



MEMORANDUM

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DATE: May 25, 2020

SUBJECT: Response to comments by Douglas Products and Packaging Company on DPR's draft Addendum to the 2006 Sulfuryl Fluoride Risk Characterization Document dated December 2018

I. Background

Douglas Products and Packaging Company (hereafter referred to as Douglas) submitted comments to the Human Health Assessment (HHA) Branch of the California Department of Pesticide Regulation (DPR) on the draft Addendum to the 2006 Sulfuryl Fluoride Risk Characterization Document in three memoranda dated March 22, 2019 (DeSesso *et al.*, 2019a), July 11, 2019 (DeSesso *et al.*, 2019b), and December 6, 2019 (DeSesso *et al.*, 2019c). This memorandum lists the Douglas comments along with DPR's detailed responses. The final Addendum referenced throughout this response refers to DPR's final May 2020 Addendum to the Sulfuryl Fluoride Risk Characterization Document. DPR sincerely appreciates the efforts taken by Douglas to review the draft Addendum. As appropriate, Douglas comments were incorporated into the final Addendum and responses to specific comments are detailed below.

II. Response to Douglas Comments submitted March 22, 2019

Request for Reconsideration to Sulfuryl Fluoride Draft Addendum to the 2006 Risk Characterization Document, Update of the Toxicology and Reference Concentrations, Human Health Assessment Branch, Department of Pesticide Regulation December 2018 (DeSesso et al., 2019a)

Douglas comment 1: Alternate Mode of Action (MOA): Potential Direct Brain Access Via the Intranasal Route. The commenter disputes the draft Addendum rationale and conclusions regarding the alternate mode of action (MOA) of direct transfer of sulfuryl fluoride or fluoride from the nose to brain and requested reconsideration of the draft Addendum's conclusion that this alternate MOA may be relevant to human exposure to sulfuryl fluoride.

DPR Response: The final Addendum to the Risk Characterization Document (RCD) for Sulfuryl Fluoride has been significantly revised since completion of the external scientific review and receipt of comments from the registrant and other stakeholders. In so doing, we have clarified that the alternate intranasal route has not been used as the basis for deriving reference concentrations (RfCs) for sulfuryl fluoride.

There are several potential pathways involved in the neurotoxic response to inhaled sulfuryl fluoride. These include the absorption into blood through the respiratory tract followed by delivery of toxic metabolites to brain (systemic mode of action) or direct entry into brain from the nasal cavity (portal of entry mode of action). In addition, it is possible that a local vascular pathway may be involved. Our analysis of available data did not allow us to clarify which of these pathways, either alone or in combination, led to neurotoxicity following inhalation of sulfuryl fluoride. Because fluoride delivery may occur by multiple routes, we included an RfC derivation methodology that did not assume any specific MOA. Our purpose in exploring the plausibility of other MOAs was to more fully account for differences in brain fluoride concentration between routes of exposure and to demonstrate that systemic circulation does not fully explain the experimental results. The final Addendum proposes three distinct RfCs for possible use as regulatory targets. These values are based on three possible MOAs: 1) systemic, 2) portal of entry at the nasal cavity (extrathoracic region), and 3) an unknown route of entry.

Douglas comment 1.1: Literature Reviews of Direct Nasal Uptake of Drugs and Chemicals from Nose-to-Brain. The commenter states that the studies reviewed by DPR do not demonstrate absorption of meaningfully effective concentrations when substances are inhaled under ambient conditions and that environmental exposure to sulfuryl fluoride differs dramatically from purposefully-focused intranasal placement of drugs.

DPR response: DPR reviewed a variety of evidence for direct central nervous system access via intranasal absorption for molecules of different sizes and charges, including manganese, insulin, albumin, oxytocin, dextran and interferon, and for living cells (microglia and mesenchymal stem cells). Details are found in Appendix E of the final Addendum. Appendix E also discusses possible pathways for fluoride from the nasal cavity to the brain, along with epidemiologic and pathologic data indicating that the olfactory region is a major target for sulfuryl fluoride and other air pollutants in humans (Ajmani *et al.*, 2016; Calvert *et al.*, 1998). A review by Merkus and van den Berg (2007) considered many reports that questioned the efficiency of intranasal route for many substances. The primary citations do not necessarily reject this pathway outright for humans. In fact, a newer review on intranasal delivery by Crowe *et al.* (2018) provides plausible support and explanation for the intranasal

route in humans. The database analyzed for the final Addendum provides evidence that fluoride accumulates in the brain at higher levels following inhalation exposure than oral exposure. Our exploration of the data was to explain that a purely systemic MOA does not fully account for the neurotoxicity of sulfuranyl fluoride.

