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DATE: September 14, 2016

SUBJECT: Responses to comments by Dr. Weisueh A. Chiu on DPR-HHAB's draft 1,3-Dichloropropene Risk Characterization Document dated August 31, 2015

Dr. Weisueh A. Chiu Dr. Chiu submitted comments on the Department of Pesticide Regulation Human Health Assessment Branch (DPR-HHAB) draft 1,3-Dichloropropene (1,3-D) Risk Characterization Document (RCD) in a memorandum dated November 17, 2015. The following paragraphs provide those comments---which were based on the charge questions posed to reviewers by DPR-HHAB---along with DPR-HHAB's detailed responses.

Please find below: Section 1. Responses to Hazard Identification and Risk Assessment Comments, and Section 2. Responses to Exposure Assessment Comments

Section 1. Responses to Hazard Identification and Risk Assessment Comments

Charge question #1: The bodyweight decrement was selected as a critical endpoint in the acute risk assessment of 1,3-D.

Data on clinical or pathologic signs were not adequate in strictly acute inhalation studies to set threshold for acute toxicity, as the high dose ranges used in those studies were designed to determine LC_{50}s. Consequently, toxicological studies not limited to a single day treatment but reporting findings shortly after the onset of exposure (usually up to 7 consecutive days) were considered for identifying acute NOELs.

As discussed on page 88 of the document (page 100 of the PDF), nine inhalation subchronic, chronic and developmental toxicity studies reported effects occurring at early time points. The

most common and sensitive effects in these studies were a reduction in body weight and/or body weight gain, observed in rats, mice and rabbits.

Dr. Chiu comment: I agree that for acute toxicity, given the available data, the selection of body weight decrement as the critical endpoint is based on sound scientific knowledge, methods, and practices.

DPR-HHAB response: No response necessary.

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Charge question #2: The effect of 1,3-D on bodyweight was assumed to be systemic in nature, implying that it had to be absorbed into the blood and distributed throughout the body before it could cause the effect. The Regional Gas Dose Ratio (RGDR) approach for systemic effects was used to adjust the dose in the animal inhalation experiments to Human Equivalent Concentrations (HEC).

As discussed on page 145, it is plausible that the body weight effect was not systemic in nature, but rather resulted from portal-of-entry impacts on the nasal passages and lung. If the body weight effect was mediated at extrathoracic sites and considered to be generalized expressions of animal stress, the acute HECs would be different. For other chemicals, respiratory irritation has been shown to lead to body weight decrements. However, there were no experimental data to support this contention for 1,3-D, and longer-term exposures resulted in clear systemic toxicity, including decreases in body weight.

Dr. Chiu comment: I agree that, in the absence of more specific mechanistic data on the mode of action for body-weight effects, the selection of the RGDR for systemic effects to convert to a HEC is appropriate and based on sound scientific knowledge, methods, and practices.

I agree that while it is possible that respiratory effects are the cause of the body weight decrements, there is no direct evidence for this hypothesis. Additionally, it should be noted that several studies via oral exposure, including some by gavage (where palatability would not be an issue), showed decreased body weights. Moreover, even for feed studies, decreased food intake may result from systemic toxicity rather than palatability. Thus, more weight is given to a systemic toxicity explanation. CalEPA should consider strengthening the justification for body

weight being a systemic effect by drawing upon the results from oral studies, or any other relevant data.

> **DPR-HHAB response**: Body weight decrements were ubiquitous in short term inhalation studies with 1,3-D. As such, we considered them to be defensible critical acute endpoints and discussed them thoroughly in the draft RCD. In the revision we included an additional study which provided further evidence for inhalation-induced short term weight decrements (Coate, 1979). Interestingly, benchmark dose analysis of the Coate body weight data generated a $BMCL_{1\sigma}$ of 6 ppm, which was substantially lower than the BMCLs in the other studies, which fell between 40 and 66 ppm. While we considered 6 ppm to be an outlier, we also emphasized that the ultimate critical value of 49 ppm could conceivably underestimate acute / short term toxicity[1].

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Charge question #3: Due to the use of the RGDR approach, the conventional interspecies uncertainty factor of 10 was reduced to 3 for the general population. The interspecies uncertainty factor of 10 was retained to protect infants or children under scenarios where exposure might be anticipated. As discussed on page 146 (page 158 of the PDF), RGDR is considered to remove the 3-fold pharmacokinetic portion while retaining the 3-fold pharmacodynamic portion of the interspecies factor.

