Response to Public Comments Received on the Carbon Tetrachloride Draft Development Support Document

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Response to Comments

April 2, 2024

TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

Response to Public Comments Received on the October 13, 2023 Proposed Carbon Tetrachloride Development Support Document

The public comment period on the draft Development Support Document (DSD) for carbon tetrachloride ended January 12, 2024. The agency received comments on the draft DSD from the Halogenated Solvents Industry Alliance, Inc. (HSIA). The TCEQ appreciates the effort put forth to provide comments on the draft DSD for carbon tetrachloride. The goal of the TCEQ is to protect human health and welfare based on the most scientifically-defensible approaches possible (as documented in the DSD), and evaluation of these comments furthered that goal. Comments are provided below, followed by TCEQ responses.

Comments from the Halogenated Solvents Industry Alliance, Inc. (HSIA)

Comment 1: *I. Carbon tetrachloride (CCl_4) Sources and Uses.* The DSD notes that CCl_4 is produced from the thermal chlorination of methyl chloride; it should also be noted that CCl_4 is produced from ethylene dichloride and perchloroethylene[1].

For CCl_4 uses, Section 2.2 of the DSD cited the USEPA's February 2017 *Preliminary Information on Manufacturing, Processing, Distribution, Use and Disposal* for Carbon Tetrachloride. EPA updated this preliminary list of CCl_4 uses in Section 1.4.2 of the Final CCl_4 Risk Evaluation, "Subcategories Determined Not to Be Conditions of Use or Otherwise Excluded."[2] We would request that the DSD be revised to reflect this more recent summary of CCl_4 uses.

Response: TCEQ appreciates this comment. Although this type of information is secondary to the primary purpose of the DSD, which is to document the derivation of toxicity factors, additional uses cited in the USEPA 2017 and USEPA 2020 references have been added.

Comment 2: *II. Derivation of the Acute 1-h ReV. A. TCEQ's derivation of the proposed acute 1-h ReV using the ten Berge (1986)[3] equation is not scientifically sound and should be reevaluated to incorporate the empirical data from the Davis (1934) study.*

[1] Marshall KA, LH Pottenger. Chlorocarbons and Chlorohydrocarbons. Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley & Sons, Inc. 2016.

[2] Risk Evaluation for Carbon Tetrachloride (Methane, Tetrachloro-); EPA-740-R1-8014 (October 2020); EPA-HQ-OPPT-2019-0499-0061, p. 39-42.

[3] ten Berge WF, A Zwart, LM Appelman. 1986. Concentration-time mortality response relationship of irritant and systematically acting vapours and gases. J Hazard Materials 13:301-309.

The final DSD for CCl_4 should reevaluate the acute 1-h ReV to be consistent with the empirical data from the Davis (1934) study[4]. In the Davis study, there were no symptoms or signs of toxicity in human subjects exposed up to 2.5 hours (which includes the 1-h time point) to 76 ppm CCl_4. As described below, these data do not support a relationship between exposure concentration and exposure duration predicted from the ten Berge equation.

The proposed acute 1-h ReV was derived from a human study by Davis (1934) with eight experiments involving various controlled CCl_4 exposures and durations. The point-of-departure (POD) for the acute 1-h ReV was from one of the experiments which resulted in a Lowest-Observed-Adverse-Effect-Level (LOAEL) of 158 ppm CCl_4 for effects indicative of acute central nervous system (CNS) depression following a 30-min exposure. The proposed DSD then used the ten Berge equation with n = 1 to adjust the exposure duration from 30-min to 1-h, thus resulting in a POD_{HEC} of 79 ppm. The uncertainty factors (UF) that were applied to the POD_{HEC} included a UF_L of 3 to extrapolate the LOAEL to a No-Observed-Adverse-Effect-Level (NOAEL).

Significantly, in another experiment in the Davis (1934) study, four human subjects were exposed to 76 ppm CCl_4 for up to 2.5 hours. None of the human subjects showed any symptoms or signs of toxicity. This empirical finding of no symptoms at 76 ppm for 2.5 hours is at odds with the LOAEL value obtained with the time duration adjustment calculation of the CNS effects reported at 158 ppm CCl_4 for 30 minutes (in one of out of four human subjects) using the ten Berge equation. This incongruity is even more extreme when the UF_L of 3 is factored in. It should also be noted that Stewart (1961) reported no acute CNS effects in human volunteers exposed to a time-weighted-average (TWA) of 49 ppm CCl_4 (range 31 to 87 ppm) for 70 minutes.[5]

Response: TCEQ acknowledges this inconsistency. In the final DSD, TCEQ now includes the observed 2.5-h and 4-h NOAEL of 76 ppm as a candidate POD and derived the 1-h reference value (ReV) based on this POD. The LOAEL to NOAEL uncertainty factor (UF_L) of 3 was removed, and the interindividual or intraspecies human uncertainty factor (UF_H) of 10 and the incomplete database uncertainty factor (UF_D) of 6 were retained to derive a 1-h reference value (ReV) of 8,200 µg/m^3 (1,300 ppb) and a short-term effects screening level (ESL) of 2,400 µg/m^3 (390 ppb).

Comment 3: *II. Derivation of the Acute 1-h ReV. A (cont'd).* The ten Berge equation describes the exposure concentration and exposure duration relationship for lethality from 20 acute inhalation studies involving predominantly rats, but also mice, guinea pigs, rabbits, dogs, and a monkey. The 20 chemicals included both local irritants and systemically acting toxicants, including CCl_4. Importantly, the ten Berge equation was only for lethality and not for other health-related endpoints, such as the acute CNS effects reported in Davis (1934). In their

[4] Davis PA. 1934. Carbon tetrachloride as an industrial hazard. JAMA. 103:962-966.

[5] Stewart RD, HH Gay, DS Erley, CL Hake, JE Petersen. 1961. Human exposure to carbon tetrachloride vapor. relationship between expired air concentration to exposure and toxicity. J Occup Med. 3:586-590.

publication, ten Berge (1986) wrote that "a general rule concerning the value of the exponent *n* [in the equation] does not exist. The exponent should always be derived *empirically* from acute inhalation toxicity experiments, in which both the concentration and exposure period are variable [italics added]."

The CNS depression from CCl_4 exposure can occur from a mechanism that involves a direct effect of CCl_4 in the brain, with the effects proportional to the CCl_4 brain concentration; this relationship has been proposed for the CNS effects of industrial solvents in general[6]. Animal studies involving acute exposures of various hydrocarbon solvents have shown a plateau effect of solvent concentration in the rat brain of up to 8 hours of exposure[7], which suggests that the ten Berge equation is not valid for exposure duration adjustments for acute CNS effects from solvent exposure.

At least for single short-term exposures to CCl_4, the empirical data from Davis (1934) do not support a relationship between exposure concentration and exposure duration predicted from the ten Berge equation since there were no symptoms or signs of toxicity in human subjects exposed up to 2.5 hours (which includes the 1-hour time point) to 76 ppm CCl_4. Based on these findings, the NOAEL for acute CNS effects in humans after a 1-h exposure is at least 76 ppm CCl_4. The derivation of the acute 1-h ReV needs to be reconsidered to be consistent with the empirical data from the Davis (1934) study.

Response: The TCEQ is now using the 2.5- and 4-h NOAEL to derive the acute 1-h ReV, and has conservatively chosen not to apply a duration adjustment to 1-h. Therefore, the ten Berge equation is no longer used for the 1-h acute ReV.

Of note, in the ten Berge (1986) publication, a value of 2.8 was derived for the exponent n, and this is similar to the exponent of 3 that TCEQ used in the duration adjustment from 4 h to 1 h. As per the *TCEQ Guidelines to Develop Toxicity Factors* (2015)[8], acute exposure duration adjustments can be made by applying Haber's rule as modified by ten Berge (1986). The mode of action (MOA) for CCl_4-related effects on the central nervous system (CNS) is not well defined,

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[6] Shugaev B. 1969. Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18: 878-888.; Baker E, T Smith, P Landrigan. 1985. The neurotoxicology of industrial solvents. Am Ind Hyg Assoc. J. 8: 207-217.

