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DATE: May 25, 2020

SUBJECT: Response to comments by Dr. Michele Bouchard on DPR's draft Addendum to the 2006 Sulfuryl Fluoride Risk Characterization Document dated December 2018

I. Background

The Department of Pesticide Regulation requested external scientific review of its draft Addendum to the 2006 Sulfuryl Fluoride Risk Characterization Document according to the 2006 California Environmental Protection Agency External Scientific Peer Review Guidelines. Dr. Michele Bouchard from the Department of Environmental and Occupational Health of the University of Montreal in Montreal, Canada was one of the assigned reviewers asked to comment on the main assumptions and conclusions of the draft Addendum (see Appendix A). We sincerely appreciate the time and effort Dr. Bouchard spent in thoroughly reviewing and commenting on the draft and all four main conclusions. This memorandum addresses those comments. The final Addendum referenced throughout this response refers to DPR's final May 2020 Addendum to the Sulfuryl Fluoride Risk Characterization Document.

Response to Comments

Conclusion 1 – *The scientific basis for the proposed RfCs depends both on the nature of the observed effects (non-neurotoxic vs. neurotoxic) and on the assumed mode of action (systemic vs. portal of entry).*

Dr. M. Bouchard, comment 1: DPR may however want to consider the following comment with regard to Table 1. It is clear from this table that the difference in DPR 2006 and DPR 2017 is simply due to a reduction of UF_{DB} from 10 to 3. However, simply when looking at Table 1 and its footnotes, it is not clear what is different between the DPR 2006 and 2017 POD_{HEC} of 122 ppm, which considers a systemic MOA versus the POD_{HEC} of 75 ppm derived from $H_{b:g}$,

which again considers a systemic MOA. Perhaps this should be clarified in the footnote to this table, although from the normalization we can deduce that is due an adjustment for animal to human differences in BW and inhalation rate in the first case and a default Hb:g of 1 in the second case.

DPR response: In recognition of this comment, Table 1 has been rearranged such that the row titled "normalization method" immediately precedes the row titled "POD_{HEC}." As a result, readers should immediately understand the various assumptions used to calculate the human equivalent concentration points of departure (POD_{HEC}).

Bouchard, comment 2: However, in the main part of the Addendum, it is not clearly stated what other parts of the brain were assessed in some studies (hippocampus, striatum, cortex). I do see this mentioned however in Appendix E (page 7). I would suggest adding the information in the main part of the Addendum.

DPR response: DPR has indicated the brain regions examined in the revised text (pg. 41) of the final Addendum.

"It is worth noting that brain vacuolation in all inhalation animal studies (rat, mouse, rabbit, dog) was mainly confined to the basal ganglia, specifically in the area of the caudate-putamen (also known as the striatum). Other brain regions, including cerebral cortex, cerebellum, medulla oblongata (except for mouse), olfactory lobe, and thalamus/hypothalamus, did not show evidence of vacuolation in response to inhaled sulfuryl fluoride."

Bouchard, comment 3: A NOAEL of 300 ppm was observed based on neurological effects (functional observational batteries (FOB), motor activity determinations, and electrophysiological parameters) (pages 4, 36-37, 45, 49). The document mentions that no effect was observed at the highest dose administered of 300 ppm (such that there is no LOAEL in this study). Nevertheless, it is said that "recent advances in risk assessment methodologies" were considered in this report. A NOAEL is retained as a POD, whereas a benchmark dose (BMD) approach would be the best methodology according to recent advances. Perhaps it should be specified that the BMD approach could not be applied for lack of dose- response curves (no effect at the highest dose administered) and no other studies available to derive a clear dose-response based on acute data.

DPR response: DPR agrees with Dr. Bouchard's comment on BMD approach. The following statement has been added to Section III.C (pg. 42) of the final Addendum.

"None of the acute inhalation toxicity studies presented in Table 9 were suitable for BMD modeling."

Bouchard, comment 4: Pages 7 to 11 and 29 report pesticide illness and human poisonings. On page 9, for the 13 case reports (death), the main symptoms are often described as pulmonary

(pulmonary congestion and edema; alveolar hemorrhage). On pages 10-11, for non-lethal cases, main symptoms are also often described as pulmonary effects. It should be made clearer at this stage that the pulmonary symptoms are observed at concentrations equal or higher than neurotoxic effects. Later on page 29, DPR do however mention that: "effects were almost always observed at the same doses as those causing neurotoxic effects". It is further mentioned that "exception was the chronic dog study, where lung inflammation and alveolar macrophage aggregation were detected at a lowest observed effect level (LOEL) of 80 ppm, while neurotoxic effects such as brain vacuolation and malacia occurred at 200 ppm". This would suggest that pulmonary effect may occur at concentrations lower than neurotoxic effects. The most interesting data reported is the non-lethal case of a 9- year old boy intoxicated following house fumigation, where basal ganglia effects were observed as in rats (page 9; Mulay et al. 2016). Perhaps the DPR could clarify if no pulmonary effects were observed in the boy case study or if they were not documented in the study.

> **DPR response:** For pesticide illness data and human poisoning reports, the exposure levels were not known to compare the doses causing pulmonary and neurotoxic effects. With respect to the case study with the 9-year old boy, pulmonary effects were not reported (pg. 18). We have specified this in the final Addendum:
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> "Documentation of serum fluoride concentration and pulmonary effects was not apparent in his medical record."

Bouchard, comment 5: Page 11 mentions that new studies on main degradates of sulfuryl fluoride, fluorosulfate and fluoride have been reviewed by the DPR. However, this information is not directly used in the derivation of the updated acute RfC based on a possible direct nasal-to-brain transfer. In the latter case, default factors were used, rather than updating PBPK modeling to consider the pharmacokinetic (PK) differences between animals and humans or in UF_{DB}, which considers sensitivity in subpopulations. Perhaps it would be helpful to the readers to clearly state the importance of this new PK information from a risk assessment standpoint and a possible new methodological approach for the derivation of RfCs.

