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Recommendations for Risk Management Under the MCP for Trichloroethene Exposures Based on Updated Toxicological Information

LSP Association, Inc.
Technical Practices Committee

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Executive Summary

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With significant contributions from
Amy Roth, PG, LSP (Wilcox and Barton, Inc.), and Jane Parkin Kullmann, PhD, DABT (WSP)

The management of health risks for trichloroethene (TCE) under the Massachusetts Contingency Plan (MCP) is typically based on non-carcinogenic effects, including developmental effects and effects on the kidneys and immune systems. The developmental effects endpoint concerns congenital heart defects (CHDs) and is principally based on a 2003 toxicological study by Johnson *et al.* which has been recognized as having a high level of uncertainty associated with its lack of scientific rigor. The purpose of this paper is to review the weight of evidence (WOE) for the association of CHDs with TCE, incorporating contemporary toxicological information. Based on this review, the LSP Association recommends that the Massachusetts Department of Environmental Protection (MassDEP) reevaluate the WOE for TCE-induced CHDs and revise its risk management framework for TCE to be based on the chronic immunological endpoint, rather than the developmental effects endpoint.

Inhalation toxicity studies that have evaluated the hypothesis that TCE can cause CHDs have not identified statistically significant associations between TCE exposure and CHDs, and the body of epidemiological evidence supporting an association between TCE exposure and CHDs is weak. In addition, all but two studies that have evaluated the teratogenicity of TCE have concluded that oral exposures to TCE are not associated with an increased risk of CHDs.

The studies that have identified an association between oral exposure to TCE and increased risk of CHDs were published by Johnson *et al.* (2003; "the Johnson study"). Despite numerous inhalation studies and other oral studies demonstrating that exposure to TCE does not induce CHDs, the United States Environmental Protection Agency (EPA), in an abundance of conservatism, relied on the findings of the Johnson study to conclude that oral and inhalation exposures to TCE are associated with increased risk of CHDs.

Because CHDs develop during a specific and very short period of time during human pregnancy (3rd to 8th week of gestation), EPA has identified women who are or could become pregnant as a sensitive subgroup and uses an acute exposure period to evaluate potential risks. MassDEP established short-term exposure limits for TCE using a Reference Concentration (RfC) and assumptions that were essentially intended to represent 'no risk' of CHDs. Importantly, a

Hazard Index of 1 (rather than 10) was applied for evaluating Imminent Hazards to women of childbearing age because developmental effects are considered by MassDEP to be 'serious health effects' that warrant Imminent Hazard risk management using the order-of-magnitude lower Hazard Index. In the case of indoor air, these agency-driven conservative action levels have resulted in the evacuation of homes, creating significant disruption and alarm among building owners and occupants.

In 2019, a toxicological study was published by DeSesso *et al.* which further evaluated the hypothesis advanced in the Johnson *et al.* (2003) study that TCE exposure is linked to CHDs, while at the same time correcting certain technical deficiencies of the Johnson study. DeSesso *et al.* demonstrated that, with a properly designed study, it could be shown that exposures to TCE via the drinking water pathway were not associated with developmental effects. In other words, more current and more rigorous scientific studies do not support the use of developmental effects as an endpoint for TCE, thus further discounting the validity of using the 2003 Johnson study as the basis for TCE risk management decisions.

In 2020, EPA conducted a WOE analysis under the Toxic Substances Control Act (TSCA) that included both the 2003 and 2019 studies and concluded that developmental effects should not be applied as a risk management endpoint for TCE. However, the EPA's Integrated Risk Information System (IRIS), as well as the most recent Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for TCE, have not yet re-evaluated the weight of evidence for TCE-induced developmental effects using the DeSesso *et al.* (2019) study. As a result, the TCE toxicity values and endpoints included in the IRIS database, which MassDEP uses to establish risk management criteria, are still based on a 2011 evaluation of the data that were available at that time, which relied heavily on Johnson *et al*.

This paper reviews the current WOE evaluation for TCE-induced CHDs using available peer-reviewed studies. Key takeaway points are:

- Epidemiological Data: These data provide a weak line of evidence supporting the association of TCE and CHDs, primarily because none of the studies have been able to determine whether exposure to TCE actually occurred or to what levels, particularly during the first trimester, which is the period of concern for CHDs. The studies also did not take into account the levels of exposure to other contaminants which may have confounded the results, and were not controlled for conditions that are known to have an association with developmental effects.
- Animal Bioassay Data: All but two out of seven animal studies involving oral exposure (Johnson *et al.* [2003] and a previous study that Johnson relied on) show no statistically significant increases in CHD with TCE exposure. All 12 animal studies involving inhalation exposure show no statistically significant increases in CHD with TCE exposure. As noted above, the Johnson study had substantial deficiencies that were addressed in a follow-up study by DeSesso *et al.* which was designed specifically to evaluate the hypothesis of TCE-induced CHDs that was advanced in the Johnson study, and which did not identify any statistically significant association between TCE exposure and CHDs. As recommended by the TSCA Science Advisory Committee (SAC), the Johnson study should not be relied upon by USEPA due to its lack of credibility; rather, EPA

should give more weight to the animal inhalation bioassays since those are more relevant to inhalation risk assessments. The SAC also noted that those studies do not support induction of cardiac defects by TCE.

- Mechanistic Data: Data indicate that metabolites of TCE (trichloroacetic acid) may cause CHDs. However, the data also indicate that the TCE exposure concentrations required to elicit such responses would be acutely toxic or exceed the solubility limit of TCE in water. Furthermore, these metabolites do not form following exposure to TCE in drinking water at concentrations below 500 milligrams per liter (mg/L), casting further uncertainty on the results reported in the Johnson study that a TCE concentration of 0.25 mg/L in drinking water was associated with TCE-induced CHDs.