[7] Hissink AM, J Krüse, BM Kulig, M Verwei, J Muijser, F Salmon, LH Leenheers, DE Owen, JHCM Lammers, AP Freidig, RH McKee. 2007. Model studies for evaluating the neurobehavioral effects of complex hydrocarbon solvents. III. PBPK modeling white spirit constituents as a tool for integrating animal and human test data. Neurotoxicol. 28: 751-760.; Hissink AM, BM Kulig, J Kruse, AP Freidig, M Verwei, H Muijser, JHCM Lammers, RH McKee, DE Owen, LM Sweeney, F Salmon. 2009. Physiologically based pharmacokinetic modeling of cyclohexane as a tool for integrating animal and human test data. Int J Toxicol. 28:498-509.

[8] Texas Commission on Environmental Quality (TCEQ). 2015. Guidelines to Develop Toxicity Factors. Office of Executive Director. RG-442. TCEQ, Austin, TX. Available from: Guidelines to Develop Inhalation and Oral Cancer and Non-Cancer Toxicity Factors (texas.gov)

but based on the published data in humans, including that of the key study (Davis, 1934), it appears to be dependent on time and concentration. For adjustment from a shorter exposure duration to a longer exposure duration a default value of 1 is used for the exponent n, and for adjustment from a longer exposure duration to a shorter exposure duration a default value of 3 is used for the exponent n.

There are also other regulatory agencies that use Haber's rule as modified by ten Berge (1986) for duration adjustment in the derivation of acute comparison values in evaluation of ambient air concentrations of chemicals. These include California's Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA),[9] the Minnesota Department of Health (MDH),[10] and the United States Environmental Protection Agency (USEPA).[11] Using the same key study (Davis, 1934), and the ten Berge equation with an exponent value of 2.5, USEPA derived Acute Guideline Exposure Levels (AEGLs)[12] for CCl_4.

Comment 4*: II. Derivation of the Acute 1-h ReV. B. The justification for an uncertainty factor (UF_D) of 6 for database inadequacy is inappropriate.* TCEQ justifies the database UF_D "because the acute toxicological database for CCl_4 indicates a potentially steep dose-response curve in humans and animal studies." It is unclear how the dose-response curve of acute CNS effects justifies an uncertainty factor that is intended to account for uncertainties related to database deficiencies. As stated in the *TCEQ Guidelines to Develop Toxicity Factors*, "The UF_D is used to account for the fact that a potential health effect may not be identified if the database is missing a particular type of study and for study quality deficiencies as well." This does not seem to be the case for CCl_4, which is a volatile chlorinated hydrocarbon that has been well documented to induce acute CNS effects and liver toxicity in both animals and humans from acute exposures. There are multiple human studies that have characterized the relationship between exposure concentration of CCl_4 and short-term exposures. The database UF_D is unwarranted for the derivation of the acute 1-h ReV.

Response: The UF_D of 6 is justified, as the database confidence is considered low to medium, as stated in Table 5 of the proposed DSD. Section 4.3 of the TCEQ Guidelines to Develop Toxicity Factors (2015) states the following: "Confidence in toxicological databases will vary depending on how much is known about each chemical's MOA and the quality of the experimental study."

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[9] Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. 2008. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels.

[10] Minnesota Department of Health Statement of Need and Reasonableness. 2001. Proposed Permanent Rules Relating to Health Risk Values Minnesota Rules, Parts 4717.8000 to 4717.8600.

[11] National Research Council. 2001. Standing Operating Procedures for Developing Acute Exposure Guideline Levels for Hazardous Chemicals. National Academy Press. Washington, D.C.

[12] Committee on Acute Exposure Guideline Levels, Committee on Toxicology, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 17. National Academies Press, Washington, D.C. 2014.

The unknown MOA for acute toxic effects of CCl_4 factors into what kind of studies are necessary in identification of the most sensitive effect. If the MOA were known, then one could predict what kinds of acute toxicity might be associated with that MOA, and therefore, one would have an indication as to whether or not there is a gap in knowledge. Because the MOA for the acute toxic effect of CCl_4 is unknown, it is not known if there is a gap in knowledge, and so it is assumed that there is a gap. Moreover, although there are studies available for derivation of an acute toxicity factor, many of these studies were conducted in a few subjects at each exposure concentration decades ago and would not be of the same quality as recently-conducted controlled human exposure studies. Because the quality of the database is considered medium at best, the quality of the studies available for derivation of an acute ReV are low to medium, and the MOA regarding CNS effects in humans is not defined, the UF_D of 6 was used for derivation of the acute 1-h ReV. The text regarding justification of a UF_D of 6 now mentions that the quality of available studies was low to medium.

Comment 5: II. Derivation of the Acute 1-h ReV. C. Recommended revision to the acute 1-hour ReV derivation. There were no symptoms or signs of toxicity in human subjects exposed to 76 ppm CCl_4 for up to 2.5 hours in the Davis (1934) study. Thus, 76 ppm can be considered a NOAEL for CCl_4 for the 1-h time point. Applying a UF_H of 10 to the POD_{HEC} of 76 ppm results in a revised acute 1-h ReV of 7.6 ppm.

Response: As per the *TCEQ Guidelines to Develop Toxicity Factors* (2015), a UF_D of 6 was applied in the derivation, because the database for development of an acute ReV was low to medium. The revised TCEQ acute 1-h ReV is 8,200 µg/m^3 (1,300 ppb) and the short-term ESL is 2,400 µg/m^3 (390 ppb).

Note that acute exposure guideline levels (AEGLs)[13] have been derived for carbon tetrachloride using the same key study (Davis, 1934). AEGLs represent threshold exposure limits (exposure levels below which adverse health effects are not likely to occur) for the general public and are applicable to emergency exposures ranging from 10 min to 8 h. Typically three levels, AEGL-1, AEGL-2, and AEGL-3 are developed for each exposure period (10 min, 30 min, 1 h, 4 h, and 8 h) and are based on increasing severity of toxic effects. AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic nonsensory effects. However, the effects are not disabling and are transient and reversible when exposure ceases. Susceptible populations include infants, children, the elderly, and persons with asthma. AEGLs may not protect against idiosyncratic responses.

AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious,

[13] Committee on Acute Exposure Guideline Levels, Committee on Toxicology, Board on Environmental Studies and Toxicology, Division on Earth and Life Studies. Acute Exposure Guideline Levels for Selected Airborne Chemicals. Volume 17. National Academies Press, Washington, D.C. 2014.

long-lasting adverse health effects or impaired ability to escape. AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience life-threatening adverse health effects or death.

Because data on CCl_4 were lacking, AEGL-1 values were not developed. The following table shows the AEGL-2 and AEGL-3 values for CCl_4. The AEGL-2 values were developed using the same key study (Davis, 1934) as was used for TCEQ's acute 1-h ReV, and duration adjustments for the NOAEL of 76 ppm for 4 h were made using the ten Berge (1986) equation with an exponent n = 2.5.

AEGL values for carbon tetrachloride

Classification	10-min	30-min	1-h	4-h	8-h	Endpoint (reference)
AEGL-1 (non-disabling)	NR [a]	NR	NR	NR	NR	Inadequate data
AEGL-2 (disabling)	27 ppm (170 mg/m^3)	18 ppm (110 mg/m^3)	13 ppm (82 mg/m^3)	7.6 ppm (48 mg/m^3)	5.8 ppm (36 mg/m^3)	NOAEL of 76 ppm for CNS effects in humans (Davis, 1934)
AEGL-3 (lethal)	700 ppm (4,400 mg/m^3)	450 ppm (2,800 mg/m^3)	340 ppm (2,100 mg/m^3)	200 ppm (1,300 mg/m^3)	150 ppm (940 mg/m^3)	Estimated LC_{01} in rats (Adams, 1952[14]); Dow chemical, 1960)

a: Not recommended. Absence of AEGL-1 values does not imply that exposures below the AEGL-2 values are without adverse effects.
AEGL, acute exposure guideline level; CNS, central nervous system; LC_{01}, lethal concentration, 1% lethality

AEGL values are used in emergency response situations involving accidental chemical releases and are not the basis for evaluation of ambient air concentrations under normal (non-emergency) conditions. **The HSIA-proposed acute 1-h ReV is equivalent to the AEGL-2 4-h level of 7.6 ppm and is just 1.7 times lower than the AEGL-2 1-h value of 13 ppm. Moreover, the HSIA-proposed acute 1-h ReV of 7.6 ppm is close to the TLV-STEL of 10 ppm (threshold limit value - short term exposure limit, American Conference of Governmental Industrial Hygienists [2012]), which is defined as a 15-min time weighted average exposure that should not be exceeded at any time during the workday.**

[14] Adams EM, HC Spencer, VK Rowe, DD McCollister, DD Irish. 1952. Vapor toxicity of carbon tetrachloride determined by experiments on laboratory animals. Arch Ind Hyg Occup Med. 6:50–66.