> **DPR response:** The new PK information suggests that direct nasal-to-brain transfer may account for the neurohistopathology of sulfuryl fluoride. Unfortunately, direct evidence for this new mode of action is lacking (see detailed discussion in Appendix E of the final Addendum). For this reason, DPR did not derive an acute reference concentration (RfC) based on this route of exposure. The importance of the new PK information was stated in the final Addendum as follows (see pg. 10):
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> "In particular, the pharmacokinetic data provide new insights into the mode of action of sulfuryl fluoride in inducing neurological effects."

Bouchard, comment 6: Page 12, the new analyzed *in vitro* data by Gollapudi et al. (2002, 2005) (not analyzed in DPR 2006) do support an extensive metabolism and suggest that

mutagenicity and clastogenicity would occur at concentrations much higher than 300 ppm (>2000 ppm). However, it is not clear if these data are presented to show the extensive metabolism and the potential toxic moieties or if it is to confirm that genotoxicity is not a critical effect.

DPR response: This Addendum was partly intended to update the toxicological database on sulfuryl fluoride. The two indicated studies were reviewed and presented in this Addendum mainly because they were not reviewed in the 2006 RCD. As noted by the reviewer, the findings of these two studies suggest that sulfuryl fluoride is genotoxic at high doses (see Section II.B). Further speculation as to the role of genotoxicity in the effects noted in this report was not deemed necessary.

Bouchard, comment 7: On page 12, it is mentioned that: "the mutation frequency at concentrations as low as 2000-4000 ppm, Analytical chemistry indicated". This sentence should be revised for punctuation. Furthermore, I would remove "as low as" because 2000-4000 ppm is not low from an exposure standpoint.

DPR response: Revised as suggested (see pg. 20 of the final Addendum):

"In both the non-activated and activated assays, there was a treatment-related increase in the mutation frequency at concentrations of 2000-4000 ppm, inclusive."

Bouchard, comment 8: On page 13, it is mentioned that DPR reviewed a special non-guideline postnatal DNT/toxicokinetic study in rat pups by Marty et al. (2015) that was submitted to both the U.S. EPA and DPR in 2015. It is also mentioned that this study was conducted to "address inadequacies in the toxicity database concerning neurotoxicity". The DNT study shows a LOAEL at 150 ppm based on elevated motor activity but with no brain lesions (Marty et al., 2015). Perhaps it should be clarified at this stage that this LOAEL of 150 ppm based on weight gain and the LOAEL of 20 ppm for elevated motor activity were considered in the updated derivation of short-term RfC. We do however understand that from Table 10 on page 38. Regarding the oncogenicity data mentioned on page 13, DPR mentions that epidemiological studies do not point to carcinogenicity of fluoride after inhalation exposure with a reference to ATSDR (2003). Perhaps it should be made clear that there are no other inhalation studies than those reviewed by ATSDR 2003 or if so results from these newer studies should be mentioned.

DPR response: Revised as suggested:

"The 20-ppm LOEL for elevated motor activity in pups was considered in the updated derivation of the short-term RfC (see Section IV.C. RfCs for Short-Term (10-14 days) Toxicity)." (See pg. 21 of the final Addendum.)

"With the exception of those studies reviewed by ATSDR, DPR is not aware of any inhalation studies on hydrogen fluoride or fluoride dust after 2003." (See pg. 22 of the final Addendum.)

Bouchard, comment 9: Page 13-26: In the new studies provided, emphasis was put on pharmacokinetics to update the RfCs. The various new data on the toxicokinetics of sulfuryl fluoride and its degradates confirm the rapid hydrolysis of the parent compound and rapid elimination following short-term inhalation as well as a rapid steady state equilibrium after repeated exposure (with a lack of accumulation upon repeated exposure). In particular, on page 14, it is mentioned that sulfuryl fluoride is rapidly metabolized at the portal of entry, so one would except [*sic*] to see information from the literature related to the degradates themselves (fluoride and fluorosulfate). Such information was added in Appendix E (Table 1), but it would support some of the assumptions to mention data from fluoride salt exposure in the main part of the Addendum.

> **DPR response:** Although fluoride was assumed to be the toxic moiety responsible for sulfuryl fluoride's neurological effects, direct evidence supporting this is lacking. DPR has discussed the uncertainty surrounding which compound(s) is the toxic species in Appendix G of the final Addendum, and indicated that other metabolites such as fluorosulfate and sulfonated adducts may also contribute to the cassette of toxicities observed with sulfuryl fluoride exposure. Given this uncertainty, DPR did not include summaries of open literature studies on fluoride salts in the main part of the Addendum. Instead, these studies were summarized and discussed in Appendices E and G, with key findings included in the main part of the Addendum when they cast light on the assumptions underlying various RfC determinations (see Section IV.A.1).

Bouchard, comment 10: On page 13, it is mentioned that "Further hydrolysis of fluorosulfate to sulfate and fluoride occurred over a longer time period". It is not clear what is meant by "over a longer time period". The time period should be mentioned.

> **DPR response:** DPR has specified the time periods in the final Addendum (see pg. 22):
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> "Further hydrolysis of fluorosulfate to sulfate and fluoride occurred over a longer time period (> 20 hours)."

Bouchard, comment 11: On page 13, elimination half-lives are mentioned but it is not mentioned from what matrix they were calculated (blood, lung)? This should be mentioned.

> **DPR response:** DPR has specified the matrix (plasma) in the final Addendum (see pg. 22).
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> "Elimination half-lives for both fluorosulfate and fluoride in the plasma were on the order of 1-2 hours and 2-3 hours, respectively."

Bouchard, comment 12: On page 13, it is mentioned that the rabbit exhibits 3-fold higher plasma levels. Perhaps, an explanation should be provided.

