Sulfuryl Fluoride

Addendum to the 2006 Risk Characterization Document Update of the Toxicology and Reference Concentrations

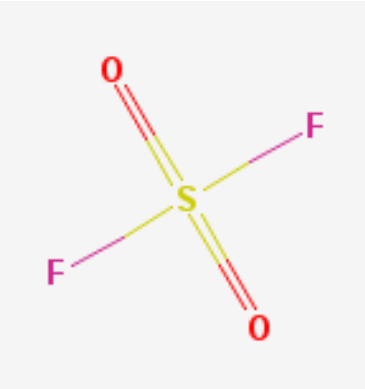

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TABLE OF CONTENTS

LIST OF TABLES VI

LIST OF FIGURES VI

LIST OF ABBREVIATIONS VII

EXECUTIVE SUMMARY 1

I. INTRODUCTION 5

I.A. REGULATORY HISTORY 6

I.B. DPR EVALUATION OF SULFURYL FLUORIDE: EVOLUTION OF THE DERIVATION OF REFERENCE CONCENTRATIONS FOR SULFURYL FLUORIDE 7

II. TOXICOLOGY PROFILE 10

II.A. PESTICIDE ILLNESSES AND HUMAN EXPOSURE 10

II.A.1. CALIFORNIA INCIDENCE 10

II.A.2. NATIONAL INCIDENCE 18

II.B. GENOTOXICITY 20

II.C. DEVELOPMENTAL NEUROTOXICITY 20

II.D. ONCOGENICITY 21

II.E. PHARMACOKINETICS 22

II.E.1. PHARMACOKINETICS IN RAT 23

II.E.2. PHARMACOKINETICS IN RABBIT 32

II.E.3. PHARMACOKINETICS IN RAT VS. RABBIT 33

II.E.4. PHARMACOKINETICS IN HUMAN 34

II.E.5. SUMMARY OF PHARMACOKINETICS 34

II.F. SULFURYL FLUORIDE PBPK MODEL 37

III. POINTS OF DEPARTURE ESTABLISHED IN ANIMAL STUDIES 40

III.A. SUMMARY OF CRITICAL TOXICOLOGICAL EFFECTS BY THE INHALATION ROUTE 40

III.B. SELECTION OF POINTS OF DEPARTURE 41

III.C. POINTS OF DEPARTURE FOR ACUTE EXPOSURE 42

IV. DERIVATION OF REFERENCE CONCENTRATIONS 44

IV.A. METHODOLOGIES FOR RFC DERIVATION 44

IV.A.1. METHODOLOGY 45

IV.A.2. EXAMPLE RFC CALCULATIONS FOR RESIDENTIAL BYSTANDERS 48

IV.A.3. EXAMPLE RFC CALCULATIONS FROM THE SULFURYL FLUORIDE PBPK MODEL 49

IV.B. RFCS FOR ACUTE TOXICITY 50

IV.C. RFCS FOR SHORT-TERM (10-14 DAYS) TOXICITY 52

IV.D. RFCS FOR SUBCHRONIC TOXICITY 54

IV.E. RFCS FOR CHRONIC TOXICITY 56

IV.F. SUMMARY OF CRITICAL NOELS, ADJUSTED PODS, AND RFCS 59

V. UNCERTAINTIES IN THE REFERENCE CONCENTRATION DETERMINATION 61

V.A. APPRAISAL OF UNCERTAINTIES ASSOCIATED WITH THE TOXICOLOGY AND HAZARD IDENTIFICATION 61

V.A.1. ACUTE TOXICITY 61

V.A.2. SHORT-TERM TOXICITY 61

V.A.3. SUBCHRONIC TOXICITY 62

V.A.4. CHRONIC TOXICITY 62

V.B. UNCERTAINTIES IN THE TOXIC METABOLITES OF SULFURYL FLUORIDE 62

V.C. UNCERTAINTIES IN THE PBPK MODEL 63

V.D. UNCERTAINTIES IN THE INTERSPECIES AND INTRASPECIES UF 64

V.E. ADDITIONAL DATABASE UNCERTAINTIES 64

V.E.1. UF_{DB} PHARMACOKINETIC UNCERTAINTY 64

V.E.2. UF_{DB} PHARMACODYNAMIC UNCERTAINTY 65

V.F. UNCERTAINTIES IN THE DERIVATION OF ACUTE REFERENCE CONCENTRATIONS FOR SULFURYL FLUORIDE 65

VI. CONCLUSION 67

VII. REFERENCES 69

APPENDIX A. PHYSICAL AND CHEMICAL PROPERTIES OF SULFURYL FLUORIDE. 78

APPENDIX B. ADDITIONAL STUDIES AND INFORMATION REVIEWED FOLLOWING COMPLETION OF THE 2006 RISK CHARACTERIZATION DOCUMENT FOR SULFURYL FLUORIDE 81

APPENDIX C. ESTABLISHING SULFURYL FLUORIDE UNCERTAINTY FACTORS FOR ACUTE AND SHORT-TERM EXPOSURE 84

APPENDIX D. BENCHMARK DOSE MODELING OF ENDPOINT DATA FOR SULFURYL FLUORIDE 92

APPENDIX E. ACCESS OF FLUORIDE TO THE BRAIN BY THE INTRANASAL ROUTE: ALTERNATIVE ENTRY PATHWAYS 122

APPENDIX F. UNCERTAINTIES RELATED TO THE PBPK MODEL 146

APPENDIX G. UNCERTAINTIES ASSOCIATED IN THE TOXIC METABOLITES OF SULFURYL FLUORIDE 153

APPENDIX H. POINTS OF DEPARTURE, UNCERTAINTY FACTORS AND REFERENCE CONCENTRATIONS ESTABLISHED BY US EPA, PMRA, AND EFSA..... 170

LIST OF TABLES

Summary Table 1. Proposed regulatory targets for sulfuryl fluoride for residential bystanders (acute reference concentrations, RfCs) ……..……...……4

Table 1. Approaches for deriving acute reference concentrations (RfCs) for sulfuryl fluoride 9

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017) 12

Table 3. Nationwide sulfuryl fluoride human case reports from open literature 18

Table 4. Sulfuryl fluoride hydrolysis using the headspace-type in vitro incubation..... 25

Table 5. Sulfuryl fluoride hydrolysis using the no-headspace in vitro incubation 26

Table 6. Tissue samples collected for sulfuryl fluoride measurement..... 34

Table 7. Peak mean tissue fluorosulfate concentration in rats and rabbits exposed by inhalation to 150 or 300 ppm of sulfuryl fluoride..... 35