A more conservative acute 1-h ReV, such as that derived by the TCEQ, is warranted for protection of the general public's health under normal ambient conditions, as well as during an emergency situation with a chemical release. The acute 1-h ReV is an air concentration at or below which is not likely to cause an adverse health effect in the general public, including sensitive subgroups such as children, the elderly, pregnant women, and people with preexisting health conditions.

The AEGL-2 values predict a threshold for disabling CNS effects from a human study (Davis, 1934). Therefore, the TCEQ will not adopt the HSIA-proposed 1-h acute ReV of 7.6 ppm which is the same or similar to AEGL-2 values (4-h AEGL-2 of 7.6 ppm or 1-h AEGL-2 of 13 ppm, respectively) for disabling CNS effects in humans, and also is similar to the 15-min TLV-STEL of 10 ppm.

Comment 6: *III. Derivation of the Acute 24-h ReV.* TCEQ proposes a database UF_D of 6 be applied in the derivation of the acute 24-h ReV for CCl_4 because "fewer 24-h studies were available than 1-h studies; and the selected POD is based on a 8-h LOAEL." It is unclear how TCEQ reached this conclusion since many of the studies referenced for the derivation of the acute 1-h ReV are also relevant (even more so) for the derivation of 24-h ReV. This includes all the animal studies as well as the human studies, with the exception of Lehmann and Schmidt-Kehl (1936). It is important to note that the two key human studies (Davis, 1934; Kazantzis and Bomford, 1960[15]) that provide exposure concentration-response data on the acute CNS effects from either controlled or workplace exposures to CCl_4 are reasonably in agreement with respect to each other when extrapolated to a 24-h exposure period using the ten Berge equation. Thus, TCEQ cannot justify a database UF_D of 6 for the derivation of the acute 24-h Rev based on the reasons provided in the DSD.

A revised 24-h ReV of 0.5 ppm is proposed by applying an UF_H of 10 and an UF_L of 3 to the POD_{HEC} of 15 ppm from the Kazantzis and Bomford (1960) study.

Response: Section 3.1.1.2 *Animal Studies* of the proposed DSD describes the animal studies that were considered for derivation of acute ReVs. As noted in the proposed DSD, inhalation exposure to 10-100 ppm CCl_4, 6–7 h/d in rats for up to 2 wks generally resulted in mild to moderate signs of liver injury (fatty degeneration). Section 3.1.1.1 *Human Studies* of the proposed DSD describes the human studies that were considered for derivation of the acute ReVs. The predominant effects seen in humans after acute inhalation of CCl_4 were CNS effects. As per the *TCEQ Guidelines to Develop Toxicity Factors* (2015), in general, sufficient human data are preferred when developing toxicity factors, unless a more sensitive endpoint has been identified in an animal study.

[15] Kazantzis G and RR Bomford. 1960. Dyspepsia due to inhalation of carbon tetrachloride vapor. Lancet. 1:360–362.

As noted previously (response to Comment 4), a UF_D of 6 is justified for derivation of the acute ReVs, as the database confidence is considered low to medium. Section 4.3 of the *TCEQ Guidelines to Develop Toxicity Factors* (2015) states the following: "Confidence in toxicological databases will vary depending on how much is known about each chemical's MOA and the quality of the experimental study." Although there are human studies available for derivation of an acute toxicity factor, many of these studies were conducted in a few subjects at each exposure concentration decades ago and would not be of the same quality of recently-conducted controlled human exposure studies.

Because the quality of the database is considered medium at best, the quality of the studies available for derivation of an acute ReV are low to medium, and the MOA regarding CNS effects in humans is not defined, the UF_D of 6 will be retained for the derivation of the acute 24-h ReV, which is 520 µg/m^3 (83 ppb). The text regarding justification of a UF_D of 6 now mentions that the quality of available studies was low to medium.

Comment 7: *IV. Derivation of the Chronic $ReV_{threshold(nc)}$. A. An uncertainty factor (UF_D) of 3 for database inadequacy is unwarranted.* The DSD states that "The database lacks an adequate multigeneration study of reproductive function by any route of exposure; therefore, a UFD was applied." There are two reasons why this UF is unwarranted. First, although the DSD cites a rat three generation reproductive toxicity conducted by Smyth (1936)[16], a more recent reproductive toxicity study has been conducted in rats were administered 0, 80, or 200 ppm CCl_4 in feed over a two-year period[17].

Given the lack of evidence of reproductive toxicity in this study, the database UF_D for lack of reproductive toxicity data from a multigeneration reproductive toxicity study is unnecessary. Moreover, the DSD points out that "developmental/reproductive toxicity due to CCl_4 exposure is not a major area of concern for the chronic evaluation because setting a toxicity factor that protects against hepatotoxicity will also protect against the reproductive/developmental effects that occur at substantially higher exposure concentrations." An UF_D of 3 is, therefore, inconsistent with DSD's conclusion. DSD's conclusion suggests that additional data on the reproductive effects would not change the outcome of the risk assessment. Taken together, the UF_D should be eliminated.

Response: The UF_D of 3 is justified and reflects a database confidence of medium to high. The proposed DSD states "The database lacks an *adequate* multigeneration study of reproductive function by any route of exposure; therefore, a UF_D was applied" (emphasis added). The studies cited by HSIA (Smyth, 1936 and Alumot, 1976) were conducted decades ago and are not considered of the same quality as a multigeneration study conducted more recently in

[16] Smyth HF, HF Smyth, CP Carpenter. 1936. The chronic toxicity of carbon tetrachloride; animal exposure and field studies. J Ind Hyg Toxicol. 18:277-298.

[17] Alumot E, E Nachtomi, E Mandel, P Holstein, A Bondi, M Herzberg. 1976. Tolerance and acceptable daily intake of chlorinated fumigants in the rat diet. Fd Cosmet Toxicol. 14:105-110.

accordance with guidelines (e.g., Organization for Economic Cooperation and Development Guideline 443[18]) and in compliance with Good Laboratory Practices.

In Section 3.1.1.3.2 *Animal Studies* of the proposed DSD it states "No adequate reproductive toxicity studies have been conducted in animals exposed by the oral route (USEPA 2010[19])." Note that this also agrees with the USEPA 2010 document (IRIS assessment for carbon tetrachloride) that describes the deficiencies of the available studies, including those of the Alumot (1976) study (refer to pp. 63-64 of the 2010 IRIS assessment). Likewise, for the inhalation route, a definitive reproductive toxicity study has not been performed (refer to p. 144 of the 2010 IRIS assessment). Lack of an adequate reproductive study is also stated in USEPA 2020 (p. 128). Moreover, the rationale for selection of a UF_D of 3 agrees with USEPA's derivation of the reference concentration (RfC) (2010, pp. 206-207).

Therefore, as per the *TCEQ Guidelines to Develop Toxicity Factors*, the UF_D of 3 is justified and will be retained for the derivation of the chronic $ReV_{threshold(nc)}$.

Comment 8: *IV. Derivation of the Chronic $ReV_{threshold(nc)}$. B. An uncertainty factor for toxicodynamic differences between animals and humans (UF_A) should be modified.* There are several studies that support adjustment of the uncertainty factor for animal to human extrapolations (UF_A) when application of a physiologically based pharmacokinetic (PBPK) model is applied for derivation of toxicity reference values. The UF_A typically consists of a toxicokinetic portion ($UF_{A\text{-}TK}$ = 3) and a toxicodynamic portion ($UF_{A\text{-}TD}$ = 3) that when combined results in a UF_A of 10. It is generally accepted that when a physiologically-based pharmacokinetic (PBPK) model is applied to the derivation of toxicity reference values, that the toxicokinetic portion of the UF_A is not needed and only the toxicodynamic portion of the UF ($UF_{A\text{-}TD}$ = 3) should be considered. However, there are several studies and regulatory guidance documents that support consideration of an adjustment of the $UF_{A\text{-}TD}$ based on chemical-specific information.[20]

[18] OECD Guideline for the Testing of Chemicals No. 443. 2018. Extended one-generation reproductive toxicity study.