DPR response: DPR's analysis indicates that the difference in renal clearance rate between rats and rabbits may be responsible for plasma fluoride difference between these two species. This reason was provided in the overview for the Pharmacokinetics Section (pg. 22):

"While the pharmacokinetic handling of sulfuryl fluoride was similar in rats and rabbits, rabbits exhibited 3-fold higher plasma fluoride levels that may be due to difference in renal clearance rate between rats and rabbits (see Appendix F)."

Bouchard, comment 13: On page 14: It is mentioned that "In the non-modified rats, the highest mean (± SD) concentration of fluorosulfate was measured in the plasma". Time points should be specified.

DPR response: DPR has specified the time point in the final Addendum (see pg. 23).

"In the non-modified rats, the highest mean (± SD) concentration of fluorosulfate measured immediately after the 4-hour exposure was in the plasma (16.8 ± 8.8 µg/mL or 169 ± 87 nmol/mL) followed by nasal tissue (6.3 ± 0.9 µg/g or 63 ± 9 nmol/g), kidney (4.0 ± 1.3 µg/g or 40 ± 13 nmol/g), lung (1.9 ± 0.6 µg/g or 19 ± 6 nmol/g), olfactory bulbs (1.1 µg/g or 11 nmol/g), lung lavage (0.22 ± 0.15 µg/mL or 2.3 ± 1.5 nmol/mL), nasal lavage (0.11 ± 0.06 µg/mL or 1.1 ± 0.6 nmol/mL), and cerebrum (below lower limit of quantitation (LLQ) = 0.5 µg/g or 5 nmol/g)."

Bouchard, comment 14: On page 14, it is mentioned that the estimated average percent of sulfuryl fluoride absorption through the upper respiratory tract is 4.9%. Perhaps the DPR could detail how this was calculated, given that inhalation is the fundamental aspect of the review.

DPR response: The average percent of sulfuryl fluoride absorption through the upper respiratory tract was calculated by the mean sulfuryl fluoride concentrations measured from the endotracheal tubes divided by the mean chamber concentration. DPR has included this detailed description in the final Addendum (see pg. 23), as follows:

"The percent of inhaled sulfuryl fluoride absorbed by the upper respiratory tract was calculated by dividing the concentration in endotracheal tubes by the concentration in the chamber for each individual rat. The mean value was 4.9% (range: 1-7%; N = 6)."

Bouchard, comment 15: On page 14, information on urinary excretion half-life of fluoride and fluorosulfate based on data from Hotchkiss et al. (2008) is mentioned. However, there is no indication of the percent of dose recovered in urine. If available, this should be added. The report simply mentions the fraction of administered fluorosulfate that is eliminated as fluoride or unchanged.

DPR response: The original report did not provide the percent of dose recovered in urine following inhalation exposure.

Bouchard, comment 16: On page 18, based on the study of Hotchkiss et al. (2011a), it is estimated that "The lack of detectable fluorosulfate in the cerebrum rendered the cerebral net free fluoride level (145 nmol/g) almost 3 times higher than the net free fluoride in plasma (49.8 nmol/mL) when samples were measured immediately after exposure to 300 ppm sulfuryl fluoride". However, fluoride levels are measured by the difference between total fluoride levels and those of fluorosulfate and at a specific time point (immediately after exposure). Given this uncertainty, caution with regard to this ratio is advised. Results are also based on a pooling from 10 samples. There could be variability in concentrations between individual blood and cerebrum samples, which will have an impact on pooled blood and cerebrum levels, thus the calculated ratio. I do however acknowledge that the high brain-to-blood partition is corroborated by other data later mentioned in the Addendum and in Appendix E. Page 22 mentions that the study of Marty et al. (2015) also reports concentrations of fluoride that are higher in brain than in plasma and on pages 22-23, it is indicated that the data of Hotchkiss et al. (2011b) in rabbits show an accumulation of fluoride in the brain (cerebrum) while fluorosulfate does not enter the brain (cerebrum) easily. Table 1 of Appendix E also presents data not mentioned in the core of the Addendum on brain and plasma concentrations after exposure to fluoride salt (NaF).

> **DPR response:** DPR acknowledges Dr. Bouchard's agreement concerning corroborating data from other inhalation studies. In Appendix E, DPR has indicated that free fluoride measured by the difference between total fluoride levels and those of fluorosulfate is comparable to measurement by the direct method at high doses. Consequently, only data from the two higher dose groups (150 and 300 ppm) in rat studies were included in the calculation of brain-to-plasma ratios.
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> The reason DPR did not include fluoride salt data in the core of the Addendum was provided in DPR's response to Dr. Bouchard's comment 9.

Bouchard, comment 17: On page 19, it is said that "Based on the similar tissue concentrations of fluorosulfate and fluoride and their rapid elimination rates in plasma, the investigators suggested that both metabolites would not accumulate in plasma or cerebrum following repeated exposures to sulfuryl fluoride". The reported rapid elimination half-life of the order of a few hours explains the rapid steady-state equilibrium observed after repeated exposure. Could this information be used to extrapolate on potential similarities between acute and other exposure scenarios in terms of risk assessment?

> **DPR response:** This information indicates that similar pharmacokinetics are operative with acute and longer term exposures, and has been used by the registrant in developing the physiologically-based pharmacokinetic (PBPK) model. In the Addendum, DPR has incorporated this knowledge to address the database uncertainty factor in Appendix C.

Bouchard, comment 18: Bottom of page 21 and top of page 22, plasma levels are mentioned in nmol/g. For clarity, I would suggest expressing in nmol/ml (although it is implicit that 1 g =

1 ml).

DPR response: In the original study report, the units were expressed in terms of plasma weight rather than volume. For this reason, DPR will retain the units as nmol/g.

Bouchard, comment 19: On page 23, the following sentence is not clear (remove fluoride levels?): "Overall, the two analytes was only markedly different in plasma and kidney fluoride levels".

DPR response: This sentence has been revised as follows in order to clarify that fluoride levels in plasma and kidney were different between rats and rabbits (see pg. 33 of the final Addendum).

"Ultimately, it was only in plasma and kidney that fluoride levels diverged markedly between rats and rabbits."