No data were available for any exposure length to assess the possibility of special sensitivity of infants or children. The 10-fold intraspecies factor was retained, as there was no indication of the range of sensitivity at any exposure length within the human population.

Dr. Chiu comment: For the general population, I agree that, in the absence of chemical specific data on toxicodynamics, the use of an interspecies UF of 3 is appropriate and based on sound scientific knowledge, methods, and practices.

For infants and children, the statement in the charge question above appears to be incorrect. In Table IV.2.a., an interspecies UF of 3 was applied even for the infants and children scenario. For

[1] The Coate (1979) data were not used to characterize acute / short term risk, as they were considered too far outside the narrow range established in the other five studies. In addition, the purity of the test article used by Coate was not characterized, prohibiting calculation of an HEC.

systemic effects, this choice is appropriate and based on sound scientific knowledge, methods, and practices. Instead, a database UF of 3 was applied for scenarios involving infants and children. This was justified by stating (page 99, footnote g) that "As no toxicity studies were conducted on young animals, this analysis had no way of assessing the possibility that infants and children might be more susceptible to the toxic effects of 1,3-D ... In addition, the lack of default surface area values [for] infants and children precluded RGDR-based calculations based on these demographics." There are several issues/inconsistencies here:

(a) There is a two-generation reproductive study in rats (Breslin *et al.*, 1987), which presumably would have been informative as to acute toxicity in the neonates and pups.

(b) The lack of default surface area calculations is not an adequate basis for justifying a database factor- it is a dosimetry issue that would apply no matter what the chemical-specific database is. Moreover, the surface area issue only applies to portal of entry effects, not the systemic effects at issue here.

Therefore, CalEPA should reconsider whether a database factor is needed for acute exposures involving infants and children or better justify this choice.

DPR-HHAB response: Dr. Chiu is correct that the statement in the charge question, "The interspecies uncertainty factor of 10 was retained to protect infants or children under scenarios where exposure might be anticipated" was incorrect. As he notes in his answer, the interspecies UF of 3, which was based on pharmacodynamic uncertainties, was retained for all populations including children, though a database UF of 3 was added in the latter case due to the absence of inhalation toxicity studies on young animals.

Dr. Chiu questions the use of the added database UF of 3 for children because very young animals were exposed without apparent effect in a 2-generation reproductive toxicity study (Breslin *et al.*, 1987). However, this study was not sufficient to ameliorate our concern that young animals had special sensitivity to this chemical, particularly as the neonates were not subjected to direct 1,3-D exposure until 5-7 weeks. (Note: during lactation, the mothers were placed in the exposure chambers without their offspring.)

We agree with Dr. Chiu's second point that the lack of child respiratory surface area values was not an adequate basis for establishing a database UF. The footnote with this

specific reference has been removed from the revised RCD. Nonetheless, the reasoning for retaining the UF stands (page 176 of the revision):

"Despite the lack of evidence for developmental or reproductive toxicity, no data were available for any exposure length to assess the possibility of special inhalation sensitivity of infants or adolescents. Consequently, an additional database uncertainty factor of 3 was designated to protect these populations under scenarios where exposure might be anticipated. Three was chosen over 10 in recognition of the relative mildness of the critical endpoints for acute, subchronic and chronic toxicity. Nonetheless, the uncertainties inherent in the choice of such a factor are recognized."

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Charge question #4: The critical chronic effect (hyperplasia of the murine nasal epithelium) was assumed to result from an extra thoracic portal of entry mode of action. As discussed on page 95 (PDF page 107), the critical chronic NOEL of 5 ppm for this effect was adjusted to human equivalent concentrations using the RGDR scalar for an extrathoracic portal of entry mode of action. However, bladder effects were also noted in the critical study, demonstrating that in addition to a portal of entry effect, 1,3-D also had systemic effects under chronic conditions. If the critical chronic value on bladder effects was used, the human equivalent concentration would have been higher.

Dr. Chiu comment: The reviewer presumes that this charge question concerns both subchronic and chronic exposures. I agree that hyperplasia of the murine nasal epithelium can be assumed to result from exposure to the extrathoracic region, and use of an RGDR for extrathoracic effects would be appropriate and based on sound scientific knowledge, methods, and practices. Given the lack of chemical-specific information on extrathoracic dosimetry, the default approach to calculating the RGDR is also appropriate and based on sound scientific knowledge, methods, and practices. The reviewer notes, however, that for other compounds, computational fluid dynamics modeling has suggested that the RGDR may be closer to 1 for extra thoracic effects (see review by EPA 2012, and references therein). CalEPA should consider discussing the more recent data (albeit not on 1,3-D) related to the RGDR for extrathoracic effects, at the very least with respect to the uncertainties they imply in the default RGDR approach.