Based on our evaluation of the updated scientific evidence referenced in this paper, we concur with the findings of EPA TSCA, the TSCA SAC, and others that the association of TCE exposure with CHDs is not scientifically supported. As a result, we recommend that MassDEP reevaluate the WOE for TCE-induced CHDs and revise its risk management framework for TCE to exclude the developmental endpoint as a basis for risk management decisions. In the absence of the developmental endpoint, risks for TCE would be managed using the chronic immunological endpoint, as found in the EPA IRIS profile for TCE. Basing risk management on the chronic immunological endpoint (rather than the acute developmental endpoint) would have the following implications for the regulation of TCE in Massachusetts:

- The RfC for TCE would not differ from those currently in use and presented within the IRIS database. The RfCs would be the same for the developmental and chronic immunological endpoints, but would be implemented differently based on the acute nature of CHDs.
- Imminent Hazard thresholds would be identified based on TCE exposures associated with a Hazard Index of 10, rather than 1, consistent with other oil and/or hazardous materials that are not associated with 'serious health effects' (310 CMR 40.0955(2)), because the risks would no longer be based on developmental effects.
- More Disposal Sites with an Active Exposure Pathway Mitigation Measure (AEPMM) would qualify for a Permanent Solution because low concentrations of TCE (e.g., 6 ug/m^3) would no longer be associated with an Imminent Hazard.
- Response actions to address Imminent Hazards for TCE would not differ from those applicable to other OHM (e.g., immediate removal from exposure would not be required).

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Health risks for trichloroethene (TCE) are managed under the Massachusetts Contingency Plan (MCP) in consideration of both carcinogenic and non-carcinogenic health effects. However, risk management for TCE is often based on non-carcinogenic effects because exposures that correspond to the MCP cumulative receptor non-cancer risk limit of a hazard index of 1 are lower than those that correspond to the cumulative receptor cancer risk limit of $1x10^{-5}$.

The non-carcinogenic health effect endpoints for TCE currently include developmental effects and effects on the immune system and kidneys. The developmental effects endpoint is principally based on a 2003 toxicological study which has been recognized as having a high level of uncertainty associated with its lack of scientific rigor. In 2019, a toxicological study was published that sought to evaluate the association of TCE and developmental effects using the same dosing regime and study design as the 2003 study, but with methodologies that reduced the uncertainties associated with the 2003 study. The 2019 study demonstrated that exposures to TCE were not associated with developmental effects, specifically congenital heart defects (CHDs).

Every five to ten years TCE periodically undergoes risk evaluation under the Toxic Substances Control Act (TSCA). In November 2020, the United States Environmental Protection Agency (EPA) released a final *Risk Evaluation for* T*richloroethene* (EPA Document #740R18008, 2020a), which considered the 2019 toxicological study for TCE, developed a weight-of-evidence (WOE) analysis for the association of TCE with developmental effects, and concluded that developmental effects should not be applied as a risk management endpoint for TCE. This conclusion was maintained in the final revision to the risk determination (EPA, 2022).

The conclusion that developmental effects should not be applied as a risk management endpoint does not change the toxicity values, risk-based values, or MCP Method 1 standards for TCE, but it does have implications for the risk management policies for TCE that are presently in place in Massachusetts, specifically as they pertain to Imminent Hazard decisions. The purpose of this paper is to review the WOE for the association of TCE with developmental effects and provide recommendations for changes to the Massachusetts Department of Environmental Protection (MassDEP) risk management policies for TCE. Based on our evaluation of the multiple lines of evidence reviewed as part of this paper, we concur with the findings of EPA TSCA, the TSCA SAC, and others that the association of TCE exposure with congenital heart defects is not scientifically supported. We therefore believe that risk management policies for TCE, which are presently based on developmental effects, should be changed.

Current State of TCE Risk Management

TCE is often identified in site characterization studies due to its long history of industrial uses and persistence in environmental media.

Selection of the Reference Concentration for TCE Inhalation

In 2011, the EPA released a Toxicological Review for TCE to support the development of toxicity values for use in health risk characterizations (EPA, 2011) in its Integrated Risk Information System (IRIS) database. Prior to 2011, EPA had last updated TCE toxicity values in 1989. In the 2011 reassessment, updated toxicity values for TCE were developed for both cancer and non-cancer health effects, and for both oral and inhalation exposure routes[1]. EPA also characterized TCE as a mutagen and as being "Carcinogenic to Humans." Risk management policies in Massachusetts are based on the toxicity values provided in the 2011 IRIS database.

In adopting the reference concentration (RfC) presented in the IRIS database for the inhalation route of exposure, EPA considered adverse non-cancer health effects observed in animal studies, including immunological effects, developmental effects, and kidney effects. Candidate RfCs based on these effects are summarized in Table 1, along with risk-based screening levels protective of these effects from potential long-term exposures via inhalation. The risk-based screening levels are based on a target non-cancer hazard index (HI) of 1.

Table 1: Summary of TCE Non-Cancer RfCs and Risk-based Screening Levels

TCE Non-cancer Toxicity Endpoint (EPA, 2011)	***Candidate RfC (micrograms per cubic meter [µg/m³] (EPA, 2011)***	***Risk-Based Screening Level (non-cancer) (µg/m³)***	
		Residential	**Commercial/Industrial**
Immune system (HI = 1)	2	**2.1**	**8.8**
Developmental (HI =1)	2	**2.1**	**8.8**
Kidney (HI = 1)	3	**3.0**	**12**
µg/m³ = micrograms per cubic meter			

Risk-based screening levels protective of potential inhalation exposures at a target cancer risk of one in one-hundred thousand (1×10^{-5}) correspond to 4.8 µg/m³ for residential exposures and 30 µg/m³ for commercial/industrial exposures. These values are higher than the corresponding non-cancer screening levels shown above. As a result, risk management decisions concerning the mitigation of potential long-term inhalation exposures to TCE are typically based on non-cancer health effects.