Bouchard, comment 20: On page 29, in the *Summary of Critical Toxicological Effects by the Inhalation Route, III.A.1 Non-Neurotoxic Effects*, some effects are mentioned but these are based on available studies in animals. There could be other effects but they were not assessed in the published studies. I would thus suggest rephrasing.

DPR response: DPR has rephrased this sentence based on this comment (see pg. 40 of the final Addendum) as follows:

"The available inhalation sulfuryl fluoride studies show that non-neurotoxic effects mainly occur in the respiratory system, kidney, and teeth."

Bouchard, comment 21: At the bottom of page 36, the same critical study and POD were used to derive all the RfCs according to the different assumptions. In this section, it is said that "No visible brain lesions have been detected in acute exposure studies in animals even at lethal doses (Miller et al., 1980)". One study is cited to support this. Perhaps it should be clarified if this is based only on one study or otherwise mention other supporting studies.

DPR response: DPR was referring to all acute studies examined in Table 9 to support the statement that there were "no visible brain lesions." The cited study by Miller *et al.*, 1980 was an LC50 study that included lethal doses. This sentence was revised for clarification (see pg. 41 of the final Addendum).

"No visible brain lesions have been detected in any animal species under acute exposure scenarios, even at lethal doses (Miller *et al.,* 1980)."

Bouchard, comment 22: Page 42 and Table 12 synthesizing the available chronic inhalation toxicity data: It is mentioned that "While the lung inflammation NOEL was lower than those based on brain vacuoles, the RfC for brain vacuolation (0.012 ppm, calculated with a total

uncertainty factor of 300) was lower than that for lung effects (0.024 ppm, calculated with a DAF of 2.7 for portal of entry action and a total uncertainty factor of 100)". Why is a DAF of 2.7 considered? All other DAFs mentioned before have a default value of 1 (see Table 8). Furthermore, it should be mentioned clearly that chronic RfCs derived from the rat and mice data and based on brain vacuoles give the same values (as can be seen from Table 12).

DPR response: DPR considers lung inflammation and alveolar macrophage aggregation as the portal of entry effects. Consequently, the dosimetric adjustment factor (DAF) was calculated using the regional gas dose ratio (RGDR) formula for the pulmonary region (the fourth row in Table 10 of the final Addendum). The calculation is detailed in Footnote b on pg. 58 in the final Addendum, as follows:

"[b]For the 2-generation rat (Sprague-Dawley) reproductive study that exhibited portal of entry effects in the pulmonary region, the mean body weight ranged between 193 g and 483 g in F_0 females, 298 g and 646 g in F_0 males, 117 g and 481 g in F1 females and 132 g and 617 g in F2 males. A default body weight of 338 g (female, chronic) for Sprague-Dawley rats was used for the ventilation calculation. Based on the default allometric equation for rats, $V_E = e^{-0.578+0.821\ln(BW)} = 0.23$ L/min. The default pulmonary region surface area for rats is 0.34 m^2 (U.S. EPA, 1994). The default minute ventilation and pulmonary surface area for adult humans are 13.8 L/min and 54 m^2, respectively (U.S. EPA, 1994). Thus, $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H = 2.7$."

DPR also states that the formation of brain vacuoles in rat and mouse studies is the basis of the chronic RfCs (see pg. 56 of the final Addendum):

"The chronic RfC of 0.012 ppm based on brain vacuolation was precisely the same in rats and mice. Because this value was close to the lowest short-term and subchronic RfCs (0.013 and 0.018 ppm, respectively), 0.013 ppm was used to evaluate risk under all three scenarios (short-term, subchronic and chronic)."

Bouchard, comment 23: In caption to Table 12, "[c]For the 18-month mouse study" should mention B6C3F1 mice and footnote d should mention the type of dog studied. For the rat, the strain is mentioned.

DPR response: Revised as recommended (see pg. 58 – 59 of the final Addendum).

Bouchard, comment 24: Table 13: Maybe it would be clearer if mentioned in a footnote that the range of values reported for the acute RfCs corresponds to lower and upper-bound values derived with the various assumptions (systemic versus portal of entry MOA or unknown MOA).

DPR response: Revised as recommended (see Table 15 on pg. 60 of the final Addendum):

"[b]This range corresponds to acute RfC values based on the various assumptions specified in Table 11."

Bouchard, comment 25: Page 53, uncertainties in the approach: Can the assumption on neurotoxicity and sensitivity of pups be verified by studies on other chemicals (or elements) affecting basal ganglia (e.g. provoking brain vacuoles)?

DPR response: There are other chemicals such as manganese and methyl bromide that impact the basal ganglia after inhalation exposure. These examples are cited in the Addendum (see Appendix E). However, the extent to which they support a mode of action for sulfuryl fluoride awaits further study.

Conclusion 2 – ***Neurotoxicity of sulfuryl fluoride can result from direct intranasal transport to the brain rather than through the respiratory system to the blood and then to the brain as discussed in Appendix E of the Addendum.***

Bouchard, comment 26: On the basis of the newly available data synthesized in the Addendum, I agree that comparison of brain-to-blood partition coefficient observed after inhalation being 20 times higher than after intravenous (IV) or oral exposure (where the compound can only enter the brain via the blood- brain-barrier, hence via a transfer from the systemic circulation) suggest a mode of entry after inhalation that is not a systemic mode-of-action. In particular, this is supported by the study of Marty et al. (2011b) in male weanling rats (PND 22) (page 21), where brain levels of fluoride are quite high compared to plasma levels, although fluorosulfate does not enter the brain as easily. Furthermore, page 23 and Tables 6-7 nicely summarize the conclusions from the newly available PK data and show the relatively high fluoride levels in cerebrum and higher brain than blood concentrations (Table 7) with limited accumulation of fluorosulfate (Table 6) for samples collected in general at the end of the exposure period. More convincingly, Table 1 and page 5 of Appendix E in particular further support this higher brain-to-plasma ratio after inhalation compared to oral, IV or intraperitoneal (I.P.) exposure; in Appendix E, data from exposure to the fluoride salt including labeled fluoride are used to support this. I would suggest adding these references related to fluoride salt (NaF and NaF^{18}) in the core of the Addendum.