Douglas comment 1.2: Anatomical Perspectives to Proposed Alternate MOA. The commenter supports the concept that fluoride from inhaled sulfuranyl fluoride in rats could be absorbed into the substantial venous plexus within the mucosa of the respiratory portion of the nasal passages. The fluoride would be carried with the venous blood to the cavernous sinus, where it would be transferred to the arterial blood in the internal carotid artery. Blood from the internal carotid artery flows mainly into the middle cerebral artery, but also in the anterior cerebral artery. The first branches of both arteries are lenticulostriate arteries that supply the basal ganglia, and thereby deliver the fluoride to that region where it could impact the caudate nucleus and putamen. The commenter also notes that because of anatomical difference between rats and humans (i.e., humans have a very small olfactory epithelial area, a small mucosal surface area lining the remainder of the nasal passages, and do not have a countercurrent exchange mechanism in the cavernous sinus), that the intranasal MOA for sulfuranyl fluoride is incomplete in humans and does not offer a viable route to the basal ganglia.

DPR response: DPR agrees that it is possible a local vascular pathway may be involved in the delivery of sulfuranyl fluoride to the brain. This pathway has been evaluated for many compounds (see Table 1 below), with low molecular weight steroids most readily passing through the blood-brain barrier in this manner. The countercurrent exchange mechanism exists in animals with a carotid rete, including cats, dogs, sheep and pigs. Even animals without a carotid rete such (rats, mice, rabbits) can transfer substances from the venous blood to the arterial blood. The commenter's suggestion that humans lack countercurrent exchange (Nunneley and Nelson, 1994) is based on a model prediction lacking experimental support. It is true that the presence of countercurrent exchange has not been directly documented in humans. However, rabbits, which possess an anatomically similar cavernous sinus/internal carotid artery complex to humans, have been shown to transport solutes from the cavernous sinus to the internal carotid (Krzyszowski and Stefanczyk-Krzyszowska, 2015; Muszak et al., 2014), rendering possible such a process in humans. Differences in airflow pattern and overall nasal anatomy between rats/rabbits and humans that could result in different nose-to-brain absorption characteristics. However, such differences do not preclude a nasal entry route for sulfuranyl fluoride in humans. One computational fluid dynamic model showed that the fraction of air diverted to the olfactory region in humans, while smaller than that in rats and rabbits, is still considered biologically meaningful (Corley *et al.*, 2009). In addition, olfactory epithelium itself is a target for many inhaled toxicants including sulfuranyl fluoride (Calvert *et al.*, 1998; Werner and Nies, 2018). See Appendix E for further discussion.

Finally, the commenter points out that there is no anatomical conduit from the nasal cavity to the basal ganglia through the either the olfactory or lymphatic route in animals. Even so, we found it difficult to explain why the concentration of sulfuranyl fluoride was elevated in the

olfactory bulb compared to the cerebrum or lung tissue of rats and rabbits following inhalation exposure (Hotchkiss *et al.*, 2011c; Hotchkiss *et al.*, 2011b). There is also evidence of basal gangliar effects from a human case report following exposure to inhaled sulfur dioxide (Mulay *et al.*, 2016).

We appreciate the commenter's thorough review of the existing science. While there are distinct differences between laboratory animal and human anatomy, DPR cannot rule out the possibility that the olfactory pathway may also be important in humans. As such, the final Addendum now includes supporting scientific information for alternative pathways for delivery of fluoride from the nasal cavity to brain (see Section IV.A.1 and Appendix E). The purpose of our exploration of the intranasal route was to explain that a purely systemic MOA does not fully account for the neurotoxicity of sulfur dioxide following inhalation.