Risk Management for TCE Based on Developmental Effects

As indicated in Table 1, the lowest candidate RfC for TCE, 2 µg/m³ (EPA, 2011), is based, in part, on a developmental effects endpoint. Although the candidate RfC for immune system effects is equal to that for developmental effects, the ways in which risk is managed for these two endpoints differ. As

[1] The updated values, including a cancer slope factor (SFo) and inhalation unit risk (IUR) for cancer risk, and reference dose (RfD) and reference concentration (RfC) for non-cancer hazards, are published in the IRIS database (EPA, 2021).

explained in this paper, risks based on potential effects to the immune system are managed as chronic exposures, whereas risks based on potential developmental effects are managed as short-term (acute) exposures and result in the identification of Imminent Hazards at relatively low indoor air TCE concentrations. As a result, careful consideration of the validity of the RfC for developmental effects is warranted.

The RfC for developmental effects is derived from the results of a study by Johnson *et al.* (2003) in which CHDs observed in the offspring of rats following oral (TCE in drinking water) exposure during the 21-day rodent gestational period were deemed a critical effect. To derive the RfC for protection against CHDs, EPA used modeling to extrapolate the TCE dose in laboratory rats to a TCE dose in humans that is believed to induce the same magnitude of toxic effect as the experimental animal species concentration (a value termed the human equivalent concentration [HEC]). EPA then modeled the HEC to be protective for 99% of the population having a 1% increased risk of CHDs ($HEC_{99,BMDL01}$), and then added a 10-fold uncertainty factor to the $HEC_{99,BMDL01}$ to obtain an RfC of 2 ug/m^3. The RfC is therefore derived using an abundance of conservatism, resulting in a value that essentially represents "no risk" of CHDs.

In addition to adoption of the highly conservative RfC of 2 µg/m^3 for TCE, EPA's concern regarding the CHDs led to two risk management circumstances that were new and unique to TCE:

1) the identification of women of child-bearing age as a potentially sensitive and at-risk sub-population; and
2) the concept that CHDs could occur during a "short period of exposure" and thus the health effect was managed as an acute exposure and the RfC was applied to the evaluation of acute exposures.

This in turn led to the development of *interim short-term exposure limits* for TCE in indoor air based on the potential for CHDs. MassDEP developed the response action levels for residential and commercial TCE inhalation exposure shown in Table 2 (MassDEP, 2014). The rationale for the development of short-term exposure levels for TCE for women of child-bearing age was predicated on the recognition that the human fetal heart is susceptible to teratogenic effects during a specific period within the first trimester (3rd to 8th week of pregnancy); hence, a short-term exposure to TCE could hypothetically induce potential CHDs, and an HI of 1 is applied as the Imminent Hazard threshold. Since women do not always recognize pregnancy prior to the 3rd week, all women who could potentially be or become pregnant are considered. For receptors other than women of child-bearing age, CHDs are not a relevant toxicity endpoint; therefore, Imminent Hazard thresholds can be based on immunological effects, for which an HI of 10 is applied.

Table 2: MassDEP Imminent Hazard Values for Various Receptors - Residential and Commercial TCE Inhalation Exposure

Exposure Scenario	***Women* Imminent Hazard Level (HI=1)[1]***	***Women* More Urgent Concern Level (HI=1)l[2]***	***All Receptors Imminent Hazard Level (HI=10)[3]***
Residential	**>6 µg/m^3**	**>20 µg/m^3**	**>20 µg/m^3**
Commercial/Industrial (8-hour workday)	**>24 µg/m^3**	**>60 µg/m^3**	**>80 µg/m^3**

* Women of child-bearing age (ages 15 to 44 according to US Center for Disease Control).

[1] Values developed by MassDEP using a hazard index (HI) of 1 but reducing the uncertainty factor in the RfC derivation by the square root of 10 (MassDEP, 2014). An HI of 1 is applicable because developmental effects are considered to be serious effects (310 CMR 40.0955(2)).

[2] Value of 20 ug/m^3 identified by MassDEP as the air concentration that would result in a dose of metabolized TCE in about 1% of people equivalent to that associated with a modeled 1% risk in the laboratory animal study used as the basis of the RfC. The commercial value is the residential value adjusted for 8 out of 24 hours of exposure (MassDEP, 2014).

[3] The developmental effects endpoint is not applicable to populations other than women of child-bearing age; therefore, an HI of 10 is applicable for effects to the immune system (310 CMR 40.0955(2)).

Based on the selection of a conservatively developed RfC that was based on developmental effects, the Imminent Hazard thresholds for women of child-bearing age are substantially lower than those for other receptors.

There are challenges associated with implementing response action thresholds for TCE that are based on concerns about CHD. Significantly, there are challenges with communicating risk management decisions to the public where a concentration of, for example, 2 µg/m^3 is considered safe for long-term exposure, but a concentration only slightly higher than 2 µg/m^3 is considered indicative of a need for immediate response actions. This can create substantial anxiety, particularly for pregnant women, because parental concerns about pregnancy and unborn children are emotionally sensitive topics; in fact, the anxiety in and of itself poses a mental health risk. This is an additional reason to thoroughly evaluate risk management policies for TCE.