[19] United States Environmental Protection Agency (USEPA). 2010. Toxicological Review of Carbon Tetrachloride. EPA/635/R-08/005F. US Environmental Protection Agency. Washington D.C. URL: Toxicological Review of Carbon Tetrachloride (CAS No. 56-23-5) (PDF) (epa.gov)

[20] WHO/IPCS. 2005. Chemical-specific adjustment factors (CSAF) for interspecies differences and human variability: guidance document for the use of data in dose/concentration-response assessment, Harmonization Project Document No. 2.; Bhat VS, ME Meek, M Valcke, C English, R Brown. 2017. Evolution of chemical-specific adjustment factors (CSAF) based on recent international experience: increasing utility and facilitating regulatory acceptance. Crit Rev Toxicol. 47:733-753.; Meek ME, A Renwick, E Ohanian, M Dourson, B Lake, BD Naumann, V Vu. 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. Toxicol. 181-182:115-120.; EPA. 2014. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. EPA/100/R-12/002F.

Additional detail regarding this comment is included in HSIA's public comments. Based on the information described in the comments, HSIA proposes to revise the default UF of 3 to 1.5 for the toxicodynamics (TD) portion of the UF_A for CCl_4.

Response: The TCEQ has reviewed the references cited by HSIA. None of these references specifically informs a chemical specific adjustment factor (CSAF) for interspecies toxicodynamics extrapolation from animal to human for CCl_4. The supplementary information in the Bhat (2017) reference only shows CSAFs for intraspecies (i.e., variability between humans) toxicokinetics extrapolation for CCl_4. If a CSAF were used for the toxicodynamics portion of the UF_A in derivation of the chronic $ReV_{threshold(nc)}$ for CCl_4 in place of the default factor of 3, the TCEQ prefers a directly applicable, peer-reviewed CSAF.

USEPA and TCEQ used the same PBPK models for derivation of chronic toxicity factors, and a UF_A of 3 was also used by USEPA in their derivation of the RfC (USEPA 2010). In Section 4.1.3 *MOA Analysis and Dose Metric* of the proposed DSD, a description is provided of the metabolism of CCl_4 and generation of highly reactive free radical metabolites that result in covalent binding and damage to cellular macromolecules, ultimately causing hepatotoxicity, the toxicodynamic response. Additionally, as described in Section 4.1.7 *Adjustments to the* POD_{HEC}, because interspecies toxicokinetic differences were accounted for by use of a PBPK model, a UF_A of 3 was used for interspecies differences in toxicodynamics. This is in agreement with derivation of the RfC, as USEPA (2010, p. 206) states "In the absence of data to quantify specific interspecies differences for cellular protective mechanisms, a UF of 3 is applied to account for species differences in pharmacodynamics." Five of the 6 members of the external peer review panel for USEPA's RFC agreed that a UF_A of 3 was appropriate, and the sixth reviewer recommended that a $BW^{0.25}$ correction be added to account for likely slower elimination of the active metabolites in humans relative to rats, thereby lowering the RfC by approximately 4-fold ($[70\ kg/0.25\ kg]^{0.25}$) (USEPA 2010, p. A-22). None of the peer reviewers suggested a toxicodynamics CSAF of 1.5.

Therefore, the UF_A of 3 will be retained for the derivation of the chronic $ReV_{threshold(nc)}$. For clarification, the description on the use of UF_A of 3 has been revised in the final DSD to include the uncertainty regarding the interspecies differences in cellular protective mechanisms.

Comment 9*: IV. Derivation of the Chronic* $ReV_{threshold(nc)}$*. C. Recommended revision to the chronic* $ReV_{threshold(nc)}$ *derivation.* Incorporating the suggested modifications to the TCEQ derivation of the chronic $ReV_{threshold(nc)}$ discussed in [Comments 7 and 8] results in a revised chronic non-cancer $ReV_{threshold(nc)}$ value of 0.178 ppm (178 ppb). Also note in Figure 1. Recommended revision to the chronic non-cancer $ReV_{threshold(nc)}$ derivation of the HSIA comments that only one PBPK model (V_{max} = 0.65, consistent with the PBPK model published by Paustenbach, 1988[21]) was used in the HSIA-derived $ReV_{threshold(nc)}$. This revised value based on non-cancer fatty

[21]Paustenbach DJ, HJ Clewell III, ML Gargas, ML et al. 1988. A physiologically based pharmacokinetic model for inhaled carbon tetrachloride. Toxicol Appl Pharmacol 96:191–211.

changes in the liver of male rats (*i.e.*, hepatotoxicity) from the Nagano (2007)[22] study is considered to be protective of cancer, as well as the reproductive/developmental effects that occur at substantially higher exposure concentrations.

Response: As per the *TCEQ Guidelines to Develop Toxicity Factors* (2015), when chronic inhalation (e.g., RfC, URF) toxicity factors or guideline levels are identified in the scientific literature or databases, they are reviewed to determine whether the approach used to develop these toxicity factors is similar to the procedures used by the TCEQ. If so, the TCEQ considers adoption of the published chronic toxicity factor or guideline level, with preference given to values that have undergone an external peer review and public involvement process. The TCEQ also considers the published values and their respective key studies as a starting place for gathering toxicity information to develop a DSD.

This is what was done for the CCl_4 DSD. The development of the chronic inhalation toxicity factor or RfC for CCl_4 (USEPA 2010) was reviewed by the TCEQ, and TCEQ followed the same general principles in the derivation of the chronic $ReV_{threshold(nc)}$ that were used for USEPA's derivation of the RfC. Note that the RfC derived by USEPA did undergo an external peer review in 2008.

As described in Section 4.1.4.2. *Benchmark Dose Modeling* of the proposed DSD, the TCEQ performed benchmark dose modeling using USEPA (2023) BMD software (desktop version 3.3.2 in Excel) to analyze data on estimated internal doses (i.e., MCA and MRAMKL) and incidence data (i.e., fatty changes of the rat liver) from the 2-yr rat bioassay (Nagano, 2007), which is the same key study used by USEPA to develop their RfC. USEPA used an older previous version of their benchmark dose modeling software (BMDS version 1.4.1 from 2007) (USEPA, 2010, p. 202). As was done by USEPA, the TCEQ also selected a default benchmark response (BMR) of 10% as the critical effect size (BMD_{10} and $BMDL_{10}$). With the newer BMDS software, the same BMD_{10} and $BMDL_{10}$ values using the same best fit models were calculated for the male rat data (MCA and MRAMKL) and for the female rat data with the highest dose dropped (MCA). Both versions of the BMDS software were unable to fit the female data with all doses included (MCA and MRAMKL). The older BMDS software did calculate a best fit model for female rat data with the highest dose dropped for the MRAMKL dose metric, but the newer BMDS software was unable to provide an adequate fit of the data.

Because the MOA for CCl_4-induced hepatotoxicity involves metabolism to reactive metabolites in the liver, human equivalent concentrations (HECs) based on the MRAMKL internal dose metric are the most proximate to the critical effect (USEPA 2010, p. 205). The TCEQ then converted the resultant $BMDL_{10}$ values based on the MRAMKL internal dose metric to HECs using the same PBPK model that USEPA used to derive the RfC. As described in Section 4.1.4.3

[22] Nagano K, T Sasaki, Y Umeda, Y, T Nishizawa, N Ikawa, H Ohbayashi, A Heihachiro, S Yamamoto, S Fukushima. 2007. Inhalation carcinogenicity and chronic toxicity of carbon tetrachloride in rats and mice. Inhal Toxicol. 19:1089–1103.