DPR-HHAB response: USEPA (2012) does indeed suggest that the extrathoracic RGDR is closer to 1 for several examined chemicals, essentially negating the default

relative minute volume-to-surface area relation between rodents and humans that underlied the RGDR determinations recommended in both the US EPA's 1994 position paper and used in the draft 1,3-D RCD to calculate human equivalent concentrations. However, due to the lack of experimental data on 1,3-D, we have elected to retain our original RGDR values for the subchronic and chronic HEC calculations (0.115 in rats and 0.198 in mice, respectively). Because these values are considerably less than one, the result is more health-protective than simply defaulting the RGDR to 1 in the absence of data. In view of Dr. Chiu's suggestion, we have added discussion on page 176 of the revised RCD to recognize the uncertainty.

Dr. Chiu comment (continued): As discussed above, a database UF of 3 was applied for scenarios involving infants and children. This was justified by stating (page 99, footnote g) that "As no toxicity studies were conducted on young animals, this analysis had no way of assessing the possibility that infants and children might be more susceptible to the toxic effects of 1,3-D ... In addition, the lack of default surface area values [for] infants and children precluded RGDR-based calculations based on these demographics." There are several issues/inconsistencies here:

(a) There is a two-generation reproductive study in rats (Breslin *et al.*, 1987), which presumably would have provided information about subchronic and chronic toxicity in the neonates and pups.

(b) The lack of default surface area calculations is not an adequate basis for justifying a database factor- it is a dosimetry issue that would apply no matter what the chemical-specific database is.

(c) Additionally, EPA (2012) recently reviewed information about children's inhalation dosimetry, which suggests that there are no significant differences with respect to extrathoracic dosimetry.

Therefore, CalEPA should reconsider whether a database factor is needed for subchronic and chronic exposures involving infants and children or better justify this choice.

DPR-HHAB response: DPR-HHAB's response to this suggestion is found above in the response to charge question #3.

Dr. Chiu comment (continued): Given these issues, CalEPA should also consider whether to include both nasal and bladder effects in its evaluation of subchronic and chronic non-cancer toxicity.

> **DPR-HHAB response:** The low RGDRs used to calculate extrathoracic human equivalent concentrations (HECs) result in far lower values than would be obtained using the systemic RGDR of 1 based on bladder histopathology. Thus regulation of 1,3-D air levels based on rodent extrathoracic endpoints would protect human populations from possible systemic effects.

Dr. Chiu comment (continued): It should be noted that in some studies, oral exposure also led to effects in the bladder (as well as cancer in the bladder). With respect to the nasal effects specifically, the reviewer does not understand why BMD modeling was not performed to derive a BMDL for the POD, rather than use of a NOEL. Specifically, the modeling could be done on the incidence of "at least slight hyperplasia." If other "supportive" effects are included (as referred to on page 93), then BMD modeling of those should be considered as well, since use of BMD modeling may lead to the conclusion that the "most sensitive" POD is a different endpoint. CalEPA should consider BMD modeling for nasal and other "supportive" effects for the subchronic and chronic assessments.

> **DPR-HHAB response:** We agree with Dr. Chiu's comment. The critical endpoints for the subchronic and chronic exposure durations were reanalyzed and are now expressed as BMCLs (16 ppm and 6 ppm, respectively) in the revised RCD. While these values do not differ greatly from the NOELs used in the draft RCD to characterize seasonal and annual risk, they emphasize that we used the most scientifically defensible means to arrive at our critical endpoints.

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Charge question #5: The linearized multistage cancer model was used for the estimation of the 1,3-D oncogenic risk. The use of this approach was supported by the apparent dose dependence of lung tumors in male mice and the evidence that 1,3-D is genotoxic *in vitro* and *in vivo*.

As discussed on pages 148-151, the incidence curve for bronchioloalveolar adenomas (9/49, 6/50, 13/49 and 22/50 at 0, 5, 20 and 60 ppm) in male mice suggests that at low concentrations 1,3-D would not induce tumors since the organism has the presumed capacity to detoxify the

chemical through metabolism and/or excretion. However, the available database is insufficient to support an oncogenic action of 1 ,3-D that operates with a threshold.