DPR response: See DPR's response to Dr. Bouchard comment 9 on why DPR decided to place the fluoride salt data in the Appendix rather than in the core of the Addendum.

Bouchard, comment 27: DPR further support their conclusions for this direct intranasal transport to the brain by the fact that effect is confined to the basal ganglia. The DPR mentions that human cases (in particular a 9-year old boy) and animal data show an effect on the basal ganglia. However, although mentioned in Appendix E, it is not clear in the main part of the Addendum what other parts of the brain were assessed in all the available studies and possible

explanations for the confined distribution in this region of the brain. This should perhaps be stated in the main part of the Addendum.

DPR response: See DPR's response to Dr. Bouchard comment 2.

Bouchard, comment 28: The third conclusion for a direct intranasal transport is that other inhaled or intranasally administered chemicals are known to access the brain (basal ganglia in particular) via a direct olfactory route or extracellular transport either directly to the basal ganglia or via the cerebrospinal fluid. In the main part of the Addendum, DPR mentions manganese (Mn) and remoxipride as examples of other small molecules that show a time course in the blood and brain as well as olfactory bulb indicative of a direct nasal transfer to the brain (page 52). This is not limited to the two examples provided in the addendum. In the main part of the Addendum, greater emphasis should be put on the relevance of this route of transport by analogy with other elements, as this is critical to the conclusions drawn, although I do acknowledge that more information is provided in Appendix E (page 8-9). Mn affects the globus palladium, a subcortical structure of the basal ganglia system. A nasal-to-brain transport of Mn by the olfactory nerve is suggested, as assessed by olfactory bulb measurements and brain-to-blood ratio after inhalation and oral exposure (Ramoju et al. 2017). Recent data on titanium dioxide also suggest such mode of transfer to the brain for inhaled particles (Chen et al. 2006; Song et al. 2015; Wang et al. 2008a, b).

DPR response: DPR did not include the data and discussion of potential direct brain access of sulfuryl fluoride via the intranasal route in the main text of the Addendum due to lack of direct evidence supporting this route, as well as for other routes and modes of action. However, Appendices E and G provide a detailed analysis of this potential mode of action.

Bouchard, comment 29: When considering this direct nasal-to-brain transfer for the neurotoxicity, the DPR did not calculate a HEC and applied instead a default uncertainty factor of 10 for animal-to-human extrapolation (UF_A). This is in line with traditional methodologies of applying a default 10-fold factor for calculating a RfC (lumped PK and PD components). Therefore, a novel possible MOA is considered (direct nasal-to-brain transfer) but UF_A applied in this case is the full default factor.

DPR response: At this time, DPR is unable to establish an RfC based solely on the intranasal pathway mode of action (MOA). Instead, DPR elected to derive RfCs based on the following three assumptions of the mode of action: 1) systemic, 2) portal of entry at nasal cavity (extrathoracic region), and 3) unknown mode of action. Using the no dosimetric adjustment for the unknown mode of action resulted in an acute inhalation RfC of 0.25 ppm. Additional details regarding this derivation can be found in Section IV.A. Methodologies for RfC Derivation starting on pg. 44 of the final Addendum.

<u>Conclusion 3</u> – ***To account for pharmacokinetic differences between laboratory animals and humans, dosimetric adjustments of air concentrations are necessary precursors to the calculation of RfCs. These are addressed in section III.D of the Addendum.***

Bouchard, comment 30: I agree with DPR that dosimetric adjustments factors (DAFs) must account for uncertainties in the mechanism of transfer of sulfuryl fluoride or rather its degradates (fluoride in particular) to the brain or the nature of the effect (systemic effect or local portal-of-entry effect). DPR clearly presents the approach used for the derivation of a human equivalent concentration (HEC), and differences in calculations depending on if the effect is systemic or portal of entry. DPR also mentions on page 4 that differences in the derived acute RfCs are due in large part to the calculation of HEC and this is evident from pages 4-5 (Table 1), on pages 31-32 and on pages 46-48 (Table 14).

DPR response: No response necessary.

Bouchard, comment 31: DPR systematically presents a comparison between RfCs derived (Table 1 and Table 14 in particular) with 1) blood:gas partitioning as a dosimetric adjustment, 2) differences in body weights and inhalation rates between animals and humans as a dosimetric adjustment or 3) PBPK when considering a systemic mode of action versus 4) RGDR (0.064 or 1 according to US EPA 1994 and 2012, respectively) when considering a portal-of-entry effect. For the derivation of such RfCs, traditional methodologies for calculating HEC for systemic effects (blood:gas partitioning or differences in BW and inhalation rates between animals and humans) and portal- of-entry effects in the extrathoracic region (RGDR) have been used. It is in line with recent methods for risk assessment to consider that a HEC derived from a duration-adjusted POD accounts for pharmacokinetic differences between animals and humans, and that the DAF used to calculate a HEC depends on the mode of action and nature of the effect.

DPR response: No response necessary.

Bouchard, comment 32: With regard to duration-adjusted POD used to calculate HEC, on page 4, it is mentioned that duration-adjusted NOAEL for neurotoxicity = 300 ppm x 6 h/24 h = 75 ppm. This is the standard adjustment to account for the 6 h inhalation instead of a continuous 24 h exposure as in humans.

DPR response: No response necessary.

Bouchard, comment 33: On page 27, regarding the PBPK model and the determination of metabolism parameter, it is said that "Fluoride is assumed to be the toxic species and all metabolic and elimination processes follow first-order kinetics, which would normally scale to the inverse of body weight (BW-0.3). However, in this model the metabolic process was scaled to BW0.3, which according to the developers better fits the data". Although it is understood that scaling is based on fits, an explanation for this should be given.