Table 8. Peak mean tissue free fluoride concentration in rats and rabbits exposed by inhalation to 150 or 300 ppm of sulfuryl fluoride..... 36

Table 9. Summary of acute toxicity studies 43

Table 10. Dosimetric adjustment approaches and associated uncertainty factors (UF) used for sulfuryl fluoride induced toxic effects 48

Table 11. Reference concentrations (RfCs) for acute exposure of residential bystanders..... 51

Table 12. Summary of short-term (10-14 days) inhalation studies..... 52

Table 13. Summary of subchronic inhalation studies 54

Table 14. Summary of chronic inhalation studies..... 57

Table 15. No-observed-effect levels (NOELs), points of departure (PODs), and reference concentrations (RfCs) for residential bystanders (infants) exposed to sulfuryl fluoride 60

LIST OF FIGURES

Figure 1. Sulfuryl fluoride episodes and illness cases (1992-2017) and pounds of sulfuryl fluoride applied by structural pest control operators reported to DPR 12

Figure 2. Medical description of 197 non-lethal cases 12

LIST OF ABBREVIATIONS

APVMA	Australian Pesticides and Veterinary Medicines Authority
ATSDR	Agency for Toxic Substances and Disease Registry
BALF	Bronchoalveolar lavage fluid
BBB	Blood-brain barrier
BMC	Benchmark concentration
BMCL	95% lower bound of BMC
BMD	Benchmark dose
BMDL	95% lower bound of BMD
BMR	Benchmark response
BW	Body weight
CAP	California Aeration Plan
CAP II	California Aeration Plan II
DAF	Dosimetric adjustment factor
DNT	Developmental neurotoxicity
dpe	Days post exposure
DPR	California Department of Pesticide Regulation
EFSA	European Food Safety Authority
ER	Emergency room
FAO/WHO	Food and Agriculture Organization / World Health Organization
FOB	Functional observational battery
GD	Gestational day
$H_{b/g}$	Blood: gas (air) partition coefficient
HDT	Highest dose tested
HEC	Human equivalent concentration
hpe	Hours post exposure
i.v.	Intravenous
IC/SCD/NESI/MS	Ion chromatography with suppressed conductivity detection and negative ion electrospray ionization mass spectrometry
ISE	Ion-selective electrodes
JMPR	Joint FAO/WHO meeting on pesticide residues
LD	Lactation day
LLQ	Lower limit of quantitation
LOQ	Limit of quantitation

LOEL	Lowest observed effect level
MD/ISE	Microdiffusion with ion-selective electrode detection
MOA	Mode of action
NAS	National Academy of Sciences
NLF	Nasal lavage fluid
NOEL	No observed effect level
OIG	Office of Inspector General
PBPK	Physiologically based pharmacokinetic
PISP	Pesticide Illness Surveillance Program
PND	Postnatal day
POD	Point of departure
POD_{ADJ}	Duration-adjusted POD
POD_{HEC}	POD expressed as human equivalent concentration
PRMA	Health Canada's Pest Management Regulatory Agency
RBC	Red blood cell
RCD	Risk characterization document
RED	Reregistration Eligibility Document
RfC	Reference concentration
RGDR	Regional gas dose ratio
$RGDR_{ET}$	Regional gas dose ratio at extrathoracic region
$RGDR_{PU}$	Regional gas dose ratio at pulmonary region
RMD	Risk management directive
SA	Surface area (m^2)
TAC	Toxic air contaminant
TK	Toxicokinetics
TRAP	Tarpaulin Removal Aeration Plan
UF	Uncertainty factor
UF_A	Uncertainty factor to account for interspecies variability (animal to human)
UF_{DB}	Database uncertainty factor
UF_H	Uncertainty factor to account for intraspecies sensitivities (within-human)
US EPA	US Environmental Protection Agency
V_E	Minute ventilation (L/min)

EXECUTIVE SUMMARY

The purpose of this Addendum is to propose regulatory targets (acute reference concentrations, RfCs) for residential bystanders for potential exposure to the fumigant sulfuryl fluoride. Determination of these values is supported by a comprehensive analysis of all available data, including studies submitted prior to the Department of Pesticide Regulation's (DPR) 2006 Risk Characterization Document (RCD) and those after completion of the 2006 RCD. The database now includes 14 additional toxicology studies, updated human illness reports, additional neurotoxicity data, and new approaches to modeling internal doses and impacts to target tissues.

Background

Sulfuryl fluoride was first registered in the US in 1959 for use as a structural fumigant. It was subsequently registered in California in 1990 as Vikane Gas Fumigant and in 1997 as Vikane® for use in structural and other non-food fumigations to control drywood termites, powder post beetles, borers, bedbugs, clothes moths, rodents, and cockroaches in dwellings, buildings, construction materials, furnishings, and vehicles. In 2005, DPR also approved the registration of sulfuryl fluoride as ProFume® for use in food commodity fumigation of grains, nuts, and dried fruits.

The insecticidal mode of action of sulfuryl fluoride is not well understood. It appears to kill termites by depleting protein and amino acid stores. The hydrolysis product fluoride disrupts carbohydrate and lipid metabolism in termites, locust and mealworm eggs by inhibiting glycolytic enzymes and increasing oxygen uptake. In mammals, fluoride may act by binding calcium, potassium and magnesium ions, thereby affecting muscle activity and other vital cellular and physiological processes. In humans, acute inhalation exposure to high concentrations of sulfuryl fluoride causes respiratory irritation, pulmonary edema, nausea, abdominal pain, central nervous system depression, numbness in the extremities, muscle twitching, seizures, renal injury, necrosis in brain tissue and death. In laboratory animals, short-term or chronic inhalation exposures impact brain, respiratory system, teeth, and kidneys. Commonly observed neurotoxic effects in mice, rats, rabbits and dogs include malacia (necrosis) and vacuolation of white fiber tracts in the basal ganglia regions of the brain.

In 2006, DPR completed an RCD on sulfuryl fluoride for structural fumigation in California. Critical no observed effect levels (NOELs) and reference concentrations (RfCs[1]) for workers and residential bystanders were established. The acute RfC for bystanders was 0.12 ppm. This RfC

[1] Reference concentrations (RfCs) are target air concentrations. They are estimates of inhalation exposures to humans that are likely to be without appreciable risk of deleterious effects. These values are calculated by dividing the critical endpoint concentrations (points of departure, POD) by the uncertainty factors appropriate to the exposure scenarios evaluated. The commonly used default uncertainty factors are 10 for interspecies sensitivity and 10 for intraspecies variability, which can be adjusted based on available data of pharmacokinetics or pharmacodynamics. Additional uncertainty factors may be applied to account for data gaps.

was based on a NOEL of 300 ppm from a two-day inhalation neurotoxicity study in adult rats and assumed an uncertainty factor of 10x to account for interspecies variability, an uncertainty factor of 10x to account for human (intraspecies) sensitivity, and an additional uncertainty factor of 10x due to the lack of data for effects of sulfuryl fluoride in the developing nervous system. The 2006 RCD concluded that the estimated acute exposure concentrations for bystanders exceeded one-tenth of the RfC, thus meeting the criteria for listing as a Toxic Air Contaminant under California's Toxic Air Contaminant Act (TAC, CA Food & Agricultural Code §14021-14027). Formal pesticide TAC listing followed extensive review by the TAC Scientific Review Panel, an outside body of scientists charged with evaluating risk assessments of substances proposed for identification as TACs. Sulfuryl fluoride is not listed as a developmental or reproductive toxicant nor as a carcinogen under California's Proposition 65 (the Safe Drinking Water and Toxic Enforcement Act of 1986).

Since the completion of the 2006 RCD, Dow AgroSciences (the registrant at the time) submitted a series of pharmacokinetic studies in laboratory animals and developed a cross-species physiologically based pharmacokinetic (PBPK) model to inform animal-to-human (interspecies) extrapolation. In addition, in consultation with DPR, the United States Environmental Protection Agency (US EPA) required the registrant to conduct a special non-guideline postnatal developmental neurotoxicity/toxicokinetic (DNT/TK) study in rats to address a significant data gap in the neurotoxicity database. This study was submitted to DPR in 2015. Based on the non-guideline DNT study findings, DPR established interim RfCs for sulfuryl fluoride for acute, short-term to subchronic, and chronic exposures for workers and bystanders in 2017 (DPR, 2017a). For bystanders, the interim acute RfC of 0.41 ppm was based on the same critical endpoint concentration set in the 2006 RCD. However, it was calculated after reducing the database uncertainty factor from 10 to 3, an adjustment supported by the data from the newly submitted DNT and pharmacokinetic studies. DPR retained the database factor of 3x to account for remaining uncertainties related to lack of early developmental neurotoxicity data and toxicity data on metabolites, as well as concerns that the available studies may not have captured the total extent of the induced neurotoxicity.

In December 2018, DPR prepared a draft Addendum to the 2006 RCD in which data from human morbidity and mortality associated with fumigation and 14 new toxicology studies were reviewed (DPR, 2018). The latter studies encompassed genotoxicity, developmental neurotoxicity, metabolism, pharmacokinetics, and PBPK modeling, all of which became available after the completion of 2006 RCD. In February 2019, DPR requested external scientific review of the draft Addendum according to the 2006 California Environmental Protection

Agency External Scientific Peer Review Guidelines[2]. Four reviewers were nominated by the University of California, Berkeley to comment on the main conclusions of the draft Addendum. The reviewers included Dr. Michele Bouchard from the Department of Environmental and Occupational Health of the University of Montreal in Montreal, Canada; Dr. Jacqueline MacDonald Gibson from the Department of Environmental Sciences and Engineering of the University of North Carolina in Chapel Hill; Dr. Chensheng Lu from the Southwest University in Chongqing, China; and Dr. Babasaheb Sonawane from Georgetown University in Washington, DC. In addition, the draft Addendum was reviewed by the Health Effects Division in US EPA's Office of Pesticide Programs and by the current sulfuryl fluoride registrant, Douglas Products and Packaging Company.

Updates included in this Addendum

- Dose response analysis using the benchmark dose (BMD) approach
- Re-examination of the default dosimetric approaches used to calculate human equivalent concentration (HEC)
- Review of literature on fluoride neurotoxicity through oral exposure
- Revised analysis of human illness reports
- Review of literature on entry of chemicals from nose to the brain via the local vascular pathway
- Additional statistical analysis on motor activity data from the non-guideline postnatal developmental neurotoxicity study (DNT) /Toxicokinetic study in rats
- Additional pharmacokinetic analysis on fluoride tissue concentrations

Proposed Revisions to the Reference Concentrations

This final Addendum incorporates comments and recommendations from the external scientific reviewers and the application of the most recent scientific advances to better refine RfCs for use as proposed regulatory targets. 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. The approaches taken and uncertainties of each resulting value are explained in detail in the following pages, including the evolution of DPR's regulatory evaluation of sulfuryl fluoride PODs from 2006 to the present. In considering all

[2] Senate Bill 1320 (Sher), Chapter 295, statutes of 1997, mandates that before any CalEPA Board, Department, or Office adopts a final version of a rulemaking, the scientific findings, conclusions, and assumptions on which the proposed rule are based must be submitted for independent external scientific peer review. This requirement is incorporated into the California Health and Safety Code Section 57004. The current Guidelines are available at https://dtsc.ca.gov/wp-content/uploads/sites/31/2018/07/Cal-EPA-ESPR-Guidelines.pdf. Under the current Interagency Agreement, the University of California, Berkeley provides nominations of qualified candidates for expert reviews of other technical work products of interest to the People of California, https://ceparev.berkeley.edu/other-peer-reviews/

comments, new data, and available approaches, DPR is proposing the following values as potential regulatory targets for sulfuryl fluoride for residential bystanders.

Summary Table 1. Proposed regulatory targets for sulfuryl fluoride for residential bystanders (acute reference concentrations, RfCs)

Parameters	Mode of Action		
	Systemic	**Portal of Entry (Extrathoracic Region)**	**Unknown**
NOEL (ppm)	300	300	300
POD_{ADJ} (ppm)	75	75	75
DAF	1[a]	1[b]	None
POD_{HEC} (ppm)	75	75	--
UF_A	3	3	10
UF_H	10	10	10
UF_{DB}	3	3	3
UF_{total}	100	100	300
RfC (ppm)	**0.75** (POD_{HEC}/UF_{total})	**0.75** (POD_{HEC}/UF_{total})	**0.25** (POD_{ADJ}/UF_{total})

Abbreviations: DAF, dosimetric adjustment factor; NOEL, no observed effect level; POD, point of departure; POD_{ADJ}, POD adjusted by duration; POD_{HEC}, human equivalent concentration; ppm, parts per million; RfC, reference concentration; UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies sensitivity; UF_{DB}, database uncertainty factor.

[a]Default DAF based on blood/gas partition coefficient ($H_{b/g}$) between rats and humans for sulfuryl fluroide induced systemic effects (U.S. EPA, 1994).

[b]Default DAF based on regional gas dose ratio (RGDR) between rats and humans for sulfuryl fluoride induced portal of entry effects at extrathoracic region (U.S. EPA, 2012a).

I. INTRODUCTION

Sulfuryl fluoride is a fumigant registered for use in California for both structural (Vikane®) and food commodity (ProFume®) fumigation. In 2006, the California Department of Pesticide Regulation (DPR) completed a risk characterization document (RCD) on sulfuryl fluoride for structural and non-food commodity fumigations (DPR, 2006a). The RCD established critical no observed effect levels (NOELs) and reference concentrations (RfCs) for workers and residential bystanders. It also concluded that the estimated acute exposures for bystanders exceeded one-tenth of the RfC, thus meeting the criteria established for listing as a pesticide toxic air contaminant under California's Toxic Air Contaminant Act (TAC, CA Food & Agricultural Code §14021-14027). The RCD also identified risks for workers exposed to sulfuryl fluoride in the air in occupational settings.

In April 2007, DPR issued a Risk Management Directive (RMD) that set target air concentrations for workers (acute and longer-term exposures) and for bystanders and residents (acute exposures). The acute RfC for bystanders was 0.12 ppm. In addition to the 10x uncertainty factors each for interspecies variability and intraspecies sensitivity, this value included an additional uncertainty factor of 10 to account for a lack of a developmental neurotoxicity study (DNT) to assess the toxicity of sulfuryl fluoride on the developing nervous system. Following issuance of the RMD, the registrant at the time, Dow AgroSciences, submitted a series of pharmacokinetic studies conducted in adult, perinatal and weanling rats, and in adult rabbits. In addition, the registrant also developed a cross-species physiologically based pharmacokinetic (PBPK) model to inform animal-to-human (interspecies) extrapolation. In consultation with DPR, US EPA required Dow AgroSciences to conduct a special non-guideline postnatal DNT/toxicokinetic study to address inadequacies in the neurotoxicity database. This study was submitted to both US EPA and DPR in 2015 (Marty *et al.*, 2015). In light of the evidence from the newer studies, along with recent advances in deriving RfCs, a draft Addendum was developed in 2018 that reevaluated the sulfuryl fluoride toxicological database and updated the critical NOELs and RfCs for bystander inhalation exposure from structural fumigation. The draft Addendum underwent external scientific review in 2019 and has now been finalized with proposed regulatory targets.

Concurrent with the advances in toxicological data, DPR continued its efforts at mitigating worker exposure to sulfuryl fluoride from structural fumigation. Interim reference concentrations (RfCs) of 2.6 ppm for female workers (8-hour exposure duration) and 0.13 ppm for short-term (1-2 week) exposure durations were proposed in 2017 (DPR, 2017a). Analysis of measured air concentrations from registrant submitted data (Barnekow and Rotondaro, 2015) indicated that worker exposures were below the 2017 interim RfCs (Stefanova-Wilbur, 2017). DPR determined that worker exposure is within the target (DPR, 2017b). DPR staff continue to monitor illness data to assess worker and bystander exposures from structural fumigations (see Section II.A.).

Additional analysis and mitigation of residential bystander exposure may be warranted based on the findings of this Addendum.

I.A. Regulatory History

1959 Sulfuryl fluoride (Vikane®) first registered as a pesticide for structural fumigation

1985 US EPA issued a reregistration guidance document for sulfuryl fluoride

1990 Sulfuryl fluoride as Vikane Gas Fumigant was registered in California and in 1997 as Vikane® for use in structural and non-food commodity fumigations to control drywood pests and non-drywood pests such as cockroaches and bedbugs

1990 Tarpaulin Removal Aeration Plan (TRAP) implemented as the industry standard for tarpaulin removal in California

1993 US EPA released the Reregistration Eligibility Document (RED) for sulfuryl fluoride

1995 TRAP revised (DPR, 1995)

2001 US EPA reevaluated the toxicology database and determined that a developmental neurotoxicity study should be required (U.S. EPA, 2001)

2001 US EPA set the worker reentry level should be 1 ppm, but determined that residential exposure would be negligible

2005 US EPA registered sulfuryl fluoride for use both as a fumigant on several additional commodities and as a structural fumigant for food processing facilities; corresponding tolerances were also established (U.S. EPA, 2005)

2005 Joint FAO/WHO Meeting on Pesticide Residues (JMPR) published sulfuryl fluoride toxicological evaluation (Samuels *et al.*, 2005)

2006 DPR completed a RCD on sulfuryl fluoride for structural fumigation (DPR, 2006a)

2006 DPR listed sulfuryl fluoride as a Toxic Air Contaminant under California's Toxic Air Contaminant Act (DPR, 2006a)

2006 US EPA changed the residential re-entry label to 1 ppm in 2006 for Vikane (U.S. EPA, 2006)

2007 DPR issued a RMD for target air concentrations for workers and for bystanders and residents based on the conclusions in the 2006 RCD (DPR, 2007)

2010 California Aeration Plan (CAP) implemented as industry standard for tarpaulin removal in California (DPR, 2010)

2013 CAP updated to CAP II, which is the current practice for tarpaulin removal in California (DPR, 2013)

2014 US EPA required Dow AgroSciences to conduct a special non-guideline postnatal DNT/toxicokinetic study to address inadequacies in the neurotoxicity database

2015 Results from the special non-guideline study were submitted to both US EPA and DPR

2017 DPR published interim RfCs for residential bystanders and workers for acute and short-term exposures. These values were based on new toxicology data submitted after the

completion of DPR's 2006 RCD, including data from the 2015 non-guideline postnatal DNT/toxicokinetic study (DPR, 2017a)

2017 DPR published a memorandum indicating the completion of worker mitigation for sulfuryl fluoride structural applications when following CAP II (DPR, 2017b)

2019 DPR invited external scientific review of its draft Addendum with proposed reference concentrations

I.B. DPR Evaluation of Sulfuryl Fluoride: Evolution of the Derivation of Reference Concentrations for Sulfuryl Fluoride

Reference concentrations (RfCs) are target air concentrations that are likely to be without appreciable risk of deleterious effects in human populations. These values are calculated by dividing the critical endpoint concentrations (expressed as points of departure, or PODs) by the specific uncertainty factors (UF) appropriate to the exposures evaluated.

To establish the critical endpoint concentration for sulfuryl fluoride, DPR considered all previous studies as well as all new submitted and open literature studies that were relevant for establishing acute, subchronic, and chronic endpoints. Results from these studies showed that the brain, respiratory system, teeth, and kidneys were impacted. Neurotoxicity was identified as the most sensitive endpoint in animals. There is also evidence for neurotoxic effects in humans after accidental exposures.

The 2006 RCD established a critical acute POD from a two-day inhalation neurotoxicity study in adult rats by Albee *et al.* (1993). Our re-analysis of the data originally reviewed in the 2006 RCD concurs with the selection of Albee *et al.* (1993) as the critical acute study. Newer studies submitted after the completion of the 2006 RCD included a developmental neurotoxicity study in which rat pups were repeatedly exposed to sulfuryl fluoride for 11 days. Developmental toxicity studies are of particular interest for detecting acute effects. However, this study did not reveal endpoints that can potentially result from an acute exposure. Several new pharmacokinetic studies with acute or short-term exposure regimens were also recently submitted to DPR. This studies were designed to address the absorption, distribution, metabolism and excretion of sulfuryl fluoride, not strictly for toxicological evaluation. Therefore, none of the new studies could be utilized to update the acute POD. As such, the current analysis relies again on the acute POD from by Albee *et al.* (1993) study as it did in the 2006 RCD.

These investigators examined a range of neurologic endpoints using functional observational batteries (FOB), motor activity determinations, and electrophysiological parameters. No effects were observed at the highest dose tested of 300 ppm (Albee *et al.*, 1993). This no-observed effect level (NOEL) of 300 ppm was considered most appropriate for estimating human risk from acute exposure to sulfuryl fluoride. Appendix H lists studies used by other regulatory agencies to establish acute, subchronic, and chronic PODs.

To calculate the acute RfC, the critical acute NOEL of 300 ppm was adjusted by the duration of the exposure in the acute inhalation study in rats (6 hours) and the expected duration of human exposure (24 hours for residential bystanders). The adjusted acute POD (POD_{ADJ}) was 75 ppm. This animal POD_{ADJ} was then converted to a human equivalent concentration (HEC) by applying dosimetric adjustment factors (DAF). The HECs (or POD_{HEC}) is the external air concentration that produces the same internal target tissue dose in humans as that achieved in laboratory animals. The choice of appropriate DAF depends on whether the effect is mediated through systemic distribution or represents a local (portal of entry) effect.

In the 2006 evaluation of sulfuryl fluoride, the animal POD_{ADJ} of 75 ppm was converted to HEC by normalizing the inhaled dose by body weight as a dosimetric adjustment from animal data to humans. The resultant HEC was 122 ppm, assuming a systemic MOA. This HEC was retained in the 2017 DPR memo, however, the UFs were adjusted downward (DPR, 2017a). A new PBPK model and a revised US EPA HEC methodology were released in 2011 and 2012, respectively, making it possible to derive HEC values using these two new approaches. In the former, the PBPK model resulted in an HEC of 326 ppm based on internal peak brain free fluoride from a systemic MOA. Using the 2012 US EPA inhalation gas dosimetry methodology, which applied a dosimetry adjustment factor of 1 for portal of entry effects in the extrathoracic region, the resulting HEC was unchanged from the POD at 75 ppm. Another approach involved application of the 1994 US EPA methodology for deriving reference concentrations, which resulted in an HEC of 4.8 ppm for portal of entry effects in the extrathoracic region or 75 ppm for systemic effects. The DPR analysis of the updated toxicology database and reanalysis of all previous data did not clarify whether sulfuryl fluoride acts through a systemic or portal of entry MOA. DPR also derived the sulfuryl fluoride RfC directly from the POD without assuming a specific MOA.

To calculate the final RfC, the HEC is divided by appropriate uncertainty factors (UFs). The commonly used default UFs are 10x to account for interspecies variability (UF_A) and 10x to account for intraspecies (human) sensitivity (UF_H). Both UFs are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x. The pharmacokinetic portion of the UF_A is typically reduced when the RfC is derived with dosimetric adjustments that account for physiological and anatomical differences between animals and humans. Additional UFs may be applied to account for gaps in the database (UF_{DB}) or for other uncertainties.

DPR's initial regulatory evaluation of sulfuryl fluoride resulted in target acute RfCs ranging from 0.048 ppm – 3.26 ppm (Table 1) (DPR, 2018). Because the same acute POD was used as the starting point for all approaches, the difference in magnitude resulted from the choice of dosimetric approach and the values of the underlying uncertainty(s). DPR has varying levels of confidence in each approach, which are further elucidated in Sections IV and V, herein. DPR's assessment of the best available science and incorporating the results from external scientific review have further refined these values to a range of 0.25 ppm – 0.75 ppm (shaded values

below), as explained further in this Addendum and supporting appendices.

Table 1. Approaches for deriving acute reference concentrations (RfCs) for sulfuryl fluoride

Parameters	Systemic MOA				Portal of Entry MOA		Unknown MOA
	DPR 2006 RfCs [a]	DPR 2017 Interim RfCs [b]	PBPK	US EPA $H_{b/g}$ [c]	US EPA 1994 RGDR[c]	US EPA 2012 RGDR[d]	
NOEL (ppm)	300	300	300	300	300	300	300
POD_{ADJ} (ppm)	75	75	--	75	75	75	75
Normalization	Inhaled dose by BW	Inhaled dose by BW	Internal peak brain free fluoride	Default DAF = 1	Inhaled dose by SA_{ET} (DAF = 0.064)	Default DAF = 1	None
POD_{HEC} (ppm)	122	122	326	75	4.8	75	--
UF_A	10	10	3	3	3	3	10
UF_H	10	10	10	10	10	10	10
UF_{DB}	10	3	3	3	3	3	3
UF-total	1000	300	100	100	100	100	300
RfC =HEC/UF (ppm)	0.122	0.41	3.26	0.75	0.048	0.75	0.25

Abbreviations: BW, body weight; DAF, dosimetric adjustment factor; $H_{b/g}$, blood:gas (air) partition coefficient; MOA, mode of action; NOEL, no observed effect level; POD, point of departure; POD_{ADJ}, POD adjusted by duration. POD_{HEC}, human equivalent concentration; ppm, parts per million; RfC, reference concentration; RGDR, regional gas dose ratio; SA_{ET}, surface area at extrathoracic region; UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies (human) sensitivity; UF_{DB}, database uncertainty factor. References: [a]DPR (2006b); [b]DPR (2017a); [c]U.S. EPA (1994); [d]U.S. EPA (2012a). Shaded cells indicate proposed regulatory targets.

II. TOXICOLOGY PROFILE

The following section includes new data and/or new analyses and provide an update to the 2006 RCD. Included are updated human exposure reports and 14 newer studies submitted by Dow AgroSciences on the toxicology of sulfuryl fluoride. The latter include studies on genotoxicity, metabolism and pharmacokinetics, PBPK modeling, and developmental neurotoxicity and toxicokinetics, as well as a discussion of database uncertainties[3]. In particular, the pharmacokinetic data provide new insights into the mode of action of sulfuryl fluoride in inducing neurological effects. A rigorous search of the open literature on sulfuryl fluoride since 2005 did not reveal additional studies that could be used to establish critical endpoints for risk assessment. However, new studies on sulfuryl fluoride's main degradates fluorosulfate and fluoride were identified and are also reviewed in this Addendum, as they contribute to a more nuanced understanding of the toxicological risks associated with sulfuryl fluoride exposure in humans.

II.A. Pesticide Illnesses and Human Exposure

II.A.1. California Incidence

Between 1992 and 2017, there were 141 episodes[4] resulting in 204 cases[5] reported to DPR's Pesticide Illness Surveillance Program (PISP) involving sulfuryl fluoride for structural fumigations (Figure 1). Twenty-three of those cases were classified as definite[6], 70 as probable[7], and 111 as possible[8]. These cases were associated with exposure to sulfuryl fluoride, or in combination with chloropicrin (warning agent), resulting from spillage, drift, and residues. For non-lethal cases, reported symptoms mainly confined to eye, respiratory system, gastrointestinal, and nervous system (Figure 2). Skin (facial and arm) irritations described as itching, burning, hives, and rash were reported in 7.6% cases. Eye irritations described as watery, dryness, itching, red, swollen, burning, and tearing were reported in 41% of cases. A symptom described as bad, bitter, foul, funny, metallic, sour or strange taste in mouth was reported in 7.1% of cases. Upper respiratory symptoms mainly occurred at nose and throat. Nose irritation described as runny, burning, dry, itchy, bleeding, pain, sneezing, stuffing, and swelling was reported in 13% of cases. Throat symptoms described as sore, irritation, burning, dry, itchy, scratchy, swelling, and pain were reported in 25% of cases. Pulmonary dysfunction described as shortness of breath, wheezing, asthma and hyperventilation was reported in 31% of cases. Other pulmonary symptoms include cough (18.3%), tightness, burning, pain, and irritation in the chest (15.2%),

[3] A complete study list appears in Appendix B of this document.
[4] Episode: an event in which a particular source appears to have exposed one or more people (cases) to pesticides.
[5] Case: Representation of an individual's exposure to a pesticide(s) that has been evaluated as definitely, probably, or possibly related to pesticide exposure.
[6] Definite: both physical and medical evidence document exposure and consequent health effects
[7] Probable: limited or circumstantial evidence supports a relationship to pesticide exposure
[8] Possible: health effects correspond generally to the reported exposure, but evidence is not available to support a relationship

and lung congestion and edema (3%). Gastrointestinal symptoms include nausea (33%), vomiting (12.2%), abdominal pain (5.6%), diarrhea (3.0%), and stomach pain (2.5%). Nervous system symptoms include headache (40.6%), dizziness (22.8%), lightheadedness (7.6%), weakness, fatigue, lethargy, and exhaustion (12.2%), and tingling or numbness in face, hand, foot, lip, mouth, and tongue (5.1%). Behavior symptoms (5.0%) include tremors, staggering, shakiness, anxiety, and agitation. Heart dysfunction includes arrhythmia (6.1%) and elevated blood pressure (1.5%). Other symptoms (<1%) reported included hypocalcemia and acidosis (data extracted from California Pesticide Illness Query https://apps.cdpr.ca.gov/calpiq/).

There were 27 cases where the individual was hospitalized or unable to perform their normal activities due to their illness and injuries from the exposure. The most severe adverse outcome was death. Sixteen deaths were reported in the DPR surveillance program between 1992 and 2017 (Table 2). The main postmortem pathological finding was pulmonary congestion and edema. For individuals who were still alive upon hospital admission and died later, their symptoms resemble those observed in non-lethal cases (Table 2). In all cases, the exact exposure levels are unknown. However, it is suspected that exposures involved very high concentrations of sulfuryl fluoride as most of the mortalities occurred when people entered houses while they were still undergoing fumigation.

A recent case report from California involved a family with four children (including a 13-month old child) who returned to their fumigated house after it was certified safe for re-entry (Barreau *et al.*, 2019). The family left the house approximately 2 hours after returning because all six members experienced symptoms of illness. Symptoms included “burning and watering eyes, itchy skin, headaches, a feeling of lungs hurting, fatigue, dry and burning throats, coughing, extreme thirst, and unusual head-banging behavior by the toddler (pg. 2)” (Barreau *et al.*, 2019). The parents returned to the house briefly on several occasions, reporting skin irritation and rash, dizziness, nausea, chest pain, and headache, persisting for hours thereafter. Evaluation by family physicians resulted in a diagnosis of "chemical exposure." The parents reported that their 13-month old child, who was beginning to verbalize at the time of the incident, “had stopped using words in the three to four months following the incident (pg. 3)” (Barreau *et al.*, 2019). Longer-term follow-up was not reported.

At the request from the Orange County Agricultural Commissioner's office, DPR conducted post-fumigation indoor air monitoring five weeks after the fumigation event noted above. While sulfuryl fluoride was not detected in 11 breathing zone samples, concentrations as high as 2.4 ppm, more than two-fold higher than the clearance level of 1 ppm, were measured in the air exhausted from a mattress air bladder (DPR, 2016). Many of the symptoms experienced by the family were consistent with exposure to sulfuryl fluoride and chloropicrin, which was used as warning agent during the fumigation. No other data are available at this time to determine whether the child’s head-banging behavior and change in speech development were associated with sulfuryl fluoride exposure.

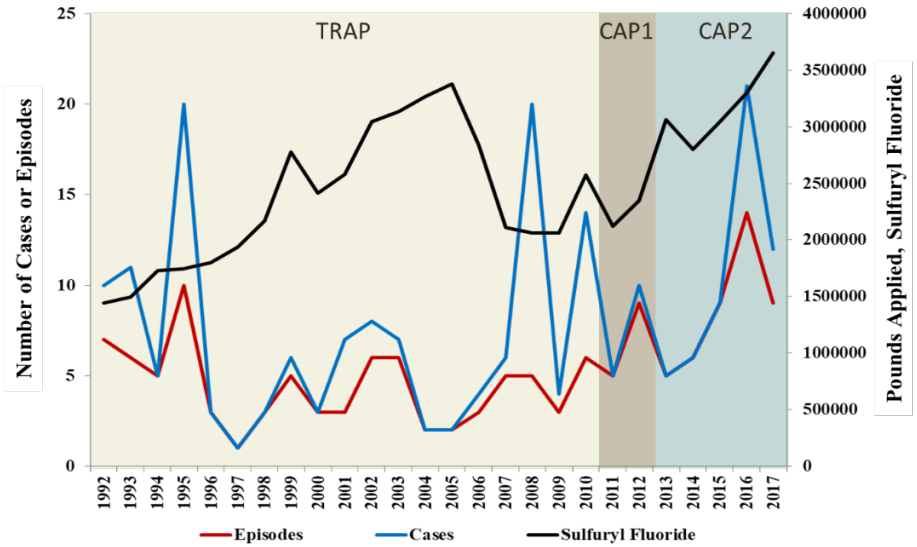

Figure 1. Sulfuryl fluoride episodes and illness cases (1992-2017) and pounds of sulfuryl fluoride applied by structural pest control operators reported to DPR TRAP-Tarpaulin Removal Aeration Plan; CAP1 – California Aeration Plan; CAP2 – California Aeration Plan 2 (II)

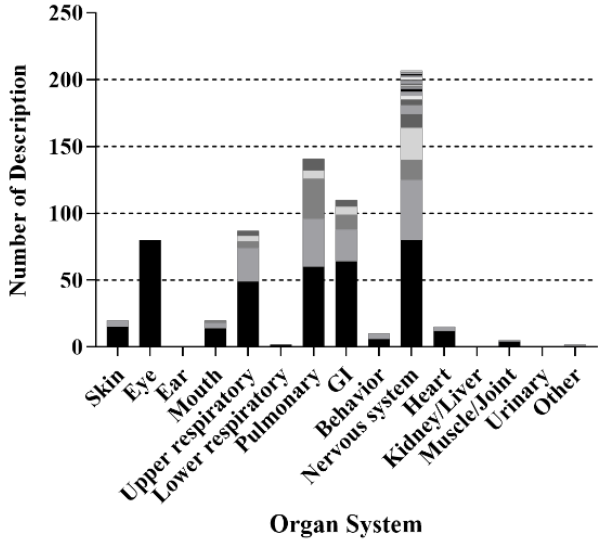

Figure 2. Medical description of 197 non-lethal cases (stacking bars with different shadings indicate discrete symptoms within same organ system)

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
1992	Male (25)	~3 hrs in the office building after clearance	NA	Dry throat, cough, brown sputum, chest tightness, eyes tearing and burning sensation	0/3	Technician entered office building day after the 24-hour aeration period. A second worker also became ill but did not lose any days from work.
1992	Female (44)	~4 hrs in the classroom after building was cleared	NA	Chest tightness, headache, sore throat, coughing, eye irritation. Persistent dry cough. Prescribed nebulizer treatment and prednisome	0/38	Teacher noticed a strong "musty odor" when she entered a classroom the morning after the school was cleared for reentry. Odor intensified after opening a file drawer. Other teachers and students also noticed the odors and developed symptoms but they did not seek care and disability days are unknown.
1992	Male (34)	~30 min outside of house under	NA	Headache, burning sensation in chest, dizziness, general	0/2	Two workers in yard of fumigated home 2 hrs after SF was injected. They noticed a

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
		fumigation		weakness, eye irritation		strong odor and became ill. A second worker did not report any days lost from work.
1993	Female (40)	Unknown	NA	Headache, exhaustion, chest pain, painful burning eyes, flu-like symptoms	0/Indefinite	Entered home 9.5 hours after it was cleared for reoccupation. Noticed odor while opening cabinets.
1993	Male (46)	Unknown	NA	Headache, nausea, eye irritation, sore throat, shortness of breath, chest pain	0/1	Entered home several hours after clearance and developed symptoms within two hours.
1994	Male (adult)	~30 min in fumigated apartment complex	NA	Nausea, upset stomach, numbness of lips, sour taste in mouth	1/5	Technician was still inside one of the units for two to three minutes when SF was injected into the building.
1994	Male (22)	Unknown	Postmortem blood: 38000 mg/L	Vomiting, diarrhea	NA	Entered while the building was under fumigation
1995	Male (40)	Unknown	Postmortem blood: 32000 mg/L	Severe pulmonary congestion and edema, diarrhea	NA	Entered while the building was under fumigation
1995	Female (adult)	Unknown	NA	Vomiting, sore throat, dizziness, chest pains, nausea, irritability	0/1	Entered home three hours after clearance. A family of three developed symptoms after a brief time inside the house. Only the mother reported a disability day.
1995	Female (37)	~8 hrs in office building after clearance	NA	Nausea, difficulty breathing, scratchy throat, eye irritation and burning sensation, blurred vision	0/3	Entered office building a day after clearance and developed symptoms within 30 minutes. Seven other workers also developed symptoms but they did not seek care.
1998	Male (22)	Unknown	NA	Vomiting, fecal incontinence, cyanotic skin	NA	Found between the house and tarps
1999	Male (37)	Unknown	NA	Felt sick, vomiting, pulmonary congestion	NA	Died within a day after crawling under fumigation tarps
1999	Male (41)	~30 min outside of fumigated home while preparing for aeration	NA	Headache, nausea, dizziness, stomach ache	0/1	Technician preparing for the aeration procedure. Took a deep breath at same time breeze was coming through the tarps and inhaled the vapors. He was able to smell the vapors through his SCBA.
2000	Female (32)	Unknown	NA	Headache, nausea, coughing, throat and chest irritation, chest tightness, eye irritation	0/1	Fifty-five hours after SF was injected, the tarp around the roof mounted air condition unit became loose creating an opening. Eight workers noticed an odor and five developed

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
						symptoms. One worker reported a disability day.
2001	Male (20)	~2 hrs in fumigated home	NA	Red, burning and swollen shut eyes, burning nose and throat, difficulty breathing, chest pains	1/Indefinite	Entered while the building was under fumigation and crawled out 2 hours later.
2002	Male (49)	Unknown	NA	Blood coming from the mouth, nose and anal regions; Pulmonary edema, pulmonary congestion, and alveolar hemorrhage	NA	Entered while the building was under fumigation with the influence of alcohol
2002	Male (41)	Unknown	NA	Vomiting, lethargy	4/Indefinite	Two men slept in the yard of a house under fumigation and may have climbed under the tarp at some point.
2002	Male (54)	Unknown	NA	Vomiting, nausea, diarrhea, lethargy, facial numbness	4/Indefinite	Two men slept in the yard of a house under fumigation and may have climbed under the tarp at some point.
2002	Male (43)	~30 min in home after clearance	NA	Headache, nausea, vomiting, burning eyes, chills, fever, extreme fatigue	0/2	Emergency responder entered home seven hours after aeration period ended and cleared for reentry.
2003	Male (12)	Unknown	NA	Itchy throat, shortness of breath, cough; History of asthma	0/7	A family of three entered home on same day it was cleared. The mother and son developed symptoms. Only the son was unable to perform normal activities.
2004	Male (33)	Unknown	NA	Headache, dizziness, blurred vision, photophobia, red, itchy and tearing eye	0/3	Worker went into crawl space under a house that was cleared for rentry and had copper 8-quinolinolate applied under a room 15 days prior.
2005	Female (37)	~3 hrs in fumigated home	Positive for fluoride	Burning and watering eyes, flushed skin, difficulty breathing, abdominal pain, vomiting, retching, hypocalcemia, hypotension, agitation, confusion, incontinence, seizure, cardiac arrest	NA	Died 3 hours later at hospital; tested positive for chloropicrin and amphetamine.
2006	Male (25)	Unknown	NA	Headache, shortness of breath, nausea, dizziness, leg weakness, abdominal pain, loss of color vision	0/7	Twenty minutes after turning on the vent tube to start the aeration procedure, a technician was exposed to the air under the tarp while removing the clips around the chimney.
2006	Female (47)	Unknown	NA	Dizziness, lightheadedness,	0/2	Smelled pesticide odor from neighbor's

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
				burning nose and eyes, coughing, lung irritation, sore throat, rash on arms and face		fumigation and immediately developed symptoms. A second person also developed symptoms but did not report any disability days.
2007	Male (25)	Unknown	NA	When found: acute loss of consciousness, slurred speech, nausea, right flank pain, weakness. During transport: defibrillated twice. At hospital: rapid heart rate, lethargy, wheezing, vomiting, hypocalcemia, acidosis, decreased kidney and liver function.	6/Indefinite	Entered while the building was under fumigation.
2008	Female (47)	Unknown	NA	Vomiting, abdominal pain, collapse	NA	Found inside fumigated house and died soon after arrival at hospital
2010	Male (48)	Unknown	NA	Metallic taste in mouth	0/1	Blowers inside a fumigated building turned on causing the tarp to blow open. Two workers were in an alley next to the building and became ill. Only one worker reported a disability day.
2010	Male (60)	Unknown	NA	Autopsy: severe atherosclerotic heart disease, atrophic brain-Alzheimer's disease, chronic obstructive pulmonary disease, chronic hypertension with atheriolonephrosclerosis, distended urinary bladder	NA	Entered the tarped area
2011	Male (45)	Unknown	Postmortem blood as positive for fluoride	NA	NA	Entered while the building was under fumigation
2011	Male (33)	Unknown	NA	Migraine headache, burning and tingling face	0/2	Emergency responder walking the perimeter of a fumigated home and was sprayed in the face by fumigant coming out of a tear in the tarp.
2011	Male (45)	Unknown	NA	Headache, wheezing, burning nose and throat	0/14	Entered home right after it was cleared for reentry. Developed symptoms an hour later.
2012	Male (32)	Unknown	Positive for	NA	NA	Entered while the building was under

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
			fluoride			fumigation; tested positive for methamphetamine.
2012	Female (34)	~8 hrs in building after clearance	NA	Severe headache, nausea, dizziness, light headed, difficulty breathing, chest heaviness, fatigue, eye irritation	0/3	Entered office building day it was cleared for reentry. The building has no windows, only two doors on opposite ends. Developed symptoms 2 hours later.
2014	Male (58)	Unknown	Positive for fluoride	NA	NA	Entered while the building was under aeration; tested positive for alcohol.
2014	Male (33)	Unknown	Positive for fluoride	Airway and vascular restriction due to ligature compression of neck	NA	Entered while the building was under fumigation; tested positive for methamphetamine.
2015	Male (34)	~1 hr inside a tented structure that began the aeration process 9 hrs earlier	Positive for fluoride	NA	NA	Entered while the building was under aeration; tested positive for methamphetamine and amphetamine.
2015	Male (35)	Unknown	NA	Headache, vomiting, dizziness, chest irritation, coughing	0/5	Worker walked perimeter of fumigated building at the start of the aeration period. Developed symptoms within 30 minutes.
2016	Male (56)	Unknown	NA	Headache, fatigue, nausea, shortness of breath, burning eyes and throat, coughing, chest pain, abdominal pain	0/8	Tarp on fumigated building had a tear that was facing a neighbor's apartment less than 20 feet away. A co-worker also became ill but did not lose any days from work. A co-worker also became ill but did report any disability days.
2016	Male (26)	~3 hrs in a fumigated home	NA	Difficulty breathing, drooling, slurred speech, non ambulatory, cardiac arrest	NA	Entered while the building was under fumigation, and died approximately two hours after arrival at the hospital
2016	Male (adult)	Unknown	NA	NA	NA	Entered while the building was under fumigation. HazMat searched the property but was unable to find the man. Decedent's body was found 2 weeks later.
2016	Male (9)	Unknown	Air bladder: sulfuryl fluoride increasing from 0.3 ppm, peaking at 2.4 ppm and	Headache, nausea, dizziness, difficulty breathing, fever, burning lungs, rash, dry, burning, and raspy throat	0/1	Entered a few hours after clearance. Family of 6 developed symptoms later that day. They left the house but continued to have symptoms each time they entered the home for at least three weeks. Repeated testing for SF by SPCO found no detectable levels in the breathing zones. DPR's Senior Industrial

Table 2. Severe sulfuryl fluoride human poisoning cases in California from DPR Pesticide Illness Surveillance Program (1992-2017)

Year	Age/Sex	Exposure*	Fluoride level	Medical description	Days Hospitalized/ Disability Days	Notes
			stabilizing at 2.2 ppm for approximately 10 seconds.			Hygienist also found no detectable levels of SF inside the house, except in a mattress air bladder in one of the bedrooms. One child was unable to attend school for one day.
2016	Male (24)	Unknown	NA	Difficulty breathing, shortness of breath, diaphoresis, altered mental status, hypocalcemia, hypomagnesemia	NA	Entered while the building was under fumigation and died several hours after arrival at the hospital
2017	Female (62)	Unknown	NA	Headache	0/1	Stayed in guest house on property