PBPK Modeling to Derive Human Equivalent Concentrations in the proposed DSD, USEPA (2010, 2020) indicated that no information is available to distinguish between a rat V_{maxC} of 0.4 or 0.65 mg/h/kg $BW^{0.70}$ as the more scientifically defensible value for this parameter. USEPA then averaged the resultant HECs from each rat model in their derivation of the RfC. Likewise, the TCEQ also averaged the resultant HECs from each rat model, using the $BMDL_{10}$ from the male rat data with the MRAMKL internal dose metric. As no information is available to distinguish between a rat V_{maxC} of 0.4 or 0.65 mg/h/kg $BW^{0.70}$ as the more scientifically defensible value, the TCEQ will not select one rat model over the other, as is proposed by HSIA.

Altogether, the TCEQ used the same key study and PBPK models that were used by USEPA in their derivation of the RfC. As described in the response to Comment 7, the UF_D of 3 is justified, reflects a database confidence of medium to high, is the same UF_D used by USEPA in their derivation of the RfC, and will be retained in the TCEQ's derivation of the chronic $ReV_{threshold(nc)}$. As described in the response to Comment 8, the UF_A is justified, reflects the uncertainty regarding the interspecies differences in cellular protective mechanisms, and is the same UF_A used by USEPA in their derivation of the RfC. Moreover, a peer-reviewed CSAF to be used in place of $UF_{A\text{-}TD}$ is not available. Therefore, the UF_A of 3 will be retained in the TCEQ's derivation of the chronic $ReV_{threshold(nc)}$. As per the TCEQ Guidelines to Develop Toxicity factors, along with a UF_H of 10, the composite uncertainty factor is 90, and the resultant chronic $ReV_{threshold(nc)}$ of 160 µg/m^3 (25 ppb) will be retained.

Comment 10: *V. Derivation of the Chronic $ESL_{nonthreshold(c)}$. A. The CCl_4-induced rodent liver tumors in Nagano (2007) occur by a non-genotoxic (threshold) mode-of-action (MOA) involving hepatotoxicity and compensatory hyperplasia.*

HSIA states that the analysis of tumors in the 2-yr bioassay of CCl_4 in mice is flawed. The increase in female mouse liver tumors at the lowest exposure level (5 ppm) in the Nagano (2007) study is not CCl_4-related when the historical control data for the BDF_1 mouse at the laboratory where the two-year inhalation study of CCl_4 was conducted and the lack of statistical significance in the combined liver adenomas and carcinomas are taken into account. In mice, there was a significant increase in liver tumors (adenomas and carcinomas) in both males and females at 25 and 125 ppm. At 5 ppm, Nagano (2007) reported a significant increase in liver adenomas in the female mice, but not for adenomas and carcinomas combined. USEPA relied upon limited information on the historical spontaneous liver tumor incidence of BDF_1 mice at the Japan Bioassay Research Center (JBRC) where the bioassay was conducted. More comprehensive historical control data from this laboratory shows that the incidence of liver tumors (adenomas, carcinomas, and combined adenomas plus carcinomas) at 5 ppm in the 2-yr study of CCl_4 was indeed within the historical range for this strain of mouse.

Response: TCEQ acknowledges that historical control tumor data are important in the evaluation of carcinogenicity studies. USEPA's *Guidelines for Carcinogen Risk Assessment*[23] state

[23] USEPA. 2005. Guidelines for Carcinogen Risk Assessment.

the following (p. 2-20 to 2-21): “The standard for determining statistical significance of tumor incidence comes from a comparison of tumors in dosed animals with those in concurrent control animals. Additional insights about both statistical and biological significance can come from an examination of historical control data… Generally speaking, statistically significant increases in tumors should not be discounted simply because incidence rates in the treated groups are within the range of historical controls or because incidence rates in the concurrent controls are somewhat lower than average. Random assignment of animals to groups and proper statistical procedures provide assurance that statistically significant results are unlikely to be due to chance alone. However, caution should be used in interpreting results that are barely statistically significant or in which incidence rates in concurrent controls are unusually low in comparison with historical controls.”

The source document for the 2-yr bioassay in mice (Nagano, 2007) states the following on p. 1098: “Notably, the incidence of hepatocellular adenomas in the 5-ppm exposed female mice (16.3%) was significantly increased by Fisher’s exact test. In addition, the tumor incidence exceeded the upper range of the JBRC historical control data (43 cases [5.1%] in 849 female Crj:BDF1 mice in seventeen 2-yr inhalation studies that have been conducted in the JBRC during the 17-yr period from 1990 to 2006, with a maximum incidence of 12% in a single study). The combined incidence of hepatocellular adenomas and carcinomas in the 5-ppm-exposed female mice (18.4%) also exceeded the upper range of the JBRC historical control data (65 cases [7.7%] in 849 female Crj:BDF1 mice, with a maximum incidence of 14% in a single study).”

As noted above, the incidence of hepatocellular adenomas in female mice exposed to 5 ppm CCl_4 was 8/49 or 16.3%, and this exceeded the maximum historical control incidence of hepatocellular adenoma (12%) in studies conducted in the same strain and sex of mice at the facility where the 2-yr bioassay of CCl_4 was conducted. Historical control hepatocellular adenoma data in female Crj:BDF1 mice also are discussed in the publication cited by HSIA (Cohen, 2023[24]). In that publication, which is not the source document but includes tables of tumor incidence data from the Nagano (2007) study, the following is stated (p. 345): “First, the historical spontaneous liver tumor incidence in BDF1 mice from 10 two-year carcinogenicity studies conducted at the JBRC showed a mean incidence of liver adenomas to be 4.4% (with a range from 2% to 8%) (Katagiri et al. 1998). Similarly, Yamate et al. (1990) noted 6/50 liver adenomas in untreated female BDF1 mice in an additional study, but it was a lifetime study (up to 150 weeks).”

Note that the incidence of hepatocellular adenomas in female mice exposed to 5 ppm CCl_4 (16.3%) exceeded the historical control incidence of hepatocellular adenomas in females of the same strain of mice conducted at the same facility (maximum incidence of 8% in Katagiri

[24] Cohen S, C Bevan, B Gollapudi, JE Klaunig. 2023. Evaluation of the carcinogenicity of carbon tetrachloride. J Toxicol Environ Health, Part B. 26:342-370.

1998[25]) and also in another study which was 150 wk in duration (incidence of 12% in Yamate 1990[26]). Also, the incidence of hepatocellular adenomas and carcinomas, combined, in female mice exposed to 5 ppm CCl_4 (18.4%) exceeded the historical control data range for these tumors combined in Katagiri (1998) (range of 2-12%, with a mean of 6.4% in ten 2-yr studies in BDF1 mice) and in the one study in Yamate (1990) (12%).

Moreover, the incidence of hepatocellular adenomas in females in the control group (2/50 or 4%) is within the range of historical control data in Katagiri 1998 (range of 2-8%, with a mean of 4.4% in ten 2-yr studies in BDF1 mice). Also, the incidence of hepatocellular adenomas and carcinomas, combined in the control females (4/50 or 8%) is within the range of historical control data in Katagiri (1998) (range of 2-12%, with a mean of 6.4% in ten 2-yr studies in BDF1 mice). The control incidences of hepatocellular adenoma and of combined hepatocellular tumors are below the one study in Yamate (1990) (12% incidence for hepatocellular adenoma; 12% incidence for combined tumors), likely due to the longer duration (150 wk) in the Yamate (1990) study. Because there is more time for tumors to develop in the Yamate (1990) study, as this study was 150 weeks in duration, instead of the standard 2-yr (104-wk) study, the tumor incidences are expected to be higher than in a 2-yr study. Overall, the incidences of hepatocellular tumors in the control group are not low in comparison to historical control incidences when one compares to studies of the same 2-yr duration, as in Katagiri (1998).

Then the following is stated in the Cohen, 2023 paper: “Finally, information provided by Dr Shoji Fukushima (personal communication), the former Director of the JBRC and the senior author on the publications by Nagano et al (2007a, 2007b), reported the range of incidences of hepatocellular adenomas in female BDF1 mice in their lab was 2–20% with a mean of 6.4% (86 out of 1,348 mice)… For combined tumors, the incidences in historical controls were in the range of 2–20% with a mean of 8.5% (114 out of 1,348).”