Table 1. Literature review on local vascular pathways

| Study (Institute, Country) | Species | Exp. System | Chemicals (MW, kDa) | Transfer | Notes |
|---|--------------|---|---|-------------------------|--|
| Krzymowski <i>et al.</i> (1992) (PAS, Poland) | Sheep Pig | <i>Ex vivo</i> (infusion) | ¹²⁵ I-LHRH (1.2) ¹²⁵ I-β-endorphin (3.4) ³ H-progesterone (0.32) ⁵¹ Cr-RBC | Yes Yes Yes No | No LHRH countercurrent transfer in anoestrous (sheep) or pro-oestrous (pig) |
| Grzegorzewski <i>et al.</i> (1995) (PAS, Poland) | Pig | <i>Ex vivo</i> (infusion) | ¹²⁵ I-oxytocin (1.0) | Yes | Transfer during a short period after ovulation and also on days 12-13 of the estrous cycle |
| Grzegorzewski <i>et al.</i> (1997) (PAS, Poland) | Pig | <i>Ex vivo</i> (infusion) | ¹²⁵ I-LHRH (1.2) | Yes | Transfer after the ovulation period (days 1-2) and on days 12-14 of the estrous cycle |
| Skipor <i>et al.</i> (1997) (PAS, Poland) | Sheep | <i>Ex vivo</i> (infusion) | ¹²⁵ I-β-endorphin (3.4) | Yes | Transfer during the early luteal phase in the breeding season, but not during seasonal anoestrus |
| Skipor <i>et al.</i> (1999) (PAS, Poland) | Sheep | <i>Ex vivo</i> (infusion) | ¹²⁵ I-LHRH (1.2) | Yes | LH is a modulatory factor |
| Krzymowski <i>et al.</i> (1999) (PAS, Poland) | Pig | <i>Ex vivo</i> (infusion vs. intranasal) | ³ H-5α-androstenol (0.27) | Yes | Less efficient transfer with intranasal route than direct infusion; overall transfer efficiency ranged from 1.7-3.7% |
| Stefanczyk-Krzyszowska <i>et al.</i> (2000) (PAS, Poland) | Pig | <i>In vivo</i> (intranasal) | ³ H-5α-androstenol (0.27) | Yes | 0.68% of pheromone dose applied to the nasal mucosa was resorbed into the venous blood. Brain radioactivity from highest to lowest: perihypophyseal vascular complex > olfactory bulb > septum > hypophysis > anterior hypothalamus > amygdala |

Table 1. Literature review on local vascular pathways

| Study (Institute, Country) | Species | Exp. System | Chemicals (MW, kDa) | Transfer | Notes |
|--|---------|--|---|-------------------|---|
| Einer-Jensen and Larsen (2000a) (USD, Denmark) | Rat | <i>In vivo</i> (intranasal) | ¹⁴ C-diazepam (0.29) ³ H-cocaine (0.30) | Yes No | Transfer of diazepam but not cocaine from the nasal cavity to the brain arterial blood |
| Einer-Jensen and Larsen (2000b) (USD, Denmark) | Rat | <i>In vivo</i> (intranasal) | Tritiated water (0.022) ³ H-tyrosine (0.18) ¹⁴ C-propranol (0.06) | Yes Yes Yes | Local transfer takes place between the venous and arterial blood in the head |
| Skipor <i>et al.</i> (2000) (PAS, Poland) | Pig | <i>Ex vivo</i> (intranasal) | ³ H-testosterone (0.29) | Yes | Absorption was 11.4%; transfer from cavernous sinus to arterial blood was 0.4%. A clear pattern for different brain region uptake was not seen (basal ganglia not assessed) |
| Skipor <i>et al.</i> (2001) (PAS, Poland) | Sheep | <i>Ex vivo</i> (infusion) | ³ H-dopamine (0.19) | Yes | Dopamine counter-current transfer from venous blood of the cavernous sinus to arterial blood was affected by reproductive cycle and estradiol treatment. |
| Skipor <i>et al.</i> (2003) (PAS, Poland) | Pig | <i>In vivo</i> (intranasal) <i>Ex vivo</i> (infusion) | ³ H-progesterone (0.32) | Yes | Intranasal: cavernous sinus to the arterial blood. Infusion: transfer is affected by the stage of estrous cycle |
| Skipor <i>et al.</i> (2004) (PAS, Poland) | Sheep | <i>Ex vivo</i> (infusion) | ¹²⁵ I-LH (30) ¹²⁵ I-prolactin (23) | No No | Molecular weight plays a major role in permeation and transfer |
| Muszak <i>et al.</i> (2014) (PAS, Poland) | Rabbit | <i>Ex vivo</i> (infusion) | ³ H-dopamine (0.19) | Yes | Brain radioactivity, highest to lowest: pia mater > pons = mammillary body > ventral tegmental area > hippocampus > corpus striatum |

Abbreviations: LHRH: Luteinizing hormone-releasing hormone; RBC, red blood cells; PAS, Polish Academy of Sciences; USD, University of Southern Denmark. ***Ex vivo* (infusion):** use an isolated head perfusion model, testing substance was administered through the angularis oculi vein with the superficial temporal vein (sheep) or the profound facial vein (pig) or profound facial/nose/labial veins (rabbit) ligated to enable direct infusion into the cavernous sinus. ***Ex vivo* (intranasal):** use an isolated head perfusion model, testing substance was infused through catheters onto the surface of the nasal mucous. ***In vivo* (intranasal):** use whole animals under anesthesia, testing substance was infused through catheters onto the surface of the nasal mucous.