Dr. Chiu comment: There are several issues with respect to the cancer assessment that could bear additional discussion/consideration:

> (a) The evidence for 1,3-D carcinogenicity is largely from oral studies- in the available inhalation studies, it appears that only benign tumors have been observed. In the absence additional evidence, benign tumors would not generally be carried forth in dose-response assessment. If they are to be used, then additional justification is necessary. Specifically, the NTP study in mice (NTP, 1985) showed bronchioalveolar adenomas and carcinomas- and this finding would be adequate justification for doing dose-response analysis of the inhalation study adenomas. CalEPA should consider including discussion of the NTP (1985) finding of lung tumors via oral exposure in their discussion of carcinogenicity- otherwise, it would be difficult to justifying performing a cancer assessment on benign-only tumors.
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> **DPR-HHAB response:** DPR-HHAB makes no distinction between purportedly benign and malignant tumors when analyzing the dose-response of oncogens. The NTP (1985) mouse study was included in the risk assessment because it was one of several studies using alternate routes of exposure (oral in the NTP case) that showed a cancerous response to 1,3-D, thus strengthening the overall case for dose-response analysis. Indeed, the data from that study suggested that an adenoma-to-carcinoma progression could occur. However, even without demonstration of progression, the mouse inhalation tumor data were considered of great toxicologic significance.

Dr. Chiu comment (continued): (b) Given that both oral and inhalation studies have shown lung tumors, it may be reasonable to consider whether the tumors are due systemic (e.g., a metabolite) rather than a portal-of-entry effect. This would impact the calculation of the HEC. See also discussion below. CalEPA should consider whether a systemic effect is more appropriate for lung tumors, and whether to modify the HEC calculation accordingly.

> **DPR-HHAB response:** On pages 117-118 of the revised RCD, HHAB discusses the weight of evidence for a portal of entry *vs.* a systemic mode of action with respect to induction of bronchioloalveolar adenomas in male mice. While our view remains that the evidence tilts toward portal of entry, a systemic mode of action could not be excluded.

Consequently, the revised RCD contains oncogenic risk calculations for both modes of action.

The discussion of portal of entry *vs.* systemic mode of action (pp. 117-118 of the revised RCD) is quoted at length here:

For multistage dose modeling, the air concentrations used in the mouse study were converted to human equivalent concentrations (HECs) assuming two different mechanistic scenarios: (1) adenomas arose following direct interaction of inspired 1,3-D with the tracheobronchial and pulmonary epithelial surfaces of the lung. This portal-of-entry scenario would be similar to the subchronic and chronic induction of nasal epithelial hyperplasia, but requiring a much higher RGDR to compute an HEC because the ratio of minute volume to involved respiratory system surface area was much less for humans than for mice; and (2) adenomas arose following absorption and circulatory redistribution to the lung of 1,3-D or its metabolites. As the second scenario invokes systemic exposure, dose scaling from mouse to human utilized a default RGDR of 1, similar to our treatment of acute toxicity. We chose to characterize lung tumorigenesis in both ways because the data did not point overwhelmingly to one or the other scenario, though we felt ultimately that the evidence tilted to the portal of entry scenario. The following observations were marshalled in support of portal of entry: (a) upper respiratory irritation occurred after acute, subchronic and chronic exposure in rodents (Cracknell *et al.*, 1987; Nitschke *et al.*, 1990) and after acute exposure in humans (section III.B.1. above); in addition, rats decreased their breathing rate at 90 ppm (Stott and Kastl, 1986), which was interpreted as evidence for sensory irritation in the upper respiratory tract; (b) pharmacokinetic studies in rats showed definitively that inspired 1,3-D reaches the lower respiratory system (Stott and Kastl, 1986); (c) 1,3-D causes tumors on contact in other mouse tissues, including forestomach upon gavage exposure and skin (papillomas) upon dermal exposure (NTP, 1985); (d) skin sensitization resulted after dermal exposure in guinea pigs (Jeffrey, 1987); (e) oral, but not inhalation, exposure in rats caused liver adenomas, suggesting that local mechanisms were operative for liver tumors (Stott *et al.*, 1995). Supporting a systemic scenario is the following evidence: (a) 1,3-D is readily absorbed by the inhalation route in both rats (Stott and Kastl, 1986) and humans (Waechter *et al.*, 1992); (b) inhalation exposure leads to epithelial hyperplasia in the mouse bladder (Stott *et al.*, 1987) and, at higher concentrations, histopathologic changes in the kidneys, stomach and liver; (c) oral

exposure in mice caused bronchioloalveolar tumors similar to those developing from inhalation exposure, suggesting that even by the inhalation route, absorption might be required for tumor development (NTP, 1985), though it is also possible that oral dosing led to inhalation exposure through reflux of volatilized or non-volatilized 1,3-D (Sells *et al.*, 2007; Damsch *et al.*, 2011a; Damsch *et al.*, 2011b).