Lines of Evidence to Evaluate the WOE for TCE-Induced CHDs

Three lines of evidence are available for evaluating the WOE for TCE-induced CHDs: epidemiological data, animal bioassay data, and mechanistic data. Each of these lines of evidence is summarized below.

Epidemiological Data

Numerous epidemiological studies have evaluated the association between TCE and developmental effects. Most of the studies have not identified any statistically significant association between TCE and CHDs (Agency for Toxic Substances and Disease Registry [ATSDR], 2019). Epidemiological studies that have been cited by EPA as a line of evidence supporting TCE-induced CHDs (EPA, 2011; EPA, 2014; EPA, 2020a) are summarized in Table 3. All of these studies have substantial limitations, which include one or more of the following:

1) no measured TCE concentrations at the point of exposure;
2) examination of solvents in general so that potential exposure to multiple chlorinated volatile organic compounds (CVOCs) was not accounted for (applies to the majority of the studies);
3) within the studies examining TCE specifically, the prevalence of CHDs is within the expected background range (i.e., no increased risk of CHDs); and
4) lack of controls for other variables that could contribute to CHDs (e.g., smoking, work-place exposures, alcohol, drug use, etc.).

In addition, all studies except the Goldberg *et al.* (1990) study examined exposure based on residency at the time of birth and none accounted for the mother's residency during the first trimester, which is the

exposure period that is relevant for TCE-induced CHDs. (Up to one-quarter of US women move residency during pregnancy [Reuters, 2019].).

A comprehensive evaluation of the existing epidemiological data for TCE was completed by Makris *et al.* (2016). The Makris evaluation defined several of the studies as either case-control studies (studies which compare a group of people who have a disease condition to a similar group of people who do not) or cohort studies (studies which follow participants over a period of time to determine how disease incidence differs). However, exposures to TCE were not directly measured in any of the studies, which leads to substantial uncertainty in being able to define whether the observed outcomes are truly associated with exposures to TCE. No studies that are cohort or case control (which are the epidemiological studies associated with the highest confidence defining an association between exposure and effect) with measured exposures to TCE have been conducted to evaluate an association between TCE exposure and CHDs.

A common way to evaluate the reliability of epidemiology studies is to apply Bradford Hill's Guidelines (Aschengrau *et al.,* 2008). Hill's Guidelines use nine viewpoints to evaluate epidemiological studies to assess whether causation can be deduced. The nine criteria are as follows:

- Strength: How strong is the association? Stronger associations are more likely to be causal.
- Consistency: Is the same effect observed in multiple settings for multiple populations?
- Specificity: Is the effect caused only by the subject exposure?
- Temporality: Is the observation of the effect after the exposure?
- Biological Gradient: Is there any apparent dose-response pattern in the study? Effects that demonstrate a dose-response are more likely to be causal.
- Plausibility: Is the effect biologically plausible given the mode of action of the toxicant?
- Coherence: Is the "cause and effect" interpretation of the study consistent with the biology of the disease?
- Experiment: Is there any experimental evidence that illustrates that removing the exposure will eliminate the effect?
- Analogy: Is there an epidemiologically or toxicologically analogous situation that supports the causal relationship for the subject exposure?

Table 3: Summary of Epidemiological Studies cited by EPA as Evidence of TCE-induced Congenital Heart Defects

Table 3 provides a summary of epidemiology studies, including those identified by Makris *et al.* (2016), and applies Hill's Guidelines to each of the epidemiological studies. Of the two studies that showed a positive association between exposure and CHDs (ATSDR [2006/2008]/Forand *et al.* [2012] and Goldberg *et al.* [1990]), strength is moderate because odds ratios (OR) were between 2 and 3 and specificity is low because TCE exposures could not be quantified on an individual level and numerous other potential causes of CHDs were not controlled for. Plausibility can be used to help determine if the strength of the effect is reasonably attributable to TCE exposure. Citing other studies, both the Forand and Goldberg studies discussed TCE as a potential cardiac teratogen, but acknowledged that the mechanism of action for TCE-induced CHDs was not well understood. Furthermore, odds ratios for CHDs were only significant

when all types of cardiac defects were combined. As discussed below in Mechanistic Studies, contemporary toxicological studies suggest that TCE exposures need to be higher than most environmental exposures before TCE can potentially cause CHDs.

The epidemiological information overall is a weak line of evidence supporting the association of TCE and CHDs, primarily because none of the studies can determine whether exposure to TCE actually occurred or to what levels. Notwithstanding that significant limitation, only two studies show a positive association. In weighing this evidence, Makris *et al.* (2016) concluded the available epidemiological studies "are not sufficient to establish a causal link between TCE exposure and CHDs in humans."

Animal Bioassay Data

Animal studies that have specifically examined the potential association of TCE and CHD (among other developmental effects) have used oral (drinking water), gavage, and inhalation routes of exposure. Table 4 provides a summary of the oral studies, and Table 5 provides a summary of the inhalation studies.