Douglas comment 1.3: Pharmacokinetic Based Reanalysis of Fluoride Brain and Blood Levels. The commenter notes that the Request for Reconsideration presents a more comprehensive assessment of fluoride pharmacokinetics than the December 2018 draft Addendum by utilizing a time-course analysis of brain and plasma fluoride levels. The commenter posits that the resulting ratios are dependent on sampling time post-exposure and exposure duration and not on exposure route. The commenter continues that a more realistic assessment of fluoride distribution in the brain versus plasma would be obtained from steady-

state exposures scenarios. Altogether, the commenter states that the results do not support a novel mode of fluoride uptake into the brain via the inhalation route but, rather, differences in fluoride uptake and elimination rates from plasma and brain tissue.

DPR response:

Complete Time-Course Analysis:

We agree that a more comprehensive analysis of fluoride pharmacokinetics would include a complete time-course determination of brain and plasma fluoride levels. In the final Addendum, we included complete time-course tissue to plasma (T/P) fluoride ratios generated from one acute inhalation study which involved continuous 3–4 hour exposures with tissue sampling initiated during the exposure and continued up to 8 hours after exposure ceased. We also included T/P ratios generated from one intravenous (i.v.) study which involved continuous infusion over several hours. These two time-course studies provided a more relevant comparison of the resulting T/P ratios than other studies which employed oral, intraperitoneal, or bolus i.v. administration.

Post-Exposure Peaks and Sampling Times:

The commenter concurred with DPR that T/P ratios are highly dependent on post-exposure sampling time. To remove any sampling time bias and provide a more standardized approach, we calculated the T/P ratios based on the highest plasma fluoride level reported in the studies. We then compared those ratios from inhalation to non-inhalation studies. By standardizing the analysis, we were able to show that fluoride pharmacokinetics differed depending on route of exposure (see Appendix E of the final Addendum). The chronic drinking water studies referred to by the commenter did not analyze daily plasma fluoride concentrations nor did they specify when the samples were collected (Jiang *et al.*, 2014a; McPherson *et al.*, 2018; Mullenix *et al.*, 1995; Shalini and Sharma, 2015). As such, the fluoride measurements could not be standardized to either peak plasma concentration or sampling time. Altogether, the T/P ratios in the chronic drinking water studies are not comparable with those that we standardized to the highest measured fluoride concentration in plasma. Our rationale and methodology for calculating T/P ratios is further detailed in Appendix E.

Steady-State Fluoride Concentrations:

On p. 27 of the March 2019 Request for Reconsideration, the commenter states that T/P fluoride ratios reach steady-state within 55 days of oral exposure in chronic drinking water studies (Jiang *et al.*, 2014a; McPherson *et al.*, 2018; Mullenix *et al.*, 1995; Shalini and Sharma, 2015). There are no pharmacokinetic data to support this assertion. There is, however, evidence to suggest that plasma fluoride levels peak during the first hour following oral ingestion, then rapidly decline due to continuous bone uptake and urinary excretion (Whitford, 1996). This cycling of high-to-low plasma concentrations is indicative of the influence of absorption, distribution, metabolism, and excretion (ADME). The T/P ratios that we calculated from the chronic drinking water studies ranged from 0.47 – 43.5 (see Table 2,

below). This variation in standardized T/P ratios does not support the contention that fluoride plasma levels reach steady-state in treated animals. Instead, the ~100-fold difference in the values may also indicate the influence of ADME. Additional support against steady-state comes from a 2-week sulfuryl fluoride inhalation study in rats. Results showed that, when measured immediately following the daily round of inhalation exposure, plasma and brain fluoride levels were comparable on Day 1 and Day 14 of the study (Hotchkiss *et al.*, 2011a). This suggests that instead of accumulating and reaching steady state, fluoride concentrations peak and then clear on a daily basis. Altogether, these results indicate that there is minimal accumulation of fluoride in the brain or plasma following repeated oral or inhalation exposures.