Dr. Chiu comment (continued): (c) If CalEPA were to draw upon the evidence of carcinogenicity from oral studies, then it is unclear why several other oral studies (beyond Stott 1995) were excluded from the detailed evaluation -*e.g.*, NTP (1985) (although this study was mentioned in the summary characterization). CalEPA should consider whether to include other oral carcinogenicity studies in its detailed evaluation.

DPR-HHAB response: Both the draft and the final versions of the 1,3-D RCD summarize all studies that produced tumors, whether by the inhalation route or any other route. Although detailed analyses did not appear in the draft RCD, they were available in the Toxicity Summary, which appear as Appendix IV in the draft and as Appendix X in the final version. This was done in order to support the more general point that 1,3-D is a carcinogen. Beyond that, we did not feel that further analyses on the relevant oral and dermal studies were called for because those exposure routes were not likely to influence the interpretation of the (route specific) inhalation-induced tumors.

Dr. Chiu comment (continued): (d) Although the issue of epichlorohydrin has been raised briefly on page 148, direct dosing of epichlorohydrin only resulted in portal of entry tumors, whereas the NTP study found evidence of systemic tumors as well. The issue of epichlorohydrin was also raised in the peer review of the NTP study, and its contributions were deemed unlikely (except perhaps with respect to forestomach tumors). Moreover, given the relative potencies and exposure levels, it is unclear that epichlorohydrin could explain the 1,3-D-induced tumors (a quantitative comparison could be made to address this point). If the NTP study is given more prominence, then CalEPA should consider modifying the text to better address the issue of epichlorohydrin.

DPR-HHAB response: Epoxidized soybean oil (2%) was added to the formulation as the stabilizing agent rather than epichlorohydrin in the mouse inhalation oncogenesis study (Stott *et al.*, 1987). Consequently, epichlorohydrin did not play a role in the genesis of inhalation-induced bronchioloalveolar tumors.

Dr. Chiu comment (continued): (e) As a side note, the comparison with the range of historical controls is completely inappropriate (page 101), as the range is related to how many studies there are. They should not be used to discount the tumors. See Elmore and Peddada (2009) (Elmore and Peddada, 2009) for discussion of the use of historical controls. CalEPA should consider modifying the text on the discussion of historical controls.

DPR-HHAB response: The following is the passage referred to by Dr. Chiu:

"Male mice exposed to 1,3-D by the inhalation route for 2 years exhibited a statistically elevated incidence of bronchioloalveolar adenomas at a nominal air concentration of 60 ppm (22/50: 44%; Table III.16) (Stott *et al.*, 1987). The incidence rate at 20 ppm (13/49: 27%) was also higher than concurrent controls (9/49: 18%) and low dose animals,(6/50: 12%) though a treatment relation was not certain, as 7 previous chronic studies showed a historical control incidence range between 7 and 32%."

We feel we did not over-interpret the historical control data, but that those data raised the possibility that adenoma incidence at the mid dose might not have been due to 1,3-D exposure. However, by subjecting the complete incidence dataset to linearized multistage analysis, we *effectively* rejected the possibility that incidence at the mid dose was independent of 1,3-D exposure.

Dr. Chiu comment (continued): (f) Metabolism is an important part of the carcinogenicity evaluation, however, the metabolism scheme shown in Figure III.1 appears incomplete- there is no mention of the CYP pathway, even though that appears to be important from a mutagenicity point of view. CalEPA should consider updating Figure III.1 to include the CYP pathway, for instance looking at the ATSDR Tox Profile (ATSDR, 2008) as well as seeing if there are any more recent references in the scientific literature.

DPR-HHAB response: We agree with Dr. Chiu and have modified Figure III.1 as suggested.

Dr. Chiu comment (continued): (g) The strongest hypothesis related to mutagenicity appears to be through epoxide formation via CYP. In this case, because the mouse lung has CYP activity, local bioactivation would be the most likely source of the active moiety, since it is unclear that an epoxide formed in the liver would be able to reach the lung (if so, then the evidence needs to be discussed). This could also explain oral carcinogenicity because circulating 1,3-D could reach

the lung and be bioactivated there. However, it is unclear what the CYP metabolic capacity of the lung for 1,3-D, so it remains to be determined whether sufficient mutagenicity can be elicited *in vivo* to explain the observed tumors. For instance, the big blue assay found no evidence of mutations in the lung after inhalation exposures (although it was noted that this study was not considered "acceptable" by CalEPA). Other evidence for genotoxicity/mutagenicity appears rather weak. Overall, there appears to be inadequate evidence to either confirm or rule out a mutagenic mode of action for lung tumors. CalEPA should consider augmenting the text to discuss this hypothesis in more detail.