DPR response: DPR has included a more detailed explanation of the registrant-submitted model in the final Addendum (see pg. 37) as follows:

"However, the comparison of the rat and rabbit metabolite levels indicated that typical first order scaling ($BW^{-0.3}$) did not fit the data. Instead, better fit was obtained by using conventional scaling to model turnover rates ($BW^{0.3}$)."

Bouchard, comment 34: On page 28 regarding model application, it is said that: "In the residential setting, plasma fluoride levels in adults peaked at ~0.2 nmol/mL on the first day and declined to less than 0.001 nmol/mL over the remainder of the 15 day period"…"In the occupational scenario, plasma fluoride levels peaked at 0.3 nmol/mL during the day, then falling to baseline levels overnight" and it is previously mentioned that "plasma fluoride levels following hypothetical exposures starting at 1 ppm sulfuryl fluoride in residential (24 hr/day for 15 days) or occupational (8 hr/day, 5 days/wk, 48 wk/year) settings". These first two sentences are not clear in relation to the described simulated exposure scenario. It should be made clear if this is an observed value or a modeled value. If exposure is constant or repeated, it is impossible that values go down. Therefore if this sentence pertains to modeling, either the exposure scenario should be clarified or this sentence.

DPR response: All of the cited values were derived from the PBPK model as indicated in the first sentence of the paragraph titled "Model Application" on pg. 38: "Dow AgroSciences later used this PBPK model to **predict** plasma fluoride levels following hypothetical exposure …" (emphasis added). The exposure concentrations varied with scenario. In the residential setting, the initial air concentration was 1 ppm, decaying exponentially thereafter. In the occupational setting, the exposure concentrations were assumed to be constant at 1 ppm without any decay over time. This information has been added in the final Addendum for clarification (pg. 38) as follows:

"In the residential setting, the exposure concentrations were assumed to start at 1 ppm and then follow an exponential decay."

"In the occupational scenario, the exposure concentrations were assumed to be constant at 1 ppm without any decay over time."

Bouchard, comment 35: Again regarding page 28 and the description of the PBPK model: Modeling is based on parameters assessed from either *in vivo* or *in vitro* data. It should be made clearer why brain levels are lower in humans than rats i.e. what parameter(s) explain(s) this and based on what data.

DPR response: Because a parameter sensitivity analysis was not included with the submitted PBPK model, it is not known what parameters(s) explain the lower brain fluoride levels in humans versus rats. However, DPR's examination of model parameters revealed that the urinary elimination (5.5 $L/hr/kg^{-0.3}$ in humans vs. 0.7 $L/hr/kg^{-0.3}$ in rats) likely plays

an important role. DPR has included the following statement in the final Addendum (see pg. 39):

"DPR also notes that the model prediction of lower internal brain fluoride concentrations in humans than rats for the same external exposure level was partially due to the difference in urinary elimination rate used in the model (5.5 L/hr/kg$^{-0.3}$ in humans vs. 0.7 L/hr/kg$^{-0.3}$ in rats). However, it is unclear how these values were derived (see Appendix F)."

Bouchard, comment 36: Finally, while I agree with the possibility of a nasal-to-brain transfer, the weight of evidence based on the data reported in the main part of the Addendum is not sufficient to fully confirm such mechanism. Therefore, consideration of a PBPK model such as the one used for Mn (Ramoju et al. 2017; c.f. Fig 1 in the article) to infer on nasal-to-brain transfer would be advised for future updates.

DPR response: DPR agrees with Dr. Bouchard that direct evidence to confirm nasal-to-brain access is lacking. Incorporation a nasal-to-brain route in the PBPK model awaits confirming experimental evidence.

Conclusion 4 – ***UFs used to calculate RfCs from HECs or duration-adjusted PODs are discussed in sections III.E, IV.E, and IV.F of the Addendum. These UFs account for inter- and intraspecies differences in sensitivities as well as the possibility that infants and children are more sensitive to sulfuryl fluoride than adults.***

Bouchard, comment 37: The consideration of default UF_A, UF_H, UF_{DB} and the pharmacokinetic (PK)/pharmacodynamic (PD) component of the UF_A is well described. I agree that DPR applied UFs to the critical HEC or POD according to standard approaches in risk assessment. DPR clearly provided (pages 2 and 30) the definition of commonly used UFs (UF_A, UF_H, UF_{DB}), along with the definition of POD and HEC (HEC = POD_{ADJ} x DAF), DAF, and PBPK model applied to systemic effects. Consistent with standard risk assessment approach (and mentioned on pages 3 and 34), DPR applied to the duration-adjusted POD or HEC the standard UF_A of 10 or 3, in the latter case if a dosimetric adjustment factor (DAF) was applied for PK differences to account for PD differences. Therefore, DPR applied an UF_A of 3 for the PD component when the PBPK model and gas:blood partitioning were considered to account for species (animal-to-human) differences in PK. DPR applied an UF_H of 10 in all cases. UF_{DB} of 3 was applied when neurotoxicity was not conducted in young animals (developing organisms), which is also in line with recent advances in risk assessment methodologies followed by major governmental agencies, suggesting that young animals are more sensitive than adults to neurotoxic effects of pesticides.

DPR response: No response necessary.

Bouchard, comment 38: On page 48, it is mentioned that there are uncertainties related to the POD of 300 ppm for the acute toxicity; the NOAEL corresponds to the highest dose administered (no effect observed at the highest dose and no critical dose). DPR mentions that motor effects were not measured at the time of peak exposure (measured at 18 h while peak exposure occurred at 2 h) and thus that critical dose may be missed since effects may be reversible. Can it be assumed that motor effects are reversible within 16 h?

> **DPR response:** What is known at present is that motor effects were not evident 18 hours after completing a regime consisting of two successive daily 6-hour inhalation exposures at 300 ppm. As testing was not conducted at the time to peak fluoride concentration of 2 hours, it is not known if an effect was manifest at that time and simply reversed in subsequent hours as the brain fluoride concentration declined, or if no effect occurred at that concentration. It is possible that an effect was missed due to the time of testing, which is noted on pg. 65 (Section V.E.2) of the final Addendum. *Note:* Reversibility of motor activity effects was indeed evident in young animals following short-term exposure (Marty *et al.*, 2015).