These ranges mentioned via personal communication in the 2023 review publication are not valid for comparison to the Nagano (2007) publication for the following reasons: a) the number of studies conducted and the years of conduct for this newly reported historical control range are not known, and b) as mentioned in Nagano (2007), the 2-yr inhalation study was reported in 1987; therefore, this study was conducted in the 1980’s. A historical control range that encompasses and/or is close to the years of study conduct is most relevant. Thus, the historical control ranges reported in Nagano (2007), Katagiri (1998), and Yamate (1990) are most relevant. Genetic drift, different experimental conditions, different sources of animals, and other factors are known to influence historical control tumor data. The addition of control data from more recently conducted studies is not appropriate and is not relevant to the timeframe

[25] Katagiri T, N Kasuke, S Aiso, H Senoh, Y Sakura, T Takeuchi, M Okudaira. 1998. A pathological study on spontaneous hepatic neoplasms in BDF1 mice. J Toxicol Pathol. 11:21-25.

[26] Yamate J, M Tajima, S Kudow, S Sannai. 1990. Background pathology in BDF1 mice allowed to live out their life span. Lab Animals. 24:332-340.

of the conduct of the study. Moreover, as stated in USEPA's *Guidelines on Carcinogen Risk Assessment* (p. 2-21): "The most relevant historical data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution."

The TCEQ is in agreement with the conclusion in the source document that the incidences of hepatocellular adenomas (16.3%) and of hepatocellular adenomas and carcinomas combined (18.4%) in female Crj:BDF1 mice exposed to 5 ppm CCl_4 exceeded the historical control range as reported in Nagano (2007), as well as Katagiri (1998) and Yamate (1990). Moreover, the incidences of hepatocellular adenomas and of hepatocellular adenomas and carcinomas combined in control female Crj:BDF1 mice are not unusually low and are within historical control ranges of 2-yr studies reported in Nagano (2007) and Katagiri (1998).

Comment 11: *V. Derivation of the Chronic $ESL_{nonthreshold(c)}$. A. The CCl_4-induced rodent liver tumors in Nagano (2007) occur by a non-genotoxic (threshold) mode-of-action (MOA) involving hepatotoxicity and compensatory hyperplasia*, cont'd.

HSIA states that the analysis of tumors in the 2-yr bioassay of CCl_4 in mice is flawed. USEPA concluded that 5 ppm represents a Lowest-Observed-Adverse-Effect-Concentration (LOAEC) for liver tumors in Nagano (2007) based on the reported results of the statistical analysis. In the publication by Nagano (2007), the difference between the 8/49 adenomas in the 5-ppm dose female group and the 2/50 adenomas in the matched controls was statistically significant at $p < 0.05$ using the Fisher's exact test. This is not entirely correct; a re-analysis of the data using the Fisher's exact test resulted in the p value = 0.05112; this may or may be considered significant at the $p = 0.05$ level of significance depending on whether the p value is rounded off. Nevertheless, the statistical consideration of the increase of liver adenomas in the 5 ppm-exposed females must be reconsidered from the perspective of these tumors being common. For common tumors, Haseman (1983) stated that the statistical significance for tumor incidences should be based on the probability of $p < 0.01$ rather than $p < 0.05$ because of the multiple comparisons and to avoid the high probability of false positives. Certainly, liver cell hepatocellular tumors in mice are a common tumor (as defined by Haseman as tumors with spontaneous incidence of >1%). This statistical standard has been adopted by the United States Food and Drug Administration (FDA)[27], and was extended to have the trend test be significant only if $p < 0.005$, rather than 0.01. The Organization for Economic Co-operation and Development (OECD)[28] has also accepted this standard of $p < 0.01$ for comparison of incidences of common tumors.

[27] FDA. Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals, Draft Guidance (2001); https://www.fda.gov/media/72296/download.

[28] OECD Guidance Document 116 on the conduct and design of chronic toxicity and carcinogenicity studies, supporting test guidelines 451, 452, and 453, 2nd Edition (2012). https://www.oecd-ilibrary.org/environment/guidance-document-116-on-the-conduct-and-design-of-chronic-toxicity-and-carcinogenicity-studies-supporting-test-guidelines-451-452-and-453_9789264221475-en.

Response: It is standard and accepted practice to perform statistical analysis on individual tumors, as well as on certain combined tumors (e.g., adenoma and carcinoma) in the same organ, as in the case of hepatocellular adenoma and carcinoma. This is in agreement with USEPA's *Guidelines for Carcinogen Risk Assessment*[29] (p. 2-19): "Statistical analysis of a long-term study should be performed for each tumor type separately. The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately but may be combined when scientifically defensible." The statistics performed by the authors of the 2-yr bioassay of CCl_4 in mice was what is included in the proposed DSD. A Fisher's exact test on the incidences of tumors was performed by the authors, which also agrees with USEPA's *Guidelines for Carcinogen Risk Assessment*. For transparency, the tables with tumor incidences (Tables 16 and 17 in rats and mice, respectively) in the proposed DSD reflect the statistical analysis performed by the authors (Nagano, 2007) of the 2-yr bioassays of CCl_4 in rats and mice. Presentation of the statistics for individual and combined tumors in this manner is standard and accepted practice. It is noted (and corrected in the final DSD) that the significance level for hepatocellular adenomas in female mice exposed to 5 ppm CCl_4 is $p \leq 0.05$.

The TCEQ is aware of statistical analyses performed as per guidelines of other agencies, such as US FDA and OECD. However, the USEPA guidelines do not specifically state or require different significance levels for common and rare tumors in rodent 2-yr carcinogenicity studies. USEPA's *Guidelines for Carcinogen Risk Assessment* state the following (p. 2-20, emphasis added): "Haseman (1983[30]) analyzed typical animal bioassays that tested both sexes of two species and concluded that, because of multiple comparisons, a single tumor increase for a species-sex-site combination that is statistically significant at the 1% level for common tumors or 5% for rare tumors corresponds to a 7–8% significance level for the study as a whole. **Therefore, animal bioassays presenting only one significant result that falls short of the 1% level for a common tumor should be treated with caution**." The 2-yr bioassay of CCl_4 in mice showed more than one significant result for liver tumors, which are considered common, and the significance level seen for the liver tumors at the mid- and high-exposure concentrations of 25 and 125 ppm for hepatocellular adenomas and carcinomas was 1% ($p \leq 0.01$).

Unlike the US EPA CCl_4 document, the TCEQ's proposed DSD does not explicitly cite a NOAEC or LOAEC regarding the tumor incidences in rats and mice. In Section 4.2 *Carcinogenic Potential* of the proposed DSD, the following is stated based on ATSDR's evaluation: "The lowest cancer effect levels were observed for mice at an exposure concentration of 25 ppm by inhalation and at a dose of 20 mg/kg-d administered orally (ATSDR 2005)." Additionally, the proposed DSD simply states the incidences and statistical analysis results for the tumors presented in Tables

[29] USEPA. 2005. Guidelines for Carcinogen Risk Assessment.

[30] Haseman JK. 1983. A reexamination of false-positive rates for carcinogenesis studies. Fundam Appl. Toxicol. 3:334-339.

16 and 17, as reported by Nagano (2007), which is considered the source document for the 2-yr bioassay of CCl_4 in rats and mice.

Furthermore, the statistical analysis and interpretation performed by the authors for the 2-yr bioassays of CCl_4 in rats and mice are in agreement with USEPA's *Guidelines for Carcinogen Risk Assessment*.

Comment 12: *V. Derivation of the Chronic $ESL_{nonthreshold(c)}$. A. The CCl_4-induced rodent liver tumors in Nagano (2007) occur by a non-genotoxic (threshold) mode-of-action (MOA) involving hepatotoxicity and compensatory hyperplasia* cont'd.

HSIA states that the analysis of tumors in the 2-yr bioassay of CCl_4 in mice is flawed. 5 ppm should be considered a NOAEC instead of a LOAEC because, in the IRIS assessment and in the 2020 final CCl_4 Risk Evaluation the USEPA failed to consider total liver tumor incidence (adenomas and carcinomas) in the 5 ppm-exposed female mice in its MOA evaluation. While there may be an increase in the liver adenomas in the 5 ppm-exposed female mice, the incidence of total liver tumors (adenomas plus carcinomas) was *not significantly* increased compared to controls (9/49 vs. 4/50, respectively). It is well known that the comparison of liver tumors needs to be made on total tumor incidence, not on adenomas or carcinomas separately.