Exposure Duration versus Exposure Route:

The commenter states that fluoride T/P ratios are highly dependent on exposure duration due to long-term accumulation in mammalian systems and differential kinetics in brain versus plasma, and that exposure route appears to have little effect on fluoride tissue distribution (p. 13, March 2019 Request for Reconsideration). To address this, we compared fluoride pharmacokinetics under chronic inhalation and chronic oral exposure scenarios. In the registrant submitted PBPK model, plasma fluoride levels were predicted to cycle daily during long-term (1 year) inhalation exposures in workers (see Figure 34, Poet and Hinderliter, 2011). If fluoride pharmacokinetics were independent of exposure route, we would expect to see a similar daily cyclic pattern of plasma fluoride levels following chronic drinking water exposure, as well as similar T/P ratios across all these studies. However, as stated above, we found a ~100-fold variation in these ratios. Without additional data from the drinking water studies, such as daily plasma and tissue fluoride concentrations and details about sample collection times, sample preparation, and analytical methodology, the studies cannot be used to support that duration and not route determines the differences in T/P ratios. Our analyses of the levels of fluoride in brain versus plasma, T/P ratios, and the available pharmacokinetic data on uptake and elimination are detailed in Appendix E of the final Addendum.

Table 2. Fluoride levels in plasma and brain and its brain-to-plasma (T/P) ratio of rats following chronic exposure to sodium fluoride in drinking water

| Study | Fluoride concentration in drinking water | Brain tissue | F-brain $\mu\text{g/g}$ (nmol/g) | F-plasma $\mu\text{g/ml}$ (nmol/ml) | T/P |
|--------------------------------|--|--------------|----------------------------------|-------------------------------------|------------------|
| McPherson <i>et al.</i> (2018) | 0 ppm, GD6-PND60 Standard chow | Whole brain | 0.35 (18.5) | 0.018 (0.95) | 19.4 |
| | 0 ppm, GD6-PND60 Low-F chow | Whole brain | 0.21 (11.1) | 0.001 (0.05) | 210 ^b |
| | 10 ppm, GD6-PND60 Low-F chow | Whole brain | 0.27 (14.3) | 0.036 (1.9) | 7.5 |
| | 20 ppm, GD6-PND60 Low-F chow | Whole brain | 0.85 (45.0) | 0.025 (1.3) | 34 |

Table 2. Fluoride levels in plasma and brain and its brain-to-plasma (T/P) ratio of rats following chronic exposure to sodium fluoride in drinking water

| Study | Fluoride concentration in drinking water | Brain tissue | F-brain $\mu\text{g/g}$ (nmol/g) | F-plasma $\mu\text{g/ml}$ (nmol/ml) | T/P |
|--|--|-------------------|----------------------------------|-------------------------------------|------|
| Shalini and Sharma (2015) | Control water (0.9 ppm), 60 days | Whole brain | 0.55 (29) | 0.048 (2.5) | 11.5 |
| | Fluoride water (10 ppm), 60 day | Whole brain | 2.61 (138.1) | 0.06 (3.2) | 43.5 |
| Jiang <i>et al.</i> (2014b) | Control, 3 months | Hippocampus | 0.24 (12.7) | 0.09 (4.8) | 2.7 |
| | | Cortex | 0.25 (13.2) | 0.09 (4.8) | 2.8 |
| | 120 mg/L, 3 months | Hippocampus | 1.14 (60.3) | 0.4 (21.2) | 2.9 |
| | | Cortex | 1.19 (63.0) | 0.4 (21.2) | 3.0 |
| ^a Mullenix <i>et al.</i> (1995) | 125 ppm, 20 weeks, Female weanlings | Cerebellum | 0.95 (50.0) | 0.64 (33.9) | 1.48 |
| | | Medulla oblongata | 0.99 (52.6) | 0.64 (33.9) | 1.55 |
| | | Hypothalamus | 0.51 (27.0) | 0.64 (33.9) | 0.80 |
| | | Striatum | 0.39 (20.5) | 0.64 (33.9) | 0.61 |
| | | Mid brain | 0.33 (15.9) | 0.64 (33.9) | 0.52 |
| | | Hippocampus | 0.55 (29.3) | 0.64 (33.9) | 0.47 |
| | | Cortex | 0.55 (29.3) | 0.64 (33.9) | 0.87 |
| | 125 ppm, 20 weeks, Male weanlings | Cerebellum | 0.65 (34.2) | 0.41 (21.6) | 1.58 |
| | | Medulla oblongata | 0.57 (30.1) | 0.41 (21.6) | 1.39 |
| | | Hypothalamus | 0.25 (13.5) | 0.41 (21.6) | 0.62 |
| | | Striatum | 0.21 (11.2) | 0.41 (21.6) | 0.52 |
| | | Mid brain | 0.23 (12.3) | 0.41 (21.6) | 0.57 |
| | | Hippocampus | 0.25 (13.4) | 0.41 (21.6) | 0.62 |
| | | Cortex | 0.52 (27.7) | 0.41 (21.6) | 1.28 |

^aThe brain concentration in this study was expressed in terms of lyophilized tissue, thus were divided by 3.3 to convert them into fresh weight concentration to calculate the brain-to-plasma fluoride concentration ratios (Whitford *et al.*, 2009). The values plotted in Fig. 11 of registrant's comment (DeSesso *et al.*, 2019a) were from a 6-week treatment of 100 ppm sodium fluoride in adult rats from the same study without the conversion to the fresh weight, which however was mistakenly cited as the 20-week treatment group. Shaded rows are T/P ratios for control rats not exposed to sodium fluoride.