Bouchard, comment 39: A major uncertainty is related to early biological alterations that were not measured in the study dating from the early 1990's. Motor effect monitored is symptomatological. The study was also conducted in mature animals and not young animals. The other uncertainty is linked to the fact that no BMD can be derived from the study of Albee et al. (1993), given that the highest dose is a no-effect dose hence NOAEL.

> **DPR response:** DPR has incorporated all plausible uncertainties, including those cited by the reviewer, into the final Addendum (see pg. 61, Section V.A.1) as follows:
>
> "The critical acute POD of 300 ppm was the high dose in a two-day inhalation neurotoxicity study in adult rats (Albee *et al.*, 1993). Although this study evaluated a range of neurobehavioral endpoints including FOB, motor activity, and electrophysiological parameters, no effects were identified at the highest dose tested. As such, we recognize that uncertainties can arise when a regulatory value is not tied to a particular effect and from which no BMD can be derived. It is possible that the critical observations were not made concurrently with peak brain fluoride concentrations. This is likely the case for motor activity determinations, which for logistical reasons were not evaluated until 18 hours after the end of the final exposure (Hotchkiss *et al.*, 2011a). It is possible that motor activity was altered during peak fluoride concentrations, then reversed at 18 hours post exposure. Elevated motor activity was detected at as low as 20 ppm in postnatal rat pups exposed to sulfuryl fluoride continuously for 11 days (PND11-PND22), but same effect was not observed when evaluated at a later time point of PND55 (Marty *et al.*, 2015). It is also possible that other markers which were not measured, such as early biological changes at the molecular level, could have elucidated a more sensitive endpoint. Finally, the Albee study was only conducted in adult animals. Therefore, the threshold for acute toxicity in young animals could be lower than the NOEL of 300 ppm based on evaluations in adult animals."

Bouchard, comment 40: Both Albee et al. (1993) and Marty et al. (2015) are studies conducted by Dow Chemicals. The main uncertainties is the lack of independent studies confirming the observed effects and the lack of studies on early biological alterations (gene expression, metabolomics, proteomics) confirming early changes at the gene, molecular or protein level, or oxidative stress markers and inflammation markers, two early changes that may be associated with levels of fluoride metabolites.

> **DPR's response:** DPR has incorporated all plausible uncertainties, including those cited by the reviewer, into the final Addendum (see response to comment 39).

Bouchard, comment 41: Although not reassessed in the Addendum but presented in the Addendum, for the subchronic RfC, as mentioned on page 50, the subchronic studies conducted in multiple species (mice, rats, rabbits) allow reducing uncertainties in the POD. A NOAEL and LOAEL can be determined. Given the available data set for the subchronic study and observed dose-response curve, one can question why a BMD approach was not considered in this case.

> **DPR response:** DPR has re-evaluated the available subchronic data and replaced no-observed effect level (NOEL) determinations with lower bound benchmark dose modeling levels (BMDLs) for endpoints that can be adequately modeled (see Sections III.B, IV.D, Appendix D, and Table 13).

Bouchard, comment 42: Again although not reassessed in the Addendum but presented in the Addendum, for the chronic RfC, the chronic POD is for the same critical effect as the subchronic POD. The chronic LOAEL equals 80 ppm while the subchronic LOAEL equals 100 ppm. The chronic NOAEL equals 20 ppm while the subchronic NOAEL equals 30 ppm. The NOAEL value is driven by the dose selection (20 and 80 ppm). Again, one can wonder why a BMD approach was not considered for the reevaluation of the POD for chronic toxicity.

> **DPR response:** DPR has re-evaluated the available chronic data and replaced NOEL determinations with BMDLs for endpoints that can be adequately modeled (see Sections III.B, IV.E, Appendix D, and Table 14).

APPENDIX A.

Request for an External Peer Review of the California Department of Pesticide Regulation's Addendum to the 2006 Risk Characterization Document for Sulfuryl Fluoride (Department of Pesticide Regulation Memorandum dated February 28, 2019)

Attachment 2

Description of Scientific Assumptions, Findings, and Conclusions
to be Addressed by the Peer Reviewers

Attachment 2

Description of Scientific Assumptions, Findings, and Conclusions to be Addressed by the Peer Reviewers

Reviewers are asked to determine whether the scientific work product is "based upon sound scientific knowledge, methods, and practices."

We request that you make this determination for each of the following issues. An explanatory statement is provided for each issue to focus the review.

For those work products which are not proposed rules, as is the case here, reviewers must evaluate the quality of the product using the same exacting standard as if it was subject to Health and Safety Code 57004, which requires highly-qualified experts to perform impartial peer reviews. This is intended to ensure that all proposed CalEPA rule-makings meet accepted standards of the relevant scientific disciplines and to prevent any influence on the rule-makings stemming from irrelevant findings, unwarranted claims, unacceptable interpretations, and personal views.

The assumptions and conclusions used to calculate updated Reference Concentrations (RfCs) for sulfuryl fluoride are discussed in Sulfuryl Fluoride: Draft Addendum to the 2006 Risk Characterization Document-Update of the Toxicology and Reference Concentrations (Addendum). These include the rationale for selection of the critical Points of Departure (PODs), the consideration of plausible routes of entry for sulfuryl fluoride, the approaches for derivation of Human Equivalent Concentrations (HECs) and the choice of appropriate Uncertainty Factors (UFs). Reviewers are requested to review the entire document and make determinations on the scientific methods used to determine each of the following assumptions and conclusions**:**

1. **The scientific basis for the proposed RfCs depend both on the nature of the observed effects (non-neurotoxic *vs*. neurotoxic) and on the assumed mode of action (systemic *vs*. portal of entry). These issues are addressed in sections III.C, III.D, and Appendix E of the Addendum.**

Non-neurotoxic effects of inhaled sulfuryl fluoride include dental fluorosis, kidney lesions, body weight changes, and thyroid hyperplasia. The mode of action for such effects is likely to be systemic, *i.e.*, mediated by absorption through the respiratory system into the blood followed by transport to target tissues. Additional non-neurotoxic effects include lesions in the respiratory tract (nasal, tracheal, and lung) that likely result from action at the portal of entry. Traditional methodologies for calculating HECs for systemic effects (blood:gas partitioning of inhaled sulfuryl fluoride) and portal of entry effects (regional gas dose ratio for the respiratory tract) are applicable to these cases for derivation of RfCs.