A LOAEC of 25 ppm and a No-Observed-Adverse-Effect-Concentration (NOAEC) of 5 ppm for mouse and rat liver tumors in the two-year inhalation study by Nagano (2007) is consistent with a MOA involving toxicity to liver cells (cell death) resulting in compensatory proliferation (hyperplasia) (Cohen, 2023). For the formation of tumors, cell injury must occur to a sufficient level to result in hepatocyte cell proliferation; this occurs only from chronic exposures. Thus, the proper weight-of-the-evidence conclusion is that CCl_4 exposures that do not initiate sufficient cytotoxicity to elicit compensatory hyperplasia do not start the cascade to tumor formation.

Therefore, a threshold should be applied for derivation of a cancer toxicity value based on liver tumors for CCl_4, starting with the same POD that TCEQ describes for female mice in Table 23 of the DSD, and applying UFs consistent with comments described in Section IV above.

Response: In the source document for the 2-yr bioassays (Nagano, 2007, p. 1100), it states that hepatic necrosis or increased levels of serum transaminases (indicators of cytotoxicity) were not seen in female mice exposed to 5 ppm and therefore it is suggested that the MOA at lower concentrations could not be simply explained as a cytotoxic-proliferative MOA, and that another MOA could be operative. In Section 4.2.3 *Carcinogenic MOA and Dose Metric* of the proposed DSD, the following is stated "Both a cytotoxic-proliferative MOA and a genotoxic MOA for CCl_4-induced hepatocarcinogenesis have been suggested. USEPA (2010, 2020)". The TCEQ acknowledges that the formation of liver tumors in mice and rats may be due (at least in part) to cytotoxicity and regenerative proliferation. USEPA (2020) and ATSDR (2005) also mention the potential for mutagenicity via lipid peroxidation-induced DNA damage that could result from the production of radicals exceeding the cell's capacity to quench radicals and/or repair alterations in DNA.

In USEPA's more recent assessment of CCl_4 (USEPA, 2020, p. 154), the following is stated: "In summary there is biological support for the involvement of the hypothesized MOA of sustained cytotoxicity and regenerative cell proliferation as key events in the hepatocellular mode of action for carbon tetrachloride exposure in the mouse. However, important uncertainties and inconsistencies exist. The hypothesized MOA by itself is not consistent with observations of increased hepatocellular adenomas in the mouse at 5 ppm. This evaluation suggests that while cytotoxicity and regenerative proliferation may strongly influence dose response at higher doses, these processes may not reflect the potential for carcinogenic action of this compound at lower doses." In this more recent assessment (USEPA, 2020), USEPA referenced the IRIS inhalation unit risk factor of 6×10^{-6} per $\mu g/m^3$ based on adrenal pheochromocytomas in male mice (USEPA, 2010) and included a cancer benchmark margin of exposure (MOE) of 300 for 1 in 10,000 cancer risk for worker populations based on a point of departure (POD_{HEC}) of 6 mg/m^3 for liver tumors in female mice (USEPA, 2020, Table 4-4, p. 189).

Note that in the proposed DSD, as was done by USEPA in their IRIS assessment (USEPA, 2010), benchmark dose modeling was performed only on incidences of combined liver tumors (hepatocellular adenoma and carcinoma combined) in rats and mice, and not on individual hepatocellular tumors. In Table 23 of the proposed DSD, the $BMDL_{10}$ for combined liver tumors in female mice is 30.78 mg/m^3 (4.89 ppm). As delineated in responses to Comments 7 and 8, TCEQ does not agree with HSIA's proposed UF_D of 1 and UF_A of 1.5 and will retain a UF_D of 3 and UF_A of 3 for derivation of chronic toxicity factors. Therefore, the composite UF for chronic toxicity factors is 90, based on a UF_H of 10, a UF_D of 3, and UF_A of 3. Thus, using the $BMDL_{10}$ for combined liver tumors in female mice and with application of a composite UF of 90, the hypothetical threshold toxicity factor for liver tumors would be 0.34 mg/m^3 (340 $\mu g/m^3$ or 54 ppb).

However, this hypothetical threshold cancer derivation is higher than the TCEQ's proposed chronic $ReV_{threshold(nc)}$ of 160 $\mu g/m^3$ (25 ppb), which is based on fatty liver changes in male rats in the 2-yr bioassay. Hepatocellular adenomas and carcinomas also were increased in male and female rats in the same 2-yr bioassay. Additionally, fatty liver was seen in male and female mice in the 2-yr bioassay. As noted in Cohen (2023), findings of fatty liver and cell death, which were seen in the 13-week studies in rats and mice, appeared to correlate with development of liver tumors in the 2-yr bioassay in both species. If the liver tumors form via a threshold MOA, then because the MOA for fatty liver changes and carcinogenesis both involve generation of reactive CCl_4 metabolites and covalent binding to macromolecules and that fatty liver precedes development of liver tumors, the chronic $ReV_{threshold(nc)}$ of 160 $\mu g/m^3$ should also protect against liver cancer. When one applies a hazard quotient of 0.3 for air permitting purposes, the chronic $ESL_{threshold(c)}$ would be 48 $\mu g/m^3$ (7.5 ppb). This toxicity factor is higher than the ambient air concentration of 2.8 $\mu g/m^3$ associated with TCEQ's inhalation unit risk factor based on adrenal pheochromocytomas in male mice, with an excess cancer risk of 1 in 100,000. Because the MOA for CCl_4-induced tumors is not entirely clear and may include a genotoxic component (which is conservatively considered to have a linear dose-response), the $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 $\mu g/m^3$ (0.44 ppb) will be used for evaluation of long-term ambient air data and for air permit reviews.

Additional text regarding consideration of a threshold MOA for liver tumors with a chronic $ESL_{threshold(c)}$ of 48 µg/m^3 (7.5 ppb) has been added to the DSD.

Comment 13: *V. Derivation of the Chronic $ESL_{nonthreshold(c)}$. B. The CCl_4-induced mouse pheochromocytomas in Nagano (2007) are not relevant to assessing human cancer risk.*

The overall evidence suggests that mouse benign adrenal pheochromocytomas that occur following CCl_4 exposure are not relevant to human cancer risk assessment.[31] While CCl_4 is among a small number of chemicals that are known to induce adrenal pheochromocytomas in mice, there is no evidence that these chemicals are associated with adrenal pheochromocytomas in humans. These tumors predominantly occur in animal carcinogenicity studies when there are other tumors or toxic effects in other organs, such as severe liver toxicity and liver carcinomas. Pheochromocytomas are uncommon tumors in mice as well as in humans.

In their two-year carcinogenicity study, Nagano (2007) reported a statistically significant increase in the incidence of benign pheochromocytomas (tumors originating in the adrenal medulla) in the male mice exposed by inhalation to 25 and 125 ppm CCl_4 and in the female mice at 125 ppm CCl_4. Benign pheochromocytomas were also observed in an oral gavage carcinogenicity study conducted in mice by the National Cancer Institute (NCI) where CCl_4 was used as a positive control for liver tumors[32]. These tumors were not increased in CCl_4-exposed rats in either study.

Medullary hyperplasia forms a continuous histological spectrum with pheochromocytomas and thus represents a diagnostic challenge, particularly for the mouse due to the size of the adrenal medulla. It is not known, however, what criteria were used by the pathologist at JBRC to distinguish between medullary hyperplasia and pheochromocytomas.

It is also apparent that the pheochromocytomas in the 2-yr study of CCl_4 in mice occurred in animals with severe body weight reduction (>30% for the > 25 ppm males and 125 ppm females) and close to 100% mortality for the male and female mice at 125 ppm. Even after 52 weeks of exposure, there was a notable reduction in body weights in the 25 and 125 ppm mice (both sexes), particularly for the 125 ppm-exposed mice (>10%). Given the severe systemic toxicity in the animals, likely related to exceedance of the maximum tolerated dose (MTD) in these studies, it is important to consider that the pheochromocytomas may be secondary

[31] Cohen SM, C Bevan, B Gollapudi, JE Klaunig. 2023. Evaluation of the carcinogenicity of carbon tetrachloride. J Toxicol Environ Health, Part B. 26: 342-370; Greim H, A Hartwig, U Reuter, AB Richter-Reichhelm, HW Thielman. 2009. Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit Rev Toxicol. 39:695-718.