Table 4: Summary of Oral Toxicity Studies Examining TCE and CHD

Study	***Species (Strain)***	***Exposure levels/duration***	***Evidence of CHDs?***
Drinking Water			
Dawson et al. 1993	Rat (Sprague-Dawley)	0, 1.5, or 1100 ppm 2 months before mating and/or during gestation	Statistically significant increase in CHDs, primarily atrial septal defects LOAEL: 1.5 ppm (0.18 mg/kg-day)
Johnson et al. 2003	Rat (Sprague-Dawley)	0, 0.0025, 0.25, 1.5, or 1100 ppm GDs 0-22	Statistically significant increase in % of abnormal hearts and % of litters with abnormal hearts LOAEL: 0.25 ppm (0.048 mg/kg-day)
DeSesso et al. 2019	Rat (Sprague-Dawley)	0, 0.25, 1.5, 500, or 1000 ppm GDs 0-22	no CHDs reported in dose groups versus controls
Gavage			
Cosby and Dukelow 1992	Mouse (B6D2F1)	0, 24, or 240 mg/kg-day GDs 1-5, 6-10, or 11-15	no CHDs reported in dose groups versus controls
Fisher et al. 2001	Rat (Sprague-Dawley)	0 or 500 mg/kg-day GDs 6-15	no CHDs reported in dose groups versus controls
Narotsky et al. 1995	Rat (F344)	0, 10.1, 32, 101, 320, 475, 633, 844, or 1125 mg/kg-day GDs 6-15	no CHDs reported in dose groups versus controls
Narotsky and Kavlock 1995	Rat (F344)	0, 1125, or 1500 mg/kg-day GDs 6-19	no CHDs reported in dose groups versus controls

GD = Gestational Day
LOAEL = Lowest Adverse Effect Level
ppm = parts per million
mg/kg-day = milligrams per kilogram per day

Table 5: Summary of Inhalation Toxicity Studies Examining TCE and CHD

Study	*Species (Strain)*	*Exposure levels/duration*	*Evidence of CHDs?*
Carney et al. 2006	Rat (Sprague-Dawley)	0, 50, 150, or 600 ppm 6 hr/day GDs 6-20	no CHDs reported in dose groups versus controls
Dorfmueller et al. 1979	Rat (Long-Evans)	0 or 1800 ppm 2 wks, 6 hr/day, 5 day/wk prior to mating and/or on GDs 0-20	no CHDs reported in dose groups versus controls
Hardin et al. 1981	Rat (Sprague-Dawley)	0 or 500 ppm 6-7 hr/day GDs 1-19	no CHDs reported in dose groups versus controls
Hardin et al. 1981	Rabbit (New Zealand white)	0 or 500 ppm 6-7 hr/day GDs 1-24	no CHDs reported in dose groups versus controls
Healy et al. 1982	Rat (Wistar)	0 or 100 ppm 4 hr/day GDs 8-21	no CHDs reported in dose groups versus controls
Schwetz et al. 1975	Rat (Sprague-Dawley)	0 or 300 ppm 7 hr/day GDs 6-15	no CHDs reported in dose groups versus controls
Schwetz et al. 1975	Mouse (Swiss-Webster)	0 or 300 ppm 7 hr/day GDs 6-15	no CHDs reported in dose groups versus controls
GD = Gestational Day ppm = parts per million			

Key takeaway points from Tables 4 and 5 are as follows:

- Twelve studies, including all of the inhalation studies, did not identify TCE-induced CHDs.
- The combined studies of Dawson *et al.* (1993) and Johnson *et al.* (2003) identified evidence of TCE-induced CHDs; these are evaluated further below.
- The DeSesso *et al.* (2019) study, which was designed specifically to evaluate the hypothesis advanced in the Johnson *et al.* (2003) study, did not identify any statistically significant association of TCE exposure and CHDs.

<u>Johnson versus DeSesso Studies</u>

The Johnson *et al.* (2003) study was performed by administering TCE in drinking water to pregnant rats at levels of 0, 0.0025, and 0.25 parts per million (ppm). The results from these dose groups were combined with results from a prior study (Dawson *et al.,* 1993), in which TCE was administered in drinking water to pregnant rats at dose levels of 0, 1.5 and 1,100 ppm, to construct a dose-response relationship for TCE-induced CHDs. The combined study, published as Johnson *et al.* (2003), has numerous limitations, including the following:

1) the use of a non-standardized dissection method whereupon identification of CHDs was determined by consensus among the investigators and the investigators were not blinded to the dose groups (it is noted that Johnson was a blinded member of the Fisher *et al.* [2001] study, listed in Table 4, which used the same non-standardized dissection techniques and did not identify a statistically significant increase in CHDs);

2) the lack of a positive control by which to evaluate the sensitivity of the non-standardized dissection/ identification method;

3) no testing for TCE concentrations in the drinking water during the performance of the bioassay to confirm the administered dose levels;

4) the pooling of nonconcurrent control data, the large number of control groups (55), and the relatively low number of dams (mothers) in treated groups (9 to 13), which may have exaggerated the statistical significance of the critical effect[2];

5) the lack of a clear, meaningful dose-response relationship for the CHDs; and

6) the omission of raw data, which were unavailable for either regulatory or public review.

With respect to limitation number five (above), when compared against pooled controls, there was only a 2-fold difference in response rate between the 0.25 ppm dose group and 1,100 ppm dose group, despite a 4,400-fold difference in exposure. CHDs were not statistically increased in the 1.5 ppm dose group, despite significant increases in the 0.25 ppm and 1,100 ppm dose groups, as shown in Table 6.

Table 6: Summary of CHDs by Quantity Observed in Each Group Reported by Johnson *et al.* (2003)

TCE (ppm)	*No. dams/ No. fetuses*	*Atrial septal defect (ASD)*	*Ventricular septal defect (VSD)*	*Malformed valves*	*Other heart defects*	*Total*	*Percent Incidence (anomalies / total fetuses)*
0	55 / 606	7	4	0	3	13	2.1%
0.025	12/ 144	0	0	0	0	0	0%
0.25	10 / 110	1	0	3	1	5*	4.5%
1.5	13 / 181	4	3	0	2	9	5.0%
1,100	9 / 105	7	5	2	0	11*	10.5%
* Statistically significant compared to control ($p < 0.05$)							

The statistics performed by Johnson *et al.* (2003) were based on total cardiovascular anomalies; when considering any single type of anomaly (e.g., atrial septal defects [ASD], ventricular septal defects [VSD]), no statistically significant findings were identified. The findings of atrial septal defects and valve aberrations were not observed in other studies that examined TCE exposure and CHDs. One possible reason for this is that Johnson *et al.* (2003) used a fixative to preserve heart tissue, but the fixative is known to make tissues friable. It is also possible that the non-standardized dissection technique, combined with the use of the fixative, resulted in some of the tissue aberrations that were not identified in other studies (DeSesso *et al.,* 2019).