DPR-HHAB response: The revised RCD now has two appendices (VI and VII) addressing the genotoxicity of 1,3-D in greater depth than in the draft RCD. The appendices state that 1,3-D epoxide is just one of several genotoxins that should be considered and that it is possible that several of these genotoxins could be involved and that different modes of action (point mutation *vs*. chromosome breakage) could be in play, depending on the test animals, exposure route and target tissue. The other genotoxins include: the parent compound 1,3-D (direct-acting, alkylating agent, TA100 mutagen); 3-chloro-2-hydroxypropanal (DNA-adduct forming chemical) formed from the hydrolysis of 1,3-D epoxide and possibly chloromethylglyoxal (presumably methylglyoxal-like in reactivity) formed from metabolic oxidation of 3-chloro-2-hydroxypropanal; 3-chloroacrolein (bifunctionally reactive alkylating agent, hisD3052 frameshift mutagen) formed presumably by P450-mediated oxidation of 1,3-D as well as by oxidation of 3-chlorallyl alcohol (the water hydrolysis product formed from 1,3-D) by alcohol dehydrogenase, catalase and (or) P450; possibly 3-chloroglycidol (presumably glycidol-like in reactivity) formed by epoxidation of 3-chlorallyl alcohol; possibly some reactive ester compound formed from 3-chloropropenoic acid (3-chloroacrylic acid); and possibly acrolein formed by the spontaneous decomposition of the S-oxide of the major mercapturic acid of 1,3-D excreted in urine.

Also as evident in the aforementioned appendices, 1,3-D and/or its metabolites can induce gene mutation in the Ames test and the mouse lymphoma TK assay, as well as induce sex-linked recessive lethals in *Drosophila* and sister-chromatid exchange in CHO cells. The importance of such positive *in vitro* results is not negated by a lack of positive effects in *in vivo* genotoxicity testing. In the case of the negative dominant-lethal testing, it only indicates that male germ cells were not affected by the specific inhalation exposure. The negative results do not address whether the target tissues for oncogenicity in the same males exhibited clastogenicity (the presumed cause of mutation in germ cells

this assay), just as the negative results ultimately do not address whether ooyctes would have been affected had the dominant-lethal testing been conducted with females instead of males. In the case of the bone-marrow polychromatic-erythrocyte (PCE) micronucleus test using gavage exposure of CD1 mice, those negative findings contrast with the positive findings after intraperitoneal injection of male B6C3F1 mice (the strain used in the NTP cancer bioassays), and by positive findings for induction of chromosome aberrations in bone-marrow cells in comparable testing also done by NTP. In addition, oral exposure of NMRI female mice (but not males) resulted in an even stronger induction of micronucleated bone marrow PCE's.

As for the negative findings in the testing in Big Blue mice, those findings may have resulted from testing that was not optimized to detect a mutagenic effect. Admittedly, the transgenic-animal testing done in 1996 may have been considered robust at that time. However, based on current OECD guidelines, the Big Blue study of 1,3-D can be faulted for a variety of crucial reasons, including too short administration time, not testing to an MTD, too short expression time, and no testing of a "full," positive control (*e.g.*, some known inhalation carcinogens causing lung cancer and [or] liver cancer).

Dr. Chiu comment (continued): (h) Given the lack of adequate mechanistic basis for a non-linear approach for cancer assessment, the use of a linearized multistage model is appropriate and based on sound scientific knowledge, methods, and practices.

DPR-HHAB response: Agreed.

Dr. Chiu comment (continued): (i) On page 149, the text states that the observed tumor dose-response data "suggest" a threshold. This is much too strong of a statement. Dose-response data with only 50 animals, and with a fairly large control rate, are completely uninformative as to a "threshold" or not. This paragraph provides no basis for questioning the multistage model, and should be deleted.

