm DCE groups 1500 ppm TCE is above limit of solubility Statistical significance based only on a per-fetus analysis, no significant increase in CCD when analyzed on a per-litter basis.

Reference	Route	Number Animals	Dose and Duration	NOAEL or LOAEL	Cardiac Effect(s) Reported and Comments
Oral Studies with TCE					
NTP 1985	gavage	Swiss CD-1 mice 20/group	100, 300, or 700 mg/kg/day TCE Throughout pregnancy	Developmental NOAEL: 700 mg/kg/day	No CCD observed
NTP 1986	gavage	Fisher 344 rats 20/group	76, 156, or 289 mg/kg/day TCE Throughout pregnancy	Developmental NOAEL: 289 mg/kg/day	No CCD observed
Dawson et al. 1993	Drinking Water	Sprague-Dawley rats 116 females in 11 groups	1.5 and 1,100 ppm TCE (0.218, or 129 mg/kg-d) 2 months before mating and/or during gestation	TCE: 1.5 ppm LOAEL	Statistically significant increase in CCD, primarily atrial septal defects, at both dose levels in groups exposed prior to pregnancy and during pregnancy, and in groups exposed to 1,100 ppm dose during pregnancy only. Statistical significance based only on a per-fetus analysis, no significant increase in CCD when analyzed on a per-litter basis. Fresh dissection technique used No significant increase in CCD in groups exposed prior to pregnancy only.
Johnson et al. 2003	Drinking Water	Sprague-Dawley rats TCE groups: 9–13female dams per group Controls: 55 dams	0, 2.5 ppb, 250 ppb, 1.5 ppm, or 1,100 ppm TCE (0, 0.00045, 0.048, 0.218, or 129 mg/kg-d) GD 0–22	TCE: 2.5 ppb NOAEL 250 ppb LOAEL	Statistically significant increase in percentage of abnormal hearts and the percentage of litters with abnormal hearts at ≥250 ppb Statistical significance is based only on a per-fetus analysis, none of the groups exhibited a statistically significant increase in CCD when analyzed on a per-litter basis Fresh dissection technique used

Reference	Route	Number Animals	Dose and Duration	NOAEL or LOAEL	Cardiac Effect(s) Reported and Comments
TCE Metabolite Studies					
Johnson et al. 1998	Drinking Water	Sprague-Dawley rats 138 females	**TCE Metabolite Study** Trichloracetic acid (TCAA), TCE, DCE, MCAA, TCEth, TCAld, DCAld, CMC, DCVC Equivalent expected if 1,100 ppm TCE broke down completely into that metabolite range: 0.15-2,730 ppm GD 1-22	TCE: 1.5 ppm LOAEL	Significantly increased incidences of fetuses with cardiac defects on a per fetus and per litter basis in TCAA group (2,730 ppm). Significant increases in fetuses with cardiac malformations observed with 1.5 or 1,100 ppm TCE or with 0.15 or 110 ppm DCE, only with pre-pregnancy plus during pregnancy treatment regimens.
Smith et al 1989	Gavage	Long-Evan rats 20-26/group	**Metabolite Study** 0, 330, 800, 1,200, or 1,800 mg/kg/day TCA GD 6-15	TCA: 330 mg/kg-day LOAEL	Statistically significant CCD in litters at 330-1800 mg/kg/day on GD 6-15. CCD included levocardia and ventricular septal defect
Smith et al. 1992	Gavage	Long-Evan rats 19-21/group	**Metabolite Study** 0, 14, 140, 400, 900, 1400, 1900, 2400 mg/kg/day DCA GD 6-15	DCA: 330 mg/kg-day LOAEL	Statistically significant CCD at 140-2,400 mg/kg/day DCA on GD 6-15 CCD included Levocardia, VSD, interventricular septal defect, and defects found between the base of the ascending aorta and right ventricle.
Epstein et al. 1992	Gavage	Long-Evans rats 4 studies: groups of 6-10 rats	**Metabolite Study** Single dose DCA – 1,900 2,400, or 3,500 mg/kg-day Treatment during various GDs to determine critical window: GD 6-8, 9-11, 12-15	DCA: 1,900 mg/kg-day LOAEL	Statistically significant CCDs at 900 mg/kg on GD 9–11, increased on GD 12–15; 2400 mg/kg, but not 3500 mg/kg of DCA led to an increase in CCDs on GD 10 and 12. No dose response CCD included interventricular defects

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