^bIn their comments, the registrant included T/P ratios for the control groups which were not exposed to fluoride in the four chronic oral studies. These ratios varied significantly (2.7 – 210; shaded values above). The variation could be due to measurement errors of plasma fluoride levels near the lower limit of quantitation (LLQ), but cannot be attributed to fluoride exposure. LLQ was not reported in the four chronic oral drinking water studies. However, plasma fluoride LLQ for similar methods in sulfuryl fluoride inhalation studies range from 1.31 – 1.42 nmol/ml.

Douglas comment 1.4: Metabolism-based analysis of sulfuryl fluoride bioavailability to brain tissue. The commenter suggests that additional evidence from metabolite quantitation across various studies supports the limited bioavailability of sulfuryl fluoride via systemic blood or directly to the brain tissue following inhalation exposures.

DPR response: Detailed discussions on the potential toxic effects of fluoride, fluorosulfate, and sulfuryl fluoride, along with the possible pathways for sulfuryl fluoride degradation *in vivo* are presented in Appendix G of the final Addendum. This discussion also includes the potential for sulfuryl fluoride exposure to result in protein adduction, specifically fluorosulfate or sulfonated adducts (e.g., FSO₂-tyrosine).

Douglas comment 2: Physiologically Based Pharmacokinetic (PBPK) Model Uncertainties Identified in Draft Addendum. The commenter disagrees with DPR's conclusion in the draft Addendum that a substantial uncertainty is associated with the PBPK model-derived fractional inhalation absorption in rabbits. The Request for Reconsideration details multiple datasets that support reducing the stated uncertainties in the draft Addendum. These studies included comparison of relative fluoride blood levels in rat and rabbit following bolus i.v. administration (Monsour et al., 1985; Whitford et al., 1991), along with sulfuryl fluoride inhalation studies in rabbits (Rick *et al.*, 2011). The commenter states that these data justify the model-derived uptake values of 45% in rabbit compared to 15% in rat (Poet and Hinderliter, 2011).

DPR response: Our analysis of the available pharmacokinetic data indicate that the 3-fold difference in plasma fluoride between rabbits and rats was mainly due to differences in renal clearance, not a differential uptake. Therefore, the assumed values of fractional inhalation absorption in rabbits based on the PBPK model remain uncertain. Rabbits exposed by inhalation to 300 ppm sulfuryl fluoride for 6 hours had a 3-fold higher plasma fluoride concentration than rats exposed under the same conditions. However, brain fluoride concentrations were similar. If fluoride enters the brain via systemic circulation, one would expect a similar magnitude of difference in brain fluoride levels. This finding lends further support to a non-systemic brain access pathway after acute inhalation exposure to sulfuryl fluoride. For further discussion, see Section II.E.3 in the final Addendum and Appendix F on the PBPK model uncertainty.

Douglas comment 3: Database Uncertainty Factor (UF_{DB}). The draft Addendum proposes the continued use of an UF_{DB} of 3x for acute exposure durations. The commenter's Request for Reconsideration regarding this factor is based on ensuring that the derivation of the UF_{DB} is consistent with US Environmental Protection Agency policy, the guidance provided to DPR by the National Research Council for quantification of uncertainties, and the need to assure that the UF_{DB} is meaningful to its purpose and scientifically justified within the quantification of risk. In addition, the commenter states that the draft Addendum uses data on sulfuryl fluoride to reduce the UF_{DB} from 3x to 1x for short-term exposures but not for acute exposures without providing sufficient rationale for this differentiation.

DPR response: DPR discussed the UF_{DB} for acute exposure durations in its 2017 memorandum (DPR, 2017), in the draft Addendum (DPR, 2018), and in the final Addendum (see Section V.E.2).