Neurotoxic effects of inhaled sulfuryl fluoride include vacuolation in the basal ganglia, altered

motor activity, tremors and electrophysiological effects. In the past, both DPR and US EPA estimated human health risks for sulfuryl fluoride based on neurotoxicity. Those assessments assumed that the neurological effects were systemic, with the active principle, fluoride, entering the brain via the blood stream after absorption through the respiratory tract. Dosimetric adjustments for systemic effects were based on the differences in body weight and inhalation rates between animals and humans. Recently, a physiologically based pharmacokinetic (PBPK) model was developed for sulfuryl fluoride in order to predict brain fluoride concentrations in animals and humans. This model also assumed a systemic route to the target tissue from the respiratory system into the blood. However, the analysis of new data suggested that the neurological effects may be mediated through a direct intranasal-to-brain route that bypasses the blood-brain barrier. This route may not be readily classifiable as systemic (blood-to-brain) or conventional portal of entry (the nasal cavity) effects. Rather, it suggests a portal of entry *subcategory* that involves absorption through the nasal cavity followed by direct access to the basal ganglia (see Conclusion 2).

2. Neurotoxicity of sulfuryl fluoride can result from direct intranasal transport to the brain rather than through the respiratory system to the blood and then to the brain as discussed in Appendix E of the Addendum.

A direct intranasal route of absorption was supported by the following observations:

a. Brain-to-plasma (T/P) ratios for fluoride following acute inhalation exposure to sulfuryl fluoride were approximately 20-fold higher than those following oral, intravenous, or intraperitoneal exposure to fluoride or sodium fluoride.

b. Brain lesions were confined to the basal ganglia after inhalation exposure to sulfuryl fluoride, but not after oral exposure to sodium fluoride.

c. Other inhaled or intranasally administered chemicals are known to access the brain (basal ganglia in particular) via a direct olfactory route.

Two possible pathways could permit direct access of sulfuryl fluoride (or its ultimate toxicant) to the central nervous system from the point of contact at the nasal epithelium. One is via the olfactory nerve through the rostral migratory stream to the subventricular zone (Appendix E). The other is via extracellular transport, either directly to the basal ganglia or through the cerebrospinal fluid. The possibility that a direct intranasal-to-brain route of absorption for sulfuryl fluoride is operative prompts the question of which methodology is most appropriate to calculate HECs and RfCs.

3. To account for pharmacokinetic differences between laboratory animals and humans, dosimetric adjustments of air concentrations are necessary precursors to the calculation of RfCs. These are addressed in section III.D of the Addendum.

Due to the uncertainties regarding how sulfuryl fluoride or its hydrolytic products gain access to the brain, different assumptions were necessary to enable dosimetric conversions.

a. Systemic (blood-to-brain) mode of action: when the neurotoxic effects were assumed to occur through a systemic mode of action, HECs were calculated using either a sulfuryl fluoride PBPK model developed by Dow AgroSciences or a default rat-to-human adjustment factor that assumed blood:gas partitioning of inhaled sulfuryl fluoride to be equal in rats and humans (*i.e.*, $H_{b/g}$-rat / $H_{b/g}$-human = 1).

b. Portal of entry mode of action (acting at the site of contact): when the neurotoxic effects were assumed to occur through a portal of entry mode of action via the nasal cavity, human equivalent concentrations were calculated using a default regional gas dose ratio (RGDR) for the extrathoracic region of 0.064 (US EPA 1994) or 1 (US EPA 2012).
c. Direct intranasal-to-brain mode of action: while a direct intranasal-to-brain route is plausible, sufficient data were not available to unequivocally support this mode of action. RfCs were therefore derived directly from duration-adjusted rat PODs, *i.e.*, without first making the dosimetric adjustments necessary for HEC calculations. This was done solely by applying a default uncertainty factor of 10 to the POD to account for interspecies differences.

4. UFs used to calculate RfCs from HECs or duration-adjusted PODs are discussed in sections III.E, IV.E, and IV.F of the Addendum. These UFs account for inter- and intraspecies differences in sensitivities as well as the possibility that infants and children are more sensitive to sulfuryl fluoride than adults.

RfCs were calculated by applying UFs to the critical HEC or POD values appropriate to the assumed mode of action for sulfuryl fluoride (see item 3 for details). The total UF (UF_{total}) was the product of all of the individual UFs. The individual UFs used to calculate the critical RfCs were as follows:

a. UF_A, animal-to-human extrapolation: This factor assumed that humans are more sensitive than laboratory animals. It defaults to 10 (3 for pharmacokinetic differences, 3 for pharmacodynamic differences) except in cases where dosimetric adjustments were made to account for pharmacokinetic differences, in which case a total UF_A of 3 was applied.

b. UF_H, intrahuman sensitivity: This factor assumed that there is a 10-fold difference in sensitivity over the entire adult human population. As with the UF_A, the default UF_H of 10 (3 for pharmacokinetic differences, 3 for pharmacodynamic differences) was applied to every assumed MOA.
c. UF_{DB}, database deficiency: This factor assumed that immature individuals (fetuses, infants and children) were 3x more sensitive than adults to the neurotoxic effects of sulfuryl fluoride. The UF_{DB} of 3 was applied when the critical neurotoxicity study was not conducted using young animals.