The DeSesso *et al.* (2019) study was designed specifically to evaluate the hypothesis advanced in the Johnson *et al.* (2003) study that TCE exposure is linked to CHDs, while at the same time correcting the deficiencies of the Johnson *et al.* (2003) study. The methods used by DeSesso to address the deficiencies of the Johnson study included the following:

[2] The pooled control group consisted of 606 fetuses, which exceeded the number of fetuses from each of the four dose groups (144 fetuses in the 0.0025 parts per million [ppm] dose group, 110 fetuses in the 0.25 ppm dose group, 181 fetuses in the 1.5 ppm dose group, and 105 fetuses in the 1,100-ppm dose group) by 3.3 to 5.8 times (Johnson *et al.,* 2003).

- following a robust design with balanced numbers of animals in each group with an adequate number of pregnant dams in each treatment group per study guidelines;
- performing the study during a single, defined time period, and obtaining animals from a single source at the same time (i.e., addressing the lack of concurrent control data in Johnson *et al.* [2003]);
- ensuring that all the TCE used was from the same lot;
- following the standard EPA-approved method for evaluating fetal hearts;
- recording maternal clinical data, as well as ovarian and uterine parameters;
- including a positive control group; and
- obtaining contemporaneous steady state blood level data, in addition to measuring TCE concentrations in water formulations throughout the gestation treatment period (i.e., addressing the lack of analytical results confirming TCE dosing concentrations in Johnson *et al.* [2003]).

CHDs identified by DeSesso *et al.* (2019) in TCE-exposed fetuses were limited to a single type (VSDs), and their numbers were not significantly different from their occurrence in control groups or from the VSDs reported in the Johnson *et al*. (2003) study. Furthermore, the incidence values for all dose groups were reported as being within the range of spontaneous background occurrences for rats reported in the published literature, suggesting that VSDs observed in both the DeSesso *et al.* (2019) and Johnson *et al.* (2003) studies were a reflection of the background incidence of VSDs. Moreover, while VSDs are the most common developmental heart defect in humans, accounting for 39% of CHDs in human infants, the vast majority (85% to 90%) of the VSDs spontaneously close during the first year of life, including nearly all small VSDs. This raises the question of whether small VSDs are a clinically relevant adverse effect. The VSDs identified in the DeSesso *et al.* (2019) study were small, consistent with the type that spontaneously close, providing further evidence that they are consistent with the background incidence of VSDs.

Mechanistic Data

Mechanistic studies include evaluations of cardiac structure and function in chick and rodent embryos and mode-of-action or key event data focused on processes and pathways that contribute to observed effects. It is hypothesized that metabolites of TCE, specifically trichloroacetic acid (TCA) and/or dichloroacetic acid (DCA), may affect cellular processes involved in septal and valvular development.

Studies involving cell culture and chick embryo exposures to TCE, TCA, or DCA have demonstrated a positive association between exposure and cellular changes that could lead to CHDs. However, the TCE exposure concentrations required to produce the observed effects are orders of magnitude higher than environmentally relevant concentrations. For example, most cell culture studies that showed positive associations as reported in ATSDR (2019) use TCE exposures that would be require TCE concentrations in drinking water of more than 3,500 milligrams per liter (mg/L), which is higher than the water solubility limit for TCE. Similarly, animal studies involving the administration of TCA to rodents which found increasing CHDs with increasing doses identified lowest observable adverse effect levels (LOAELs) of 300 milligrams per kilogram per day (mg/kg-day) and higher, which translate to TCE concentrations in water of 1,500 mg/L and higher (ATSDR, 2019).

Mechanistic studies of TCE-induced CHDs were critically evaluated by Urban *et al.* (2020), who applied National Toxicology Program guidance for conducting systematic reviews to 75 in vivo and in vitro studies. Urban concluded that "the database generally shows a lack of support for an association between in utero exposure to TCE and CHDs in humans. The very limited and inconsistent evidence that is supportive of an association is generally limited to data from chickens and may reflect species difference and/or sensitivities in the in ovo model involving direct injection. When considering the consistent findings demonstrating a lack of a relationship between TCE in utero exposures and CHDs across most mammalian models, combined with the lack of generalizability of the experiments in chickens (e.g., exposure scenarios, biological differences with humans, etc.), the mechanistic evidence stream is considered to be consistent with the human and animal evidence streams, which collectively demonstrate a lack of association between in utero exposures to TCE and CHDs." Urban *et al.* (2020) also noted that the in ovo model lacks several of the protective mechanisms that exist in the mammalian fetus in utero, including maternal metabolism, excretion and the placental barrier. In addition, Urban *et al.* (2020) noted that the doses of TCE required to elicit responses on the in ovo model translate to doses that would exceed the LD_{50} of TCE in animal models.

The DeSesso *et al.* (2019) study also evaluated TCE and TCA in maternal blood. The study found that at all dose levels, TCE was non-detect in maternal blood, and TCA was only detected in maternal blood at the 500 ppm and 1,000 ppm dose levels. By comparison, TCE and TCA concentrations measured in maternal blood in inhalation studies were two to ten times higher than the levels measured after drinking water exposure in the DeSesso *et al.* (2019) study. These findings are plausible given the first-pass hepatic metabolism of TCE that occurs following oral exposure, as opposed to inhalation exposure. There are two significant conclusions that can be drawn from these findings:

1) Inhalation exposure studies should be more sensitive to TCE-induced CHD than the drinking water studies. However, even with the detection of TCE and TCA in maternal blood following inhalation exposures at higher concentrations than those measured in maternal blood following drinking water exposure, inhalation toxicity studies did not identify a statistically significant relationship between TCE exposure and CHDs.