[32] Weisburger EK. 1977. Carcinogenicity studies on halogenated hydrocarbons. Environ Health Perspect. 21:7-16.

effects of the systemic toxicity. Therefore, the mouse pheochromocytomas should not be considered in the assessment of human cancer risk.

Response: In Nagano (2007, p. 1101), the authors describe the criterion for selection of the exposure concentrations in the 2-yr bioassays in rats and mice, and exposure concentration selection was based on the results of 13-wk studies in each species, which is appropriate. The authors state that the selection of exposure concentrations was based on no more than a 10% decrease in body weight in comparison to concurrent controls in the 13-wk studies. As reviewed in van Berlo (2022)[33], the concept of MTD originated with the National Cancer Institute and is defined as follows: "The MTD is defined as the highest dose of the test agent given during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity." As reviewed in van Berlo (2022), the National Toxicology Program has basically the same definition for MTD. In the *USEPA Guidelines for Carcinogen Risk Assessment* (2005, p. 2-17), it states "With regard to the appropriateness of the high dose, an adequate high dose would generally be one that produces some toxic effects without unduly affecting mortality from effects other than cancer or producing significant adverse effects on the nutrition and health of the test animals." As reviewed in van Berlo (2022), generally, a 10% decrement in body weight in comparison to controls in a subchronic study is a criterion used for selection of the high dose/exposure concentration in a 2-yr bioassay. **The MTD criterion based on body weight change is to avoid false negative results, and not false positive results**. It is well documented that in carcinogenicity studies conducted with dietary restriction, the resultant lower body weights are associated with a lower tumor burden. Moreover, the purpose of the 2-yr carcinogenicity assay is hazard and risk assessment in the evaluation of whether a substance is or is not carcinogenic.

In Nagano (2007, p. 1097), the decreased survival rates in male and female mice in the 25 and 125 ppm CCl_4 groups was causally related to the significantly increased number of mice that died of hepatocellular tumors. Based on the descriptions of MTD in the preceding paragraph, the study design was appropriate, as the longevity of the animals was impacted by the carcinogenicity of CCl_4. At the end of the 104-week study, when compared to controls mean terminal body weights were 32% and 22% lower in males and females in the 25 ppm CCl_4 group, respectively, and were 39% and 31% lower in males and females in the 125 ppm CCl_4 group, respectively (Table 1 of Nagano, 2007). **The authors also noted that there were no statistically significant differences in food consumption in any CCl_4 group**. Therefore, the decreased body weight was likely due to cachexia related to the development of tumors and metastases, as the hepatocellular carcinomas were highly metastasized. Palpation for masses was performed in the 2-yr bioassay in mice, and the authors state that the incidences of palpable liver masses, which first appeared at Week 43 in a male and Week 41 in a female, were increased in 125 ppm-exposed mice. The authors also note that the hepatocellular tumors occurred earlier in any CCl_4-exposed group of mice than in the control group, and that tumor onset also

[33] van Berlo D, M Woutersen, A Muller, M Pronk, J Vriend, B Hakkert. 2022. 10% Body weight (gain) change as criterion for the maximum tolerated dose: a critical analysis. Regul Toxicol Pharmacol. 134:105235.

occurred sooner with increased CCl_4 exposure concentrations (i.e., an inverse relationship of time at tumor onset with CCl_4 exposure concentration).

TCEQ acknowledges that pheochromocytomas are uncommon in humans and mice. In 2-yr rodent bioassays, pheochromocytomas occur most frequently in male rats, and less frequently in male and female mice (Greim, 2009)[34]. In the previously cited historical control study (Yamate, 1990, see response to Comment 10) only 1/50 male BDF1 mice had a pheochromocytoma. Given how uncommon pheochromocytomas are in mice, the incidences seen in males in the 25-ppm group (32%) and in males and females in the 125-ppm group (64% and 44%, respectively) are remarkably high. In the 2-yr study of CCl_4 in mice, as well as in other studies mentioned in Greim (2009), pheochromocytomas may occur with different tumors in other organs; however, this is not a rationale for dismissal of pheochromocytomas in hazard identification and risk assessment for a given substance. Moreover, a tumor type being uncommon in animal carcinogenicity studies and rare in humans are also not justifications for dismissal of the tumor type for use in hazard identification and risk assessment for a given substance. HSIA questions the criteria used by the pathologist at JBRC to distinguish between medullary hyperplasia and pheochromocytomas, but in the source document (Nagano, 2007) a photomicrograph of an adrenal pheochromocytoma seen in a male mouse exposed to 125 ppm CCl_4 is shown (Figure 6, p. 1099) and the tumors are described in the text as follows: "The adrenal tumor was benign and characterized by massive proliferation of adreno-medullary cells compressing the adjacent adreno-cortical tissue." As per this description, it appears that the findings seen in the adrenal medulla were tumors (i.e., pheochromocytomas), and are consistent with the criteria described in Greim (2009, p. 697), where proliferation of chromaffin cells resulting in compression of adjacent adrenocortical tissue would be considered a tumor. Although the pheochromocytomas seen in mice may not be concordant with a risk of pheochromocytomas in humans, Greim (2009, p. 714) acknowledges that the database for a conclusion regarding site concordance is inadequate. Moreover, site concordance is not an assumption or criterion for hazard identification and risk assessment of a given substance, especially when the MOA is uncertain.

Therefore, TCEQ will retain the dose-response assessment for carcinogenicity associated with adrenal pheochromocytomas in mice. Because the MOA for development of adrenal pheochromocytomas in mice exposed to CCl_4 is not known, as per *TCEQ Guidelines to Develop Toxicity Factors* (2015) and USEPA's *Guidelines for Carcinogen Risk Assessment* (2005), a linear non-threshold extrapolation was performed that resulted in a unit risk factor of 3.6×10^{-6} per $\mu g/m^3$, which corresponds to a chronic $ESL_{nonthreshold(c)}$ of 2.8 $\mu g/m^3$ (0.44 ppb) at a no significant risk level of 1 in 100,000 excess cancer risk. Note that USEPA derived a similar inhalation unit risk factor of 6×10^{-6} per $\mu g/m^3$ based on adrenal pheochromocytomas in mice (USEPA, 2010 IRIS Risk Assessment document; USEPA 2020).

[34] Greim H, A Hartwig, U Reuter, A-B Richter-Reichhelm, H-W Thielman. 2009. Chemically induced pheochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit Rev Toxicol. 39:695-718.

Comment 14: : *V. Derivation of the Chronic $ESL_{nonthreshold(c)}$. C. Recommended revision to the chronic $ESL_{nonthreshold(c)}$ derivation.*

Using the female mouse liver tumor data from the Nagano (2007) study, along with a threshold MOA, results in a revised chronic cancer $ESL_{nonthreshold(c)}$ of 0.345 ppm (345 ppb).

Response: The TCEQ will not adopt the HSIA-proposed $ESL_{nonthreshold(c)}$ of 2,170 µg/m^3 (345 ppb). Refer to responses to comments 10 to 13. TCEQ will also not adopt the HSIA-proposed chronic $ReV_{threshold(nc)}$ of 1,120 µg/m^3 (178 ppb) based on non-cancer fatty changes in the liver of male rats that HSIA considers to be protective of cancer (refer to comment 9 and response).

The final chronic toxicity factors derived by TCEQ are as follows:

- Chronic ReV = 160 µg/m^3 (25 ppb)
- $^{chronic}ESL_{threshold(nc)}$ = 48 µg/m^3 (7.5 ppb)
- URF = 3.6×10^{-6} per µg/m^3
- $^{chronic}ESL_{nonthreshold(c)}$ = 2.8 µg/m^3 (0.44 ppb)

The $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/m^3 (0.44 ppb) is the critical long-term health-based air monitoring comparison value (AMCV) for the evaluation of long-term ambient air data as this value is lower than the chronic ReV. The long-term ESL for air permit reviews is the $^{chronic}ESL_{nonthreshold(c)}$ of 2.8 µg/m^3 (0.44 ppb).