Briefly in its 2006 RCD, DPR established an acute POD from a study with adult rats. The acute RfC was calculated based on this POD and a total uncertainty factor that included an UF_{DB} of 10 for lack of a developmental neurotoxicity study. In 2017, a special DNT study was submitted for DPR's consideration. This study exposed rat pups to sulfuryl fluoride for 11 days during PND10 – PND21 (Marty et al. 2015). The results from the DNT study showed that fluoride concentrations in pup brains were close to those measured in adult animals (Hotchkiss et al., 2011a). Similar findings were reported in earlier studies that covered different developmental periods (fetuses and pups exposed indirectly during

gestation or via lactation; Marty et al., 2011a; Marty et al., 2011b). Based on these collective results, DPR reduced the pharmacokinetic component of the UF_{DB} from 3x to 1x for acute exposures. However, there were remaining uncertainties regarding the possible sensitivity of immature organisms to sulfuryl fluoride. The DNT study covered a limited postnatal developmental period and there was no study in which pups were exposed to sulfuryl fluoride during the early postnatal days. DPR acknowledges the difficulty in conducting any inhalation exposure study on such young animals (DPR, 2017). Even so, the sensitivity of that age group remains unknown, and therefore represents a database gap that should be accounted for. In addition, motor activity in the DNT study was not measured immediately after sulfuryl fluoride exposure when fluoride (the putative toxic species) concentrations were shown to peak in the brain. Considering all these uncertainties, the 3x pharmacodynamic component was retained, resulting in a final UF_{DB} of 3x. Therefore, the acute RfC values of 0.25 ppm (unknown mode of action) and 0.75 ppm (systemic and portal of entry effect), based on the POD of 300 ppm from the acute neurotoxicity study with adult rats, utilized an UF_{DB} of 3x (see Table 11 in the Final Addendum).

The short-term RfC was based on a POD for decreases in motor activity in postnatal pups, which would allow the UF_{DB} of 3x for adult-derived endpoints to be reduced to 1x in recognition of the decreased database uncertainty associated specifically with young animals. Therefore, the short-term RfC values of 0.042 ppm (systemic or portal of entry effects) and 0.013 ppm (unknown mode of action) based on the POD of 5 ppm from the DNT study with rat pups utilized an UF_{DB} of 1x (Table 12 in the Final Addendum).

III. Response to Douglas Comments submitted July 11, 2019

Supplement to Request for Reconsideration to Sulfuryl Fluoride Draft Addendum to the 2006 Risk Characterization Document, Update of the Toxicology and Reference Concentrations, Human Health Assessment Branch, Department of Pesticide Regulation December 2018 (DeSesso et al., 2019b), Appendix 1 Information Request 5: Special Developmental Neurotoxicity and Toxicokinetic (DNT/TK) Study (Marty et al. 2015): Supplemental Information Details

Douglas comment: On April 10, 2019, DPR met with Douglas and its representatives to review and discuss the March 2019 Request for Reconsideration. During this meeting, DPR requested additional information regarding some of the technical points presented. This additional document is designed to provide DPR with the requested information. The commenter also provides supplemental information regarding the special non-guideline developmental neurotoxicity/toxicokinetic (DNT/TK) study conducted for sulfuryl fluoride (Marty et al., 2015) and the role this study should play in informing selection of a scientifically appropriate database uncertainty factor for acute sulfuryl fluoride exposures to residents/bystanders (infants).

DPR response: DPR sincerely appreciates the time and effort of the commenter to provide additional information. Many of the details have been included in the final Addendum and appendices. Specifically, an analysis of whether toxic species other than fluoride (e.g., sulfonated adducts) could impact motor activity and other neurological effects is now detailed in Appendices E and G. An exploration of the basal ganglia as a target site for inhaled sulfur fluoride across all tested species as well as in one human case is now detailed in Appendix E. The commenter's point about the appropriateness of using animals receiving low fluoride diets for use in toxicological evaluations was helpful. We revised the critical endpoint so that it reflects only animals receiving normal laboratory diet. As to the application of results from the special non-guideline DNT/TK study (Marty et al., 2015), we thank you again and refer you to our response to Douglas comment #3, above.