2) It is unlikely that TCA would have been present in maternal blood at the 0.25 ppm and 1.5 ppm TCE drinking water concentrations evaluated in the Johnson *et al.* (2003) study.

Given the findings of the inhalation toxicity studies, which should be the most sensitive to TCE-induced CHDs, and the lack of TCA in maternal blood at exposures below 500 ppm TCE in drinking water, it can be concluded that TCE is not a dosimetrically plausible teratogen at the TCE concentrations used in the drinking water studies performed by Johnson *et al.* (2003) and DeSesso *et al.* (2019). This increases the uncertainty of whether the CHDs observed in the Johnson *et al.* (2003) study were actually attributable to TCE exposures.

<u>Weight of Evidence Evaluation Summary for TCE-Induced CHDs</u>

The overall weight of evidence for TCE-induced CHDs indicates the following:

- Epidemiological studies are generally negative or equivocal, with only two studies showing a positive association. Using Hill's Guidelines, the two studies with positive associations exhibit moderate strength but low specificity because neither of the studies could determine if people were actually exposed to TCE, what concentrations people were exposed to, or whether mothers of babies with CHDs were living within the 'exposure area' during the first trimester.

- None of the animal studies involving inhalation exposure, and only two animal studies involving oral exposure, show statistically significant increases in CHD with TCE exposure. The principal study that shows a statistically significant increase in CHD with TCE exposure (Johnson *et al.* [2003]) has substantial deficiencies that were addressed in a follow-up study (DeSesso *et al.* [2019]), which demonstrated no statistically significant increase in CHDs with TCE exposure.
- Metabolites of TCE (e.g., TCA) may cause CHDs. However, the TCE exposure concentrations required to elicit such responses are not environmentally relevant (i.e., the concentrations would be acutely toxic or exceed the solubility limit of TCE in water). Furthermore, it has been demonstrated that TCA concentrations in maternal blood are higher following inhalation exposures than following oral exposures to TCE, yet inhalation studies have shown no statistically significant TCE-related increases in CHDs. Therefore, it is not dosimetrically plausible for metabolites of TCE to cause CHDs following the lower (less than 500 mg/L) oral doses of TCE that were administered in the Johnson *et al.* (2003) and DeSesso *et al.* (2019) studies. Furthermore, the dosimetry information indicates that it is unlikely that the populations which may have been exposed to environmentally relevant doses of TCE (i.e., considerably lower than those administered in the toxicological studies) in the epidemiology studies would have increased CHDs as a result of TCE exposures.

Contemporary Assessment of TCE by EPA TSCA and Other States

When EPA was developing the Draft TSCA Risk Evaluation (EPA, 2020b), the TSCA Science Advisory Committee (SAC) provided comments and recommendations (EPA, 2020c) before it became final. The SAC commenters collectively identified the following lines of evidence that TCE risks should not be managed using TCE-induced CHDs:

- With the exception of the flawed Johnson *et al.* (2003) study, the results of all other animal studies demonstrate a lack of association between TCE exposure and CHDs, including the study by DeSesso *et al.* (2019) which was specifically designed to evaluate the findings of Johnson *et al.* (2003) after addressing its deficiencies. The DeSesso study followed validated laboratory practice methods for dissection and evaluation in contrast to the unvalidated dissection and evaluation methods used by Johnson. The SAC also suggested that the Johnson study lacked credibility and should not be relied upon by EPA, and that EPA give more weight to the animal inhalation bioassays since those are more relevant to inhalation risk assessments. The animal inhalation studies do not support the induction of cardiac defects by TCE.
- Available TCE metabolite studies (with DCA or TCA), which the EPA concludes provide the strongest and most consistent lines of evidence in support of the TCE-CHD relationship, do not in fact support the association between TCE exposure and CHDs. The DCA/TCA dose levels in those studies in which CHDs were reported were so high that extrapolating to equivalent TCE administered doses results in dose levels that are known to be lethal in rats. This also likely explains why the TCE metabolite studies are inconsistent with the in vivo animal studies that fail to demonstrate a TCE-CHD relationship. The SAC noted that these studies have limited relevance to assessing environmental risks of TCE exposures and that they should not be given a high weight. Review of mechanistic studies performed by Urban *et al.* (2020) supports these conclusions that the chicken in ovo model, which is the only model that identifies an association between TCE and CHDs, is not relevant to human exposures.
- Evidence supporting EPA's conclusion that TCE "at sufficient doses" has the potential to cause CHDs in humans is at odds with toxicokinetic data on TCE in rats. As demonstrated in studies

with rats, exposure by drinking water does not achieve systemic doses that are comparable with inhalation or oral gavage, as evidenced by considerable differences in blood or plasma levels of TCE and TCA, respectively. Given that TCE developmental studies by oral gavage (Fisher *et al.,* [2001]) and inhalation (Carney *et al.,* [2006]) routes fail to show an increase in CHDs, even at systemic doses that are considerably higher than can be achieved by the drinking water route, the findings of Johnson *et al.* (2003) do not appear to exhibit a biologically plausible effect.

