Texas Commission on Environmental Quality Response to Public Comments Received on the September 2015 Proposed Ethylene Dichloride Development Support Document

The public comment period for the September 2015 Proposed Development Support Document (DSD) for ethylene dichloride (EDC) ended in December 2015, with an extension period that ended in February 2016. The Toxicology Division (TD) received comments from the Hazardous Air Pollutants (HAP) Task Force, a testing consortium of US manufacturers of EDC, on February 22, 2016. The TD of the Texas Commission on Environmental Quality (TCEQ) appreciates the effort put forth by the HAP Task Force to provide technical comments on the proposed EDC DSD. The goal of the TD and 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. A summary of the comments from the HAP Task Force and TCEQ responses are provided below. The full comments and the request for an extension to the comment period are provided in the Appendix. TCEQ responses indicate what changes, if any, were made to the DSD in response to the comments.

Comment 1:

HAP Task Force:

Acute toxicity:

Hotchkiss *et al.* (2010) is adequately described in the draft DSD, but it was not adequately assessed in supporting development of the 1-h and 24-h ReVs. This data set was collected under a specific US Environmental Protection Agency ("EPA") guideline designed to address acute inhalation hazards, *e.g.*, from accidental release *(Acute Toxicity with BAL and Histopathology (Inhalation)* [OPPTS 870.1350]), and conducted under good laboratory practices (GLP), both of which underscore its reliability. Accordingly, the Hotchkiss *et al.* (2010) data set on acute inhalation hazards of EDC merits high confidence; application of an UF_D of 6 is overly conservative, and the recommendation for an additional data set to support this one is not appropriate and would represent an unnecessary use of animals.

TCEQ Response:

The TCEQ agrees that the Hotchkiss et al. (2010) study was a well conducted and reliable study, and as such the quality of the study was rated as high in the DSD. The UF_D, however, encompasses both the quality of the study used to derive the POD and the confidence in the overall database. Hotchkiss et al. (2010) is the only study that evaluated respiratory effects following EDC exposure, and only a single species was examined. Section 5.4 of the TCEQ guidelines states that case-specific factors could affect the database confidence category, such as whether the available studies examined the likely most sensitive effects (TCEQ 2015). Additional studies may have revealed a more sensitive species or respiratory endpoint. EDC also shows a very steep dose-response curve with respect to mortality depending on the species examined, as detailed in the evaluation conducted by California's OEHHA (2000), and this also appears to be true with respect to severity of effect. For example, mild respiratory effects were observed in rats following a single exposure of 100 ppm (Hotchkiss et al. 2010), while death

occurred following multiple exposures to 100 ppm in rabbits and 300 ppm in rabbits and rats (Rao et al. 1980). This consideration combined with limitations in the database (see above) justifies a UF_D of 6.

Comment 2:

HAP Task Force:

Reproductive and Developmental Toxicity:

Payan *et al.* (1995), which evaluated the dose-response for maternal and developmental toxicity in rats following oral and inhalation exposure to EDC, was not included in this assessment. Payan *et al*. (1995) concluded that, although there was maternal toxicity at the highest doses (both inhalation and oral), EDC did not demonstrate selective toxicity to the developing embryo/fetus. These results should be included in the DSD. The Extended One-Generation Reproductive Toxicity Study (EOGRTS) reported by Charlap (2015), although not published, was conducted according to the recent EOGRTS OECD 443 guideline and under GLP, and therefore is considered a reliable data set. The results should be more fully described in the DSD.

TCEQ Response:

The TCEQ appreciates this additional information and has added the Payan et al. (1995) study to the DSD. In regards to the Charlap (2015) study, although it was unpublished and examined the effects of EDC following oral administration, this study was included in the DSD. However, inhalation data are preferred over oral data when developing inhalation reference values (TCEQ 2015), and therefore a stronger emphasis was placed on the inhalation studies.

Comment 3:

HAP Task Force:

Genotoxicity:

There is a large database of information available to assess the potential for EDC to induce genotoxic effects, including both *in vitro* and *in vivo* approaches. Although *in vitro* data with appropriate metabolic activation are mostly positive, there is a significant amount of negative *in vivo* genotoxicity/mutagenicity data.

In particular, two *in vivo* studies not mentioned in the current draft DSD may be new information for TCEQ to consider. One is a published report of an *in vivo* transgenic rodent mutation assay, conducted in *lacZ* mice (MutamouseTM) (Hachia and Motohashi, 2000). The other one is only available as a final report currently, but was conducted according to the (then-draft) OECD guideline and under GLP (Hotchkiss *et al.* 2014). Both of these studies demonstrate no *in vivo* induction of genotoxic effects: one with gene mutation as measured in transgenic MutaMouseTM *(lacZ);* the other with DNA damage as measured by the Comet assay, which evaluates primary DNA damage that precedes both gene mutation and chromosomal effects.

While the earlier work, conducted in mice, pre-dates the guidelines, it did employ many of the aspects of current guideline requirements for OECD 488, although not all of them. Nonetheless,

it is an adequate study that demonstrates no increase in gene mutation *(lacZ)* in the target tissue of liver (or testes) from mice exposed to a single injection of 150 mg EDC/kg bw, or to repeated injections for a total dose of 280 mg EDC/kg body weight:

"No increase was detected in the frequency following DCE administration of single doses of up to 150 mg/kg or of consecutive injections of up to 280 mg/kg." (Hachia and Motohashi, 2000).

The more recent study was conducted to elucidate MOA for the mammary tumors induced in rats following EDC exposure, reported by Nagano *et al.* (2006); it was sponsored by the 1,2-Dichlorethane REACH Consortium and conducted at the Dow Toxicology and Environmental Research and Consulting laboratory, led by Dr. J.A. Hotchkiss. That Consortium has agreed to provide the full report to TCEQ, so only a few critical aspects of the data set will be addressed here. This OECD 489 guideline, GLP study included 4 weeks of repeated inhalation exposure to 160 ppm EDC, a level higher than the one resulting in mammary tumors in the Nagano *et al.* (2006) study. The Comet assay did not demonstrate any increase in Comet-related DNA damage in the target mammary tissue; the positive control (methylnitrosourea, MNU) demonstrated an increased Comet response in mammary tissue, confirming both the appropriate conduct of the assay in mammary tissue and the validity of the negative Comet results for the EDC-treated mammary tissue. Although no non-target tissues were evaluated for DNA damage, the presence of increased levels of EDC-induced S-(2-guanylethyl) glutathione adduct (GEG) in mammary tissue and in liver confirmed internal exposure to EDC. One additional genotoxicity-related endpoint was evaluated, the endogenously present 8-hydroxy-2 ’-deoxyguanosine (8-OH-dG) adduct, which can be increased following induction of oxidative stress; levels of this adduct were not different in the exposed tissues compared to controls in both mammary and liver tissue. These data provide clear evidence that internal exposure to EDC at these levels did not result in any increase in a very sensitive measure of DNA damage in the target, mammary tissue, while a positive control did result in an increase in DNA damage.

TCEQ Response:

The TCEQ appreciates this summary and has added some information to Section 4.2.8 of the DSD in response to these comments. This supports the conclusion already stated in the DSD that the MOA for EDC-induced tumors has not been demonstrated to be mutagenic. However, extensive new detailed information was not added as the data would not affect the MOA analysis or the use of a linear low-dose extrapolation method (see response to Comment 5).

Comment 4:

HAP Task Force:

Carcinogenicity:

As stated in in our December 4, 2015 letter requesting an extension of the comment period, there are several bioassays reported for EDC, but the Task Force recommends relying on the Nagano *et al.* (2006) peer-reviewed published data set describing an inhalation bioassay conducted in rats and mice. This is based on the fact that, given the volatility of EDC, inhalation exposure is the most likely exposure route for worker and general populations, and that the Nagano *et al.* (2006) study was conducted in accordance with OECD 451/453 and under GLP. In fact, the rat data on

mammary tumors from this data set provide the preferred dose-response data set to use to set cancer-related chronic values for EDC.

The DSD should not rely on the mouse liver hemangiosarcoma data for the following reasons:

- The authors of Nagano *el al.* (2006) state: "Neither hemangiosarcomas in the liver of male mice nor malignant lymphomas in the lymph node of female mice were likely to be causally related to the DCE [1,2-dichioroethane, or EDC] exposure, since no significant dose response relationship was found in the observed incidences of hemangiosarcomas or malignant lymphomas in the DCE-exposed groups." Therefore, other cancer endpoints should be evaluated (see below).
- The data for hemangiosarcoma of the liver in male mice did not demonstrate a normal doseresponse *(e.g.,* increased induced tumors with increased exposure); in particular, the number of hemangiosarcomas of the liver did not increase with increasing exposure from 30 ppm to 90 ppm -- for this 3-fold increase in exposure, the number of tumors actually decreased (6/50 at 30 ppm vs 5/50 at 90 ppm). This anomaly is a significant constraint to the usefulness of the data on this tumor, especially as a basis for a quantitative assessment such as ESL development.
- This is not the same endpoint as the NCI oral cancer bioassay used by EPA previously, although the draft DSD states (at p. 33) that it is the same: "USEPA (1991) used the same cancer endpoint in male rats to derive their URF, although based on a different route of exposure *(i.e.,* the oral exposure study by NCI 1978), which strengthens the use of this candidate cancer endpoint." The NCI bioassay identified circulatory hemangiosarcomas in male rats, not hemangiosarcomas of the liver in male mice.

A better choice would be to base the chronic ESL_{cancer} for EDC on the rat mammary tumor data reported by Nagano et al. (2006), for the following reasons:

- Rat mammary tumors were identified in the NCI oral bioassay and the Nagano et al. (2006) inhalation bioassay,
- Rat mammary tumor data from the Nagano et al. (2006) study demonstrated a better dose response out of all the tumor endpoints analyzed,
- The rat mammary tumor data lend themselves to quantitative analysis as the most sensitive response, since it was the only tissue where the tumor incidence increased with a statistically significant concentration-response that was above the historical control range at 40 and 160 ppm, and showed a statistically significant increase at 160 ppm in comparison to the concurrent control.

TCEQ Response:

The TCEQ appreciates this consideration of the critical endpoint used for the derivation for the cancer-based risk value. After detailed review of the available data and due consideration of relevant information (e.g., PBPK results showing the non-linear relationship between EDC dose and liver metabolite concentrations), the TCEQ concluded that the data for combined mammary tumors in rats is more scientifically defensible for use as the critical endpoint. The DSD has been revised accordingly.

Comment 5:

HAP Task Force:

Mode-of-Action for Cancer:

The recent MOA study (Hotchkiss *et al.* 2014), which evaluated several potential MOA endpoints in female rats following a 4-week exposure to 160 ppm EDC, provides important insight and context *vis-à-vis* a potential MCA for mammary gland tumors induced by exposures to EDC.

The negative results in rat mammary tissue from the *in vivo* Comet assay raise the possibility that EDC likely induces mammary tumors in EDC-exposed rats by a non-genotoxic MOA, although the specifics of this MOA cannot be determined from the results of the current study. Nevertheless, we would like TCEQ to consider the results of the mechanistic study, especially the negative *in vivo* Comet assay, in developing a chronic (cancer) ESL value, as it would influence the choice of approach in the dose-response (non-linear versus linear).

TCEQ Response:

As previously mentioned, additional information was added to the DSD in response to these comments, which supports the conclusion already stated in the DSD, that the MOA for EDC-induced tumors has not been demonstrated to be mutagenic. On the other hand, currently available information is also inadequate to demonstrate a non-mutagenic MOA, which would be required (along with dose-response data for a non-mutagenic precursor key event in the carcinogenic MOA) to deviate from a default linear low-dose extrapolation.

Comment 6:

HAP Task Force:

Section 3.1.2.2.1 (lines 10- 11)

The study by Hotchkiss *et al.* (2010) was conducted according to regulatory guidance for an enforceable consent agreement ("ECA") between the HAP Task Force and the US Environmental Protection Agency ("EPA"), not testing for the HPV Chemical Program. This is a critical point because a special testing protocol was developed for the ECA in order to provide toxicity data on the respiratory system associated with accidental release and acute exposure of hazardous air pollutants ("HAPs"). The testing of EDC using this protocol is one of the reasons why an uncertainty factor of 6 for database uncertainty is unjustified for the 1-hour and 24-hour ReVs (see comments below for section 3.1.5 and 4.1.6).

TCEQ Response:

See the response to Comment 1.

Comment 7:

HAP Task Force:

Section 3.1.2.3 (pages 12-13):

The rabbit teratology study by Rao *et al.* (1980) reported maternal toxicity at both 300 ppm (3/19) and at 100 ppm (4/21) EDC.

In the rat teratology study by Rao *et al.* (1980), there was marked mortality in the 300 ppm group (10/16). Only one of the surviving females was pregnant at the time of Cesarean section and all of her implantations were resorbed. The embryotoxicity seen in this one animal was considered secondary to the maternal toxicity that occurred. Also, exposure of rats to 100 ppm EDC did not affect the *incidence* (and not the rate as stated in the proposed DSD) of pregnancy.

The developmental toxicity by Payan *et al.* (1995) should be added in this section. In this study, rats were exposed by inhalation to 0, 150, 200, 250, or 300 ppm EDC during GD 6 to 20.

On line 36 of page 12, the study by Charlap (2015) is an *extended* one-generation reproductive toxicity (EOGRT) study (OECD 443). This is a relatively new type of protocol that includes the option for developmental neurotoxicity and developmental immunotoxicity. On line 37 of page 12, it should be noted that Charlap (2015) was sponsored by the HAP Task Force in satisfaction of the ECA. In addition, the summary of Charlap (2015) on page 13 is incomplete. It should include the results of the developmental endpoints (including thyroid hormone levels) and the developmental neurotoxicity portion of the study.

As PBPK modeling was used to extrapolate the oral doses in the Charlap (2015) EOGRT study to inhalation exposure, it would be informative to include the oral reproductive and developmental toxicity studies on EDC, specifically Lane *et al.* (1982) and Payan *et al.* (1995).

TCEQ Response:

The TCEQ has updated the DSD in regards to the corrections listed above. As for additional information, inhalation data are preferred over oral data when developing inhalation reference values (TCEQ 2015), and therefore a stronger emphasis was placed on the inhalation studies.

Comment 8:

HAP Task Force:

Section 3.1.4.2 (page 14) and Section 3.2.4.2 (pages 17-18):

Based on EPA (2005) criteria which are used by TCEQ in its TCEQ Guidelines to Develop Toxicity Factors (2015) document, EDC is considered soluble, not very soluble.

Gargas *et al.* (1989) reported that the blood:air partition coefficients for EDC were 19.5 for humans and 30.4 for rats. Thus, the statement in the proposed DSD that data are lacking for the blood:air partition coefficients is incorrect.

TCEQ Response:

The TCEQ appreciates this information and has changed the characterization of the solubility of EDC to soluble, and added human and rat blood:air partition coefficients for EDC, citing D'Souza et al. (1987). However, TCEQ guidelines state that if the animal/human ratio of the blood:gas partition coefficients is greater than 1, a default value of 1 is used (TCEQ 2015). Therefore, the POD_{HEC} remains equal to the POD_{ADJ} of 2.0833 ppm.

Comment 9:

HAP Task Force:

Section 3.1.5 (pages 14-15) and Section 3.2.5 (page 18):

TCEQ justifies an uncertainty factor of 6 for database uncertainty (UFD) because "only a single study looked at possible nasal/respiratory effects, and additional studies could provide more insight into this target region." However, the study by Hotchkiss *et al.* (2010) was conducted under the ECA between the Task Force and EPA, using a specific guideline specified in the ECA -- *Acute Toxicity with BAL and Histopathology (Inhalation) [OPPTS 870.1350]*. The objective of this study was to determine sublethal effects on the respiratory system associated with accidental release and acute exposure of HAPs. Hotchkiss *et al.* (2010) is a well-conducted, GLP, acute inhalation bioassay that evaluates a comprehensive array of endpoints, including an adequate evaluation of point-of-entry (POE) respiratory tract effects; this study established an unequivocal NOAEL. Thus, we submit that it would be unreasonable for TCEQ to add a factor of 6 for derivation of the acute ReVs because "additional studies could provide more insight into this target organ." In these circumstances this would be an unnecessary use of animals for toxicity testing.

Furthermore, TCEQ's Guidelines to Develop Toxicity Factors (2015) document states in Table 3-5 (page 81) that the UF_D is for "Extrapolation from valid results in laboratory animals when the data are "incomplete" and "intended to account for the inability of any single laboratory animal study to adequately address all possible adverse outcomes in humans." TCEQ also lists in Table 4-2 (page 118) the minimum database for high confidence for UF_D of 1: two inhalation bioassays in different species, and two prenatal developmental species in different species. EDC meets all of these test requirements for an UFD of 1 (with a confidence of high), in addition to the specialized acute toxicity study that is intended specifically to provide inhalation data on accidental release scenarios.

TCEQ Response:

See the response to Comment 1 and Comment 11.

Comment 10:

HAP Task Force:

Section 4.1.5.1 (pages 24-25):

The following equation is written incorrectly: $POD_{ADJ} = POD_{HEC} \times (D/24\ h) \times (F/7\ d)$.

The correct equation is: POD_{ADJ} = POD x (D/24 h) x (F/7 d)

TCEQ Response:

Consistent with this comment, the error has been corrected.

Comment 11:

HAP Task Force:

Section 4.1.5.2 (page 25):

Gargas *et al.* (1989) reported that the blood:air partition coefficients for EDC were 19.5 for humans and 30.4 for rats. Thus,

$POD_{HEC} = POD_{ADJ}$ X $((H_{b/g})_A / (H_{b/g})_H)$

= 2.0833 ppm x (30.4/19.5)

= 3.25 ppm

TCEQ Response:

The TCEQ appreciates this additional information, and has added human and rat blood:air partition coefficients for EDC, citing D'Souza et al. (1987). However, TCEQ guidelines state that if the animal/human ratio of the blood:gas partition coefficients is greater than 1, a default value of 1 is used (TCEQ 2015). Therefore, the POD_{HEC} remains equal to the POD_{ADJ} of 2.0833 ppm.

Comment 12:

HAP Task Force:

Section 4.1.6 (page 25):

TCEQ cannot justify an uncertainty factor of 6 for database uncertainty (UF_D) based on Table *5-2* (page 147) in TCEQ's Guidelines to Develop Toxicity Factors (2015) document. For a UF_D of 1, the following is required: two chronic bioassays in different species, one two-generation reproductive study, and two developmental toxicity species in different species.

EDC meets all of these test requirements for an UF_D of 1, with a confidence of high.

TCEQ Response:

As stated in the TCEQ Guidelines, "The minimum database confidence levels given in Table 5-2 for RfD/RfC derivation cannot represent the completeness of the overall database for a given chemical as many important details and considerations are not addressed, and use of Table 5-2 solely for this purpose would represent a significant oversimplification of scientific judgment necessary for the UF_D value selection process. Therefore, Table 5-2 should not be the sole consideration in selecting a UF_D value for a chemical." Such is the case for EDC. Although there are multiple studies available for EDC, very few identified levels of toxicity below that which

caused mortality. As with acute exposures, EDC shows a steep dose-response curve in regard to severity of effect following chronic exposures, with mild liver/kidney effects at 50 ppm and high toxicity/mortality at 250 ppm (Spreafico et al. 1980). This requisites due consideration when selecting the UF_D given the studies available. Additional studies could identify more sensitive endpoints, and unlike the Hotchkiss et al. (2010) study, the Spreafico et al. (1980) key study quality is medium. These combined database considerations justify a UF_D of 6.

Appendix – Request for an extension of the comment period received on December 4, 2015, and official comments received on February 19, 2016, from the HAP Task Force.

HAP Task Force
P.O. Box 331, Millwood, VA 22646 (540) 837-1602

December 4, 2015

Toxicology Division, MC 168
Texas Commission on Environmental Quality
P.O. Box 13087
Austin, TX 78711

Re: Draft Development Support Document ("DSD") for Ethylene Dichloride ("EDC")

Dear Sir or Madam:

I write on behalf of the HAP Task Force, a testing consortium of US manufacturers of EDC. Last year the Task Force provided information to the Commission ("TCEQ") in connection with its intent to prepare a DSD for EDC, and earlier this year we provided you the final report of an extended one-generation drinking water reproductive toxicity study on EDC sponsored by the Task Force in satisfaction of an enforceable consent agreement with the US Environmental Protection Agency ("EPA").

The Task Force has reviewed with interest the draft DSD for EDC. In light our preliminary review, we respectfully request an extension of the comment period by 60 days so that we may analyze recent mode-of-action ("MOA") data on EDC in female rat mammary tissue, which we believe may be very informative as to appropriate dose-response modeling decisions in developing a chronic ESL_{cancer} value for EDC. This is important because we note that TCEQ used Benchmark Concentration ("BMC") modeling to derive a point of departure ("POD"), and the BMCL10 based on the mouse liver hemangiosarcomas was calculated to be 9.02 ppm. In deriving the unit risk factor, however, TCEQ did not adjust the POD value for exposure duration (discontinuous exposure in the animal study to continuous exposure) and to account for animal-to-human differences. These adjustments are a necessary step in the derivation of cancer potency factors, as discussed in the *TCEQ Guidelines to Develop Toxicity Factors* (2015).

TCEQ used hemangiosarcomas of the liver in male mice as the critical endpoint for developing the chronic ESL_{cancer} value for EDC. This tumor endpoint is not the appropriate endpoint based on the following:

- The authors of Nagano *et al.* (2006) state: "Neither hemangiosarcomas in the liver of male mice nor malignant lymphomas in the lymph node of female mice were likely to be causally related to the DCE [1,2-dichloroethane, or EDC] exposure, since no significant dose response relationship was found in the observed incidences of hemangiosarcomas or malignant lymphomas in the DCE-exposed groups." Therefore, other cancer endpoints should be evaluated (see below).

- This is not the same endpoint as the NCI oral cancer bioassay used by EPA previously, although the draft DSD states (at p. 33) that it is the same: "USEPA (1991) used the same cancer endpoint in male rats to derive their URF, although based on a different route of exposure (*i.e.*, the oral exposure study by NCI 1978), which strengthens the use of this candidate cancer endpoint." The NCI bioassay identified circulatory hemangiosarcomas in male rats, not hemangiosarcomas of the liver in male mice.

A better choice would be to base the draft chronic ESL_{cancer} for EDC on the rat mammary tumor data reported by Nagano *et al.* (2006), for the following reasons:

- Rat mammary tumors were identified in the NCI oral bioassay and the Nagano *et al.* (2006) inhalation bioassay.
- Rat mammary tumor data from the Nagano *et al.* (2006) study demonstrated a better dose-response than the male mice liver hemangiosarcomas as well as other tumor endpoints analyzed.
- The rat mammary tumor data lend themselves to quantitative analysis as the most sensitive response, since it was the only tissue where the tumor incidence increased with a statistically significant concentration-response that was above the historical control range at 40 and 160 ppm, and showed a statistically significant increase at 160 ppm in comparison to the concurrent control.

More importantly, new data are available regarding the potential MOA in female rat mammary tissue. For example, a recent GLP *in vivo* Comet assay has shown that that EDC did not cause strand breaks in female rat mammary cells. This study also evaluated other MOA endpoints and thus could be very useful in conducting a more accurate quantitative cancer assessment to establish a more scientifically appropriate unit risk factor. Given a 60-day extension of the comment period, we believe that we could assess these new findings regarding their elucidation of the MOA and provide the results to TCEQ for evaluation.

Respectfully submitted,

Peter E. Voytek /WCN

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Manager

H A P
Task Force

P.O. Box 331, Millwood, VA 22646 (540) 837-1602

February 19, 2016

Toxicology Division, MC 168
Texas Commission on Environmental Quality
P.O. Box 13087
Austin, TX 78711

Re: Draft Development Support Document ("DSD") for Ethylene Dichloride ("EDC")

Dear Sir or Madam:

I write on behalf of the HAP Task Force, a testing consortium of US manufacturers of EDC. The Task Force appreciates the extension of the comment period on the draft DSD for EDC granted by the Commission ("TCEQ"), and is pleased to offer these comments for your consideration.

EDC is a data-rich chemical, with acute, sub-acute, subchronic, and chronic data from laboratory animals available for consideration in setting toxicity and risk values. While the draft DSD on EDC does a good job of describing much of the available data, several important studies (some published in peer-reviewed literature and some unpublished) were either missed entirely or were not appropriately assessed in the draft DSD. Some of these gaps or inappropriate assessments resulted in uncertainty factors ("UF") that are overly conservative, given the available data; some of the available data contributes to an improved understanding of genotoxicity potential and mode-of-action (MOA) of EDC, especially for carcinogenicity mechanisms. These points are discussed below, followed by some more detailed specific comments.

Acute toxicity:

Hotchkiss *et al.* (2010) is adequately described in the draft DSD, but it was not adequately assessed in supporting development of the 1-h and 24-h ReVs. This data set was collected under a specific US Environmental Protection Agency ("EPA") guideline designed to address acute inhalation hazards, *e.g.*, from accidental release (*Acute Toxicity with BAL and Histopathology (Inhalation)* [OPPTS 870.1350]), and conducted under good laboratory practices (GLP), both of which underscore its reliability. Accordingly, the Hotchkiss *et al.* (2010) data set on acute inhalation hazards of EDC merits high confidence; application of an UF_D of 6 is overly conservative, and the recommendation for an additional data set to support this one is not appropriate and would represent an unnecessary use of animals.

Reproductive and Developmental Toxicity:

Payan *et al.* (1995), which evaluated the dose-response for maternal and developmental toxicity in rats following oral and inhalation exposure to EDC, was not included in this assessment. Payan *et al.*

(1995) concluded that, although there was maternal toxicity at the highest doses (both inhalation and oral), EDC did not demonstrate selective toxicity to the developing embryo/fetus. These results should be included in the DSD. The Extended One-Generation Reproductive Toxicity Study (EOGRTS) reported by Charlap (2015), although not published, was conducted according to the recent EOGRTS OECD 443 guideline and under GLP, and therefore is considered a reliable data set. The results should be more fully described in the DSD.

Repeated Exposure and Chronic Toxicity Value Calculations:

There are some errors in the formulae as stated in the draft DSD, including on how the POD_{HEC} is calculated. In addition, there are published values for blood:air partition coefficients for EDC that should be cited and used.

Genotoxicity:

There is a large database of information available to assess the potential for EDC to induce genotoxic effects, including both *in vitro* and *in vivo* approaches. Although *in vitro* data with appropriate metabolic activation are mostly positive, there is a significant amount of negative *in vivo* genotoxicity/mutagenicity data.

In particular, two *in vivo* studies not mentioned in the current draft DSD may be new information for TCEQ to consider. One is a published report of an *in vivo* transgenic rodent mutation assay, conducted in *lacZ* mice (Mutamouse™) (Hachia and Motohashi, 2000). The other one is only available as a final report currently, but was conducted according to the (then-draft) OECD guideline and under GLP (Hotchkiss *et al.*, 2014). Both of these studies demonstrate no *in vivo* induction of genotoxic effects: one with gene mutation as measured in transgenic MutaMouse™ (*lacZ*); the other with DNA damage as measured by the Comet assay, which evaluates primary DNA damage that precedes both gene mutation and chromosomal effects.

While the earlier work, conducted in mice, pre-dates the guidelines, it did employ many of the aspects of current guideline requirements for OECD 488, although not all of them. Nonetheless, it is an adequate study that demonstrates no increase in gene mutation *(lacZ)* in the target tissue of liver (or testes) from mice exposed to a single injection of 150 mg EDC/kg bw, or to repeated injections for a total dose of 280 mg EDC/kg body weight:

"No increase was detected in the frequency following DCE administration of single doses of up to 150 mg/kg or of consecutive injections of up to 280 mg/kg." (Hachia and Motohashi, 2000).

The more recent study was conducted to elucidate MOA for the mammary tumors induced in rats following EDC exposure, reported by Nagano *et al.* (2006); it was sponsored by the 1,2-Dichlorethane REACh Consortium and conducted at the Dow Toxicology and Environmental Research and Consulting laboratory, led by Dr. J.A. Hotchkiss. That Consortium has agreed to provide the full report to TCEQ, so only a few critical aspects of the data set will be addressed here. This OECD 489 guideline, GLP study included 4 weeks of repeated inhalation exposure to 160 ppm EDC, a level higher than the one resulting in mammary tumors in the Nagano *et al.* (2006) study. The Comet assay did not demonstrate any increase in Comet-related DNA damage in the target mammary tissue; the positive control

(methylnitrosourea, MNU) demonstrated an increased Comet response in mammary tissue, confirming both the appropriate conduct of the assay in mammary tissue and the validity of the negative Comet results for the EDC-treated mammary tissue. Although no non-target tissues were evaluated for DNA damage, the presence of increased levels of EDC-induced S-(2-guanylethyl) glutathione adduct (GEG) in mammary tissue and in liver confirmed internal exposure to EDC. One additional genotoxicity-related endpoint was evaluated, the endogenously present 8-hydroxy-2'-deoxyguanosine (8-OH-dG) adduct, which can be increased following induction of oxidative stress; levels of this adduct were not different in the exposed tissues compared to controls in both mammary and liver tissue. These data provide clear evidence that internal exposure to EDC at these levels did not result in any increase in a very sensitive measure of DNA damage in the target, mammary tissue, while a positive control did result in an increase in DNA damage.

Carcinogenicity:

As stated in in our December 4, 2015 letter requesting an extension of the comment period, there are several bioassays reported for EDC, but the Task Force recommends relying on the Nagano *et al.* (2006) peer-reviewed published data set describing an inhalation bioassay conducted in rats and mice. This is based on the fact that, given the volatility of EDC, inhalation exposure is the most likely exposure route for worker and general populations, and that the Nagano *et al.* (2006) study was conducted in accordance with OECD 451/453 and under GLP. In fact, the rat data on mammary tumors from this data set provide the preferred dose-response data set to use to set cancer-related chronic values for EDC.

The DSD should not rely on the mouse liver hemangiosarcoma data for the following reasons:

- The authors of Nagano *et al.* (2006) state: "Neither hemangiosarcomas in the liver of male mice nor malignant lymphomas in the lymph node of female mice were likely to be causally related to the DCE [1,2-dichloroethane, or EDC] exposure, since no significant dose response relationship was found in the observed incidences of hemangiosarcomas or malignant lymphomas in the DCE-exposed groups." Therefore, other cancer endpoints should be evaluated (see below).

- The data for hemangiosarcoma of the liver in male mice did not demonstrate a normal dose-response (*e.g.*, increased induced tumors with increased exposure); in particular, the number of hemangiosarcomas of the liver did not increase with increasing exposure from 30 ppm to 90 ppm -- for this 3-fold increase in exposure, the number of tumors actually decreased (6/50 at 30 ppm vs 5/50 at 90 ppm). This anomaly is a significant constraint to the usefulness of the data on this tumor, especially as a basis for a quantitative assessment such as ESL development.

- This is not the same endpoint as the NCI oral cancer bioassay used by EPA previously, although the draft DSD states (at p. 33) that it is the same: "USEPA (1991) used the same cancer endpoint in male rats to derive their URF, although based on a different route of exposure (*i.e.*, the oral exposure study by NCI 1978), which strengthens the use of this candidate cancer endpoint." The NCI bioassay identified circulatory hemangiosarcomas in male rats, not hemangiosarcomas of the liver in male mice.

A better choice would be to base the chronic ESL_{cancer} for EDC on the rat mammary tumor data reported by Nagano *et al.* (2006), for the following reasons:

- Rat mammary tumors were identified in the NCI oral bioassay and the Nagano *et al.* (2006) inhalation bioassay,
- Rat mammary tumor data from the Nagano *et al.* (2006) study demonstrated a better dose-response out of all the tumor endpoints analyzed,
- The rat mammary tumor data lend themselves to quantitative analysis as the most sensitive response, since it was the only tissue where the tumor incidence increased with a statistically significant concentration-response that was above the historical control range at 40 and 160 ppm, and showed a statistically significant increase at 160 ppm in comparison to the concurrent control.

Mode-of-Action for Cancer:

The recent MOA study (Hotchkiss *et al.*, 2014), which evaluated several potential MOA endpoints in female rats following a 4-week exposure to 160 ppm EDC, provides important insight and context *vis*-à-*vis* a potential MOA for mammary gland tumors induced by exposures to EDC.

The negative results in rat mammary tissue from the *in vivo* Comet assay raise the possibility that EDC likely induces mammary tumors in EDC-exposed rats by a non-genotoxic MOA, although the specifics of this MOA cannot be determined from the results of the current study. Nevertheless, we would like TCEQ to consider the results of the mechanistic study, especially the negative *in vivo* Comet assay, in developing a chronic (cancer) ESL value, as it would influence the choice of approach in the dose-response (non-linear versus linear).

Additional details on several of the above points are found in the Appendix. We greatly appreciate the opportunity to provide these comments and hope that they are useful to TCEQ.

Respectfully submitted,

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Appendix

Specific Comments:

Section 3.1.2.2.1 (lines 10-11)

The study by Hotchkiss *et al.* (2010) was conducted according to regulatory guidance for an enforceable consent agreement ("ECA") between the HAP Task Force and the US Environmental Protection Agency ("EPA"), not testing for the HPV Chemical Program. This is a critical point because a special testing protocol was developed for the ECA in order to provide toxicity data on the respiratory system associated with accidental release and acute exposure of hazardous air pollutants ("HAPs"). The testing of EDC using this protocol is one of the reasons why an uncertainty factor of 6 for database uncertainty is unjustified for the 1-hour and 24-hour ReVs (see comments below for section 3.1.5 and 4.1.6).

Section 3.1.2.3 (pages 12-13)

The rabbit teratology study by Rao *et al.* (1980) reported maternal toxicity at both 300 ppm (3/19) and at 100 ppm (4/21) EDC.

In the rat teratology study by Rao *et al.* (1980), there was marked mortality in the 300 ppm group (10/16). Only one of the surviving females was pregnant at the time of Cesarean section and all of her implantations were resorbed. The embryotoxicity seen in this one animal was considered secondary to the maternal toxicity that occurred. Also, exposure of rats to 100 ppm EDC did not affect the *incidence* (and not the rate as stated in the proposed DSD) of pregnancy.

The developmental toxicity by Payan *et al.* (1995) should be added in this section. In this study, rats were exposed by inhalation to 0, 150, 200, 250, or 300 ppm EDC during GD 6 to 20.

On line 36 of page 12, the study by Charlap (2015) is an *extended* one-generation reproductive toxicity (EOGRT) study (OECD 443). This is a relatively new type of protocol that includes the option for developmental neurotoxicity and developmental immunotoxicity. On line 37 of page 12, it should be noted that Charlap (2015) was sponsored by the HAP Task Force in satisfaction of the ECA. In addition, the summary of Charlap (2015) on page 13 is incomplete. It should include the results of the developmental endpoints (including thyroid hormone levels) and the developmental neurotoxicity portion of the study.

As PBPK modeling was used to extrapolate the oral doses in the Charlap (2015) EOGRT study to inhalation exposure, it would be informative to include the oral reproductive and developmental toxicity studies on EDC, specifically Lane *et al.* (1982) and Payan *et al.* (1995).

Section 3.1.4.2 (page 14) and Section 3.2.4.2 (pages 17-18)

Based on EPA (2005) criteria which are used by TCEQ in its TCEQ Guidelines to Develop Toxicity Factors (2015) document, EDC is considered soluble, not very soluble.

Gargas *et al.* (1989) reported that the blood:air partition coefficients for EDC were 19.5 for humans and 30.4 for rats. Thus, the statement in the proposed DSD that data are lacking for the blood:air partition coefficients is incorrect.

Section 3.1.5 (pages 14-15) and Section 3.2.5 (page 18)

TCEQ justifies an uncertainty factor of 6 for database uncertainty (UF_D) because "only a single study looked at possible nasal/respiratory effects, and additional studies could provide more insight into this target region." However, the study by Hotchkiss *et al.* (2010) was conducted under the ECA between the Task Force and EPA, using a specific guideline specified in the ECA -- *Acute Toxicity with BAL and Histopathology (Inhalation)* [OPPTS 870.1350]. The objective of this study was to determine sublethal effects on the respiratory system associated with accidental release and acute exposure of HAPs. Hotchkiss *et al.* (2010) is a well-conducted, GLP, acute inhalation bioassay that evaluates a comprehensive array of endpoints, including an adequate evaluation of point-of-entry (POE) respiratory tract effects; this study established an unequivocal NOAEL. Thus, we submit that it would be unreasonable for TCEQ to add a factor of 6 for derivation of the acute ReVs because "additional studies could provide more insight into this target organ." In these circumstances this would be an unnecessary use of animals for toxicity testing.

Furthermore, TCEQ's Guidelines to Develop Toxicity Factors (2015) document states in Table 3-5 (page 81) that the UF_D is for "Extrapolation from valid results in laboratory animals when the data are "incomplete" and "intended to account for the inability of any single laboratory animal study to adequately address all possible adverse outcomes in humans." TCEQ also lists in Table 4-2 (page 118) the minimum database for high confidence for UF_D of 1: two inhalation bioassays in different species, and two prenatal developmental species in different species. EDC meets all of these test requirements for an UF_D of 1 (with a confidence of high), in addition to the specialized acute toxicity study that is intended specifically to provide inhalation data on accidental release scenarios.

Section 4.1.5.1 (pages 24-25)

The following equation is written incorrectly: $POD_{ADJ} = POD_{HEC} \times (D/24\ h) \times (F/7\ d)$.

The correct equation is: $POD_{ADJ} = POD \times (D/24\ h) \times (F/7\ d)$

Section 4.1.5.2 (page 25)

Gargas *et al.* (1989) reported that the blood:air partition coefficients for EDC were 19.5 for humans and 30.4 for rats. Thus,

$POD_{HEC} = POD_{ADJ} \times ((H_{b/g})_A / (H_{b/g})_H)$

$= 2.0833\ ppm \times (30.4/19.5)$

$= 3.25\ ppm$

Section 4.1.6 (page 25)

TCEQ cannot justify an uncertainty factor of 6 for database uncertainty (UF_D) based on Table 5-2 (page 147) in TCEQ's Guidelines to Develop Toxicity Factors (2015) document. For a UF_D of 1, the following is required: two chronic bioassays in different species, one two-generation reproductive study, and two developmental toxicity species in different species.

EDC meets all of these test requirements for an UF_D of 1, with a confidence of high.

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