With regards to the statistical and biological significance of the motor activity results in the special non-guideline DNT/TK study, our analysis of the data was discussed in the DPR 2017 memorandum. To further clarify, we found that the statistical discrepancy lies with the motor activity analysis in males at post-natal day (PND) 22. The study authors found a statistically significant difference only between controls and animals dosed with 20 ppm, while US EPA found significantly elevated motor activity at both 20 and 150 ppm. For its part, DPR used multiple statistical approaches for this dataset. DPR first examined total session activity data (ambulatory counts summed across the entire 48-min session), finding that 20 ppm values were significantly higher than controls ($p = 0.0184$). DPR then examined the activity data for each epoch and treated the epoch as a fixed effect in a linear regression model. Again, DPR found significant differences in motor activity counts between control and 20 ppm animals ($p = 0.00004$). Furthermore, DPR dichotomized the motor activity data by scoring animals as positive when they showed elevated motor activity in two or more epochs, then used chi-squared testing to analyze the difference in occurrence frequency among the four groups. DPR again found significantly elevated frequencies in animals scoring positive at 20 ppm compared to controls ($p = 0.0065$). Finally, DPR used linear regression with mixed effects treating epochs as repeated measures to test the difference in habituation rate among the four groups, finding no significant dose effect. DPR thus concluded that the elevated motor activity values in the 20 ppm treated group were statistically different than controls. This conclusion agrees with study authors' analysis (Marty et al., 2015).

DPR cannot rely on the null results reported for the Functional Observational Battery (FOB) open field test because ambulatory activity in the open field test was assessed subjectively and because FOB endpoints are usually less sensitive than motor activity in detecting treatment-related effects (Raffaele et al., 2010). The null results of FOB and motor activity determinations at PND55 cannot be used to disregard the statistically significant findings of elevated motor activity at PND22. Finally, the lack of dose responsiveness at 150 ppm is possibly due to non-linear toxicokinetics, with systemic toxicity overriding the stimulatory responses in the brain at that dose (DPR, 2017). The reduced body weight gain between PND17 – 21 in both male and female pups treated with 150 ppm is a sign of toxicity. In

reviewing functional assays for neurotoxicity testing, Dr. Virginia Moser (US EPA, retired) has stated, “For many behavioral measures, nonmonotonic or inverted U-shaped curves are not uncommon, often due to feedback regulation of the nervous system ... It is important to note that at higher doses, effects may be more generalized and not necessarily due to a direct action on the nervous system” (p. 42, Moser, 2011).

IV. Response to Douglas Comments submitted December 6, 2019

Additional Supplement to Request for Reconsideration to Sulfuryl Fluoride Draft Addendum to the 2006 Risk Characterization Document, Update of the Toxicology and Reference Concentrations, Human Health Assessment Branch, Department of Pesticide Regulation December 2018 (DeSesso et al., 2019c).

Douglas comment RE: US EPA 1994 Regional Gas Dose Ratio Approach. The commenter states that the 1994 US EPA Regional Gas Dose Ratio (RGDR) approach has been superseded and that using this approach is not consistent with current science. Rather, the US EPA (2012) dose adjustment factor approach is based on updated scientific literature and understanding of the adjustment factor and uses more advanced methods, including computational fluid dynamics (CFD) and physiologically based pharmacokinetic (PBPK) models.

DPR response: We approached the calculation of the sulfuryl fluoride reference concentrations (RfCs) using several different methodologies to reflect the most recent advances in animal to human equivalency and the currently available data. As explained above, we proposed three distinct RfCs for possible use as regulatory targets. These values are based on three possible modes of action: 1) systemic, 2) portal of entry from the nasal cavity, and 3) an unknown mode of action. The final Addendum uses the default dose adjustment factor (DAF) for portal of entry effects in the extrathoracic region as recommended by US EPA in 2012 to calculate one of the candidate human equivalent concentrations (see Section IV.A.1).

Douglas comment RE: Comparison of Rodents to Primates for Alternate Mode of Action (Direct Nose to Brain Mechanism). The commenter states that the direct intranasal transport of a compound such as fluoride to the brain is possible in rats and may result in accumulation of the compound in the basal ganglia to a limited extent. However, in humans, neither direct intranasal transport nor the resultant accumulation of a compound such as fluoride in the basal ganglia would occur. Therefore, the alternate mode of action is not viable in humans, and should be eliminated as an option for the acute RfC.

DPR response: See our responses to Douglas comment 1.2 above regarding the intranasal transport of fluoride to the brain and its accumulation in the basal ganglia. Other possible pathways that may deliver fluorosulfate or sulfonated adducts (e.g., FSO₂-tyrosine) are discussed in Appendices E and G.

Karen Morrison

May 25, 2020

Page 13

Douglas comment RE: Pharmacokinetic Perspective of Fluoride Plasma to Brain Ratio.

The commenter states that fluoride plasma to brain ratios do not support an alternate mode of action. Instead, the collective data show that the relative uptake of fluoride into brain tissue compared with plasma is primarily a function of exposure duration and not route, and therefore is a consequence of differences in pharmacokinetics.

DPR response: See our response to Douglas comment 1.3, above.

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