- Available human epidemiological data do not provide a reliable line of evidence for the association between TCE exposure and CHDs. There is a high risk of bias associated with exposure characterization and confounding factors, as well as inconsistent results in the available epidemiological evidence stream. The SAC further noted that the epidemiological studies relied on most heavily by EPA as supporting a link between TCE exposure and CHDs had not accounted for whether exposures to TCE had occurred during the window of critical cardiac development.

The TSCA Risk Evaluation was initially finalized in November 2020 (EPA, 2020a), and the risk determination component of the document was revised again and finalized in 2022 (EPA, 2022). In both versions of the Final TSCA risk evaluation, EPA re-evaluated the WOE for CHDs, accounting for "the conflicting results of previous WOE assessments." The Risk Evaluation concluded that while CHDs were associated with a lower point of departure (POD) for TCE, "there is lower confidence in the dose-response and extrapolation of results from those studies." EPA cited their charge under TSCA to use the best available science and weight of scientific evidence, and confidence in the POD. They noted that the framework for risk evaluation does not require the EPA to use the lowest number available, rather that public health is best served when EPA relies upon the highest quality information for which EPA has the highest confidence. Based on those considerations, the EPA relied upon the adverse immunological effects (specifically immunosuppression) for acute exposures, and on autoimmunity for chronic exposures, as the critical endpoints for determining whether or not a condition of use presented unreasonable risks. The TSCA Risk Evaluation therefore provides a position by EPA that downplays the significance of CHDs as a critical effect for TCE and suggests other health effects endpoints should be used to inform risk management decisions.

Consistent with the scientific process of consistently evaluating new and valid data, four states have already determined that CHDs should not necessarily be considered in the management of TCE.

- In 2016, the Indiana Department of Environmental Management (IDEM) concluded that "an accelerated response [for TCE] is not scientifically supportable based upon current information" and noted that application of accelerated response levels for TCE have been controversial and problematic as a policy (IDEM, 2016).
- In 2019, the Missouri Department of Natural Resources (MDNR) indicated that its revisions to Missouri's Risk Based Corrective Action screening tables would base values for TCE solely on chronic exposure because the agency believed that scientific support was lacking for the short-term exposure levels related to the Johnson *et al.* (2003) study (MDNR, 2019).
- In 2021, Georgia's Environmental Protection Division (EPD) published vapor intrusion guidance which states, "EPD recognizes the lack of regulatory and scientific consensus related to short-term inhalation exposure of pregnant women to elevated concentrations of trichloroethene (TCE) and the potential correlation with fetal cardiac development. EPD will evaluate the need for any accelerated response actions due to TCE on a site-specific basis" (GAEPD, 2021).

- Within the past two years, Ohio EPA withdrew its 2016 guidance document on recommendations for response action levels and timeframes for vapor intrusion sites. That guidance document had included specific response action levels and timeframes for TCE based on the developmental effects endpoint that forms the basis of the RfC for TCE.

Implications for Risk Management of TCE at MCP Sites

MassDEP's current approach to managing the risks associated with TCE at MCP sites is articulated in guidance issued in *US EPA Trichloroethylene Toxicity Values and Office of Research and Standards Recommendations Regarding Remediation Targets and Timeframes to Address Potential Developmental Risks* (MassDEP, 2014a). The risk management approach set forth in this document is based on scientific information that was current in 2014 and which centered on CHD as a critical effect; this evidence was evaluated by MassDEP in *Assessing the Congenital Cardiac Toxicity of Trichloroethylene: Key Scientific Issues* (MassDEP, 2014b). As stated in that evaluation, "the NAS recommended further low dose studies to replicate the effects observed in the critical (Johnson et al. [2003]) study. Until such studies are conducted, ORS concurs with US EPA that the current available weight of the scientific evidence on TCE-induced congenital cardiac toxicity is sufficient to warrant concern and the critical study is a reasonable basis for developing toxicity numbers."

The toxicological study published by DeSesso *et al.* (2019) is exactly the kind of high-quality study that MassDEP's Office of Research and Standards (ORS) cites as a reason to reconsider the weight of evidence for TCE-induced CHDs. As discussed above, that study evaluated the potential for TCE to induce CHDs following low-dose exposures, and clearly demonstrated that TCE exposure was not associated with a statistically significant increase in CHDs.

With respect to the body of human, animal, and mechanistic evidence, as described in this paper, the WOE for TCE-induced CHDs is weak and lacks support from epidemiological studies, animal bioassays, or mechanistic studies at environmentally relevant exposure levels. When the current toxicological study by DeSesso *et al.* (2019) was considered within the body of the WOE, EPA (TCSA) concluded that there was not support for using CHDs as a risk management endpoint for TCE. In addition to EPA, four states have determined that the management of TCE should not be based on concern about CHDs.

In consideration of the current and high-quality scientific evidence provided in this paper, we recommend that MassDEP consider the WOE for TCE-induced CHDs presented herein and revise its risk management framework for TCE to be based on the chronic immunological endpoint. This would result in the following:

- The RfC for TCE would not differ from those currently in use and presented within the IRIS database. The RfCs would be the same for the developmental and chronic immunological endpoints, but would be implemented differently based on the acute nature of CHDs.
- Imminent Hazard thresholds would be identified based on TCE exposures associated with a Hazard Index of 10, rather than 1, consistent with other oil and/or hazardous materials that are not associated with 'serious health effects' (310 CMR 40.0955(2)), because the risks would no longer be based on developmental effects.
- More Disposal Sites with an Active Exposure Pathway Mitigation Measure (AEPMM) would qualify for a Permanent Solution because low concentrations of TCE (e.g., 6 ug/m^3) would no longer be associated with an Imminent Hazard.

- Response actions to address Imminent Hazards for TCE would not differ from those applicable to other OHM (e.g., immediate removal from exposure would not be required).

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