DPR-HHAB response: We feel that a study employing 50 animals/dose can provide valuable data regarding the possibility of a threshold mechanism for oncogenesis. In fact, the incidence data are not inconsistent with an operative threshold. For this reason, we have left this discussion largely intact in the revised RCD. We did, however, feel that calculating conditional MOEs assuming a threshold of 5 ppm added a quantitative aspect

to the discussion in view of the available data that may not be supported. Consequently, we removed all of these calculations in the revised RCD. The final paragraph of the discussion of the threshold possibility now reads (p. 180):

"For the reasons discussed above, we considered multistage modeling, with its low-dose linear constraints, to be the most appropriate approach to evaluating the oncogenic risk of 1,3-D. The resultant risk values for many non-occupational and occupational scenarios, expressed as the probability of cancer in humans exposed under specified conditions, were above the negligible risk standard of 10^{-6}. Nonetheless, a further uncertainty regarding mode of action exists. If it emerged that the oncogenic action of 1,3-D was entirely as a non-genotoxic promoter that operated with a threshold, then a tumor NOEL and resultant MOEs may be the more appropriate risk metric. However, since a threshold mechanism was not identified for this compound, and since insufficient animals were available to establish a threshold, oncogenic MOEs were not calculated."

Dr. Chiu comment: (j) With respect to the first two lines on page 151, the use of a tumor NOEL is an inappropriate basis for a non-genotoxic carcinogen. If a non-genotoxic mode of action is established, then that means a key precursor event (i.e., a necessary effect to induce cancer) has been identified. Thus, a BMD for the key precursor event (such as cytotoxicity or hyperplasia) would be the correct basis for determining the MOE, since it is presumed that protecting against the precursor event would prevent cancer. CalEPA should consider modifying the text to address this issue.

DPR-HHAB response: See the preceding response.

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Section 2. Responses to Exposure Assessment Comments

Charge question #1: Due to a lack of 1,3-D air monitoring data, DPR estimated certain agricultural handler exposures to 1,3-D using both 1,3-D data and data obtained from chloropicrin exposure studies. As described in "Table IV.4 Data Sources for Exposure Scenarios" (page 104), DPR applied the surrogate data approach for generating exposure estimates of five agricultural handler scenarios: shallow shank application method with tarp, drip application method with and without tarp, application using hand-wand, and tarp remover.

- As discussed on page 105 (page 117 of the PDF), the surrogate approach employed 1,3-D breathing-zone air concentrations for the applicator using a shallow shank (broadcast and without the use of a tarp); these air concentrations were adjusted using a ratio of surrogate data (page 108). The surrogate data ratio consists of the 95th percentile of the measured chloropicrin breathing zone air concentrations for the scenario of interest for 1,3-D exposure assessment over the 95th percentile of the measured chloropicrin breathing-zone air concentrations for the applicator using shallow shank.
- The chloropicrin air concentrations utilized in the ratio were corrected for recovery and adjusted to the same application rate.
- The 1,3-D breathing-zone air concentration for the scenario of interest was derived by multiplying the measured I ,3-D breathing-zone air concentrations for the applicator using a shallow shank (broadcast and without the use of a tarp) and the appropriate ratio.

Dr. Chiu comment: This is not my area of expertise, but the approach seems reasonable. However, CalEPA should consider whether it would be useful to provide additional justification for the use of chloropicrin data in the form of a comparison of relevant physicochemical properties.

> **DPR-HHAB response:** The surrogate ratio approach using chloropicrin is a reasonable first approximation of the 1,3-D air worker breathing zone air concentrations. Chloropicrin and 1,3-D do differ in their physical and chemical properties, and those differences produce differing patterns in mass loss following the application. However, both chloropicrin and 1,3-D tend to show small flux immediately following the application. For the majority of applications the maximum flux for both chloropicrin (Barry, 2014) and 1,3-D (Knuteson, 1992b; Knuteson, 1992a; Knuteson *et al.*, 1995; Gillis, 1998; Knuteson and Dolder, 2000; van Wesenbeeck and Phillips, 2000) occur 6 or more hours following application. In some studies the maximum flux occurs 24 hours or more following the application. The magnitude of flux will more likely be dominated by the application method itself, as application methods are reasonably similar between fumigants. The comparable small initial flux for most chloropicrin and 1,3-D applications supports this assumption and by extension, the surrogate ratio approach.

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Charge question #2: DPR employed a scaling approach for estimating residential bystanders exposures to 1,3-D due to shallow shank, deep shank, and drip application methods. As

discussed in "Residential Bystander Exposure Estimates (Edge of Buffer Zone)" (page 118), the 1,3-D air concentrations at 100 feet downwind from shallow shank, deep shank, or drip applications were generated using Industrial Source Complex Short-Term Model version 3 (ISCST3) with a nominal flux of 100 µg/m^2/s for all applications and all field sizes. This modeling approach allows for scaling of the air concentration from a given application rate of 1,3-D employed in the modeling to the maximum rate allowed.

Dr. Chiu comment: This is not my area of expertise, but the approach seems reasonable and based on sound scientific knowledge, methods, and practices.

DPR-HHAB response: No response necessary.

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Charge question #3: DPR evaluated the lifetime exposure to 1,3-D by individuals residing in a high 1,3-D use area using stimulated 1,3-D air concentration coupled with stochastic human exposure assessment models: Monte Carlo Annual-Based Lifetime Exposure model (MCABLE) and High-End Exposure version 5, Crystal Ball (HEESCB). As discussed in "Residential Bystander Exposure from Ambient Air (Modeling)" (page 122), long-term ambient air concentrations of 1,3-D are not available for estimating the lifetime exposure of residential bystanders. Hence, simulated air concentrations coupled with stochastic (*i.e.*, probabilistic) human exposure assessment models were used.

- The simulated air concentrations of 1,3-0 were generated by SOil Fumigant Exposure Assessment System (SOFEA©) version 2 (SOFEA-2) (page 122).

- The exposure estimates were generated using two stochastic human exposure assessment models: MCABLE and HEE5CB (page 123); the main differences between these models are the volume of data used per simulation (11664 values in HEE5CB versus 1.16 million values in MCABLE) (page 131) and residential-mobility assumptions employed for estimating exposures (page 129-131). HEE5CB has a more restrictive assumption than MCABLE in the time that an individual lives (i.e., residency) and spends (i.e., mobility) within different townships in a high I,3-D use area.

- In some cases, SOFEA-2 may have under-predicted the concentrations of I,3-D (Table V. 6 [page 164]). To minimize the potential impact of the air concentration under-

predictions, only the simulation air concentrations with annual average values equal to or higher than the observed mean value were included in the human exposure modeling (page 163).

Dr. Chiu comment: This is not my area of expertise, but the approach seems reasonable and based on sound scientific knowledge, methods, and practices. I support the use of Monte Carlo simulations for capturing uncertainty/variability in exposure assessments.

DPR-HHAB response: No response necessary.

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The Big Picture

Issue "a": (a) Are there any scientific issues not mentioned above that are part of the scientific basis of the draft risk assessment? If so, please comment on whether these are based on sound scientific knowledge, methods, and practices.

Dr. Chiu comment: Most of these were already addressed above. A few additional comments:

(a) Perhaps this is due to my unfamiliarity with pesticide registration, but it is unclear to me why more published literature was not included- in particular, there was not reference to a literature search to identify relevant studies published in the open scientific literature. While these will not mostly be "guideline" studies, any risk assessment should evaluate the totality of the evidence. Even if no additional studies were identified, this would usually be documented. CalEPA should consider performing and documenting a literature search to identify additional studies that may be relevant to this evaluation.

DPR-HHAB response: We agree with Dr. Chiu and have added the following passage to the Introduction section of the revised RCD:

"The following human health assessment concentrates exclusively on risks emanating from

inhalation exposure projected to occur in California under occupational, bystander and ambient scenarios. While the great majority of studies relevant to this evaluation were done by the Registrant to satisfy federal registration requirements, we also conducted a search for open literature publications relevant to the characterization of mammalian inhalation toxicity using PubMed (http://www.ncbi.nlm.nig.gov/pubmed/) and the search terms "telone", "telone II" and "1,3-dichloropropene". Following an initial screen to remove duplicates, the combined searches identified 91 potentially relevant studies published between 1976 and 2011. Two additional publications were identified following a search of the NTP database (http://ntp.niehs.nig.gov/). Screening of these studies did not reveal data that added significantly to the Registrant data already on file at DPR."

Dr. Chiu comment: (b) Additionally, in order to better inform risk management decision, CalEPA should consider whether to model the tarp remover scenario both with and without respiratory protection.

DPR-HHAB response: We agree with the reviewer that further investigation on the tarp remover exposure scenarios is needed. For the purpose of this risk assessment, our intention is to inform the Risk Manager of potential concerns, if any, on activities associated with agricultural handers. During the mitigation phase, additional work will be performed to refine the exposure estimates---*e.g.*, using computer modeling---for developing appropriate health protection measures.

Issue "b": Taken as a whole, is the scientific portion of this proposal based upon sound scientific knowledge, methods, and practices?

Dr. Chiu comment: Yes, taken as a whole, the scientific portion of this proposal is based upon sound scientific knowledge, methods, and practices. I anticipate that addressing my comments above would not lead to drastic changes in the overall conclusions.

DPR-HHAB response: No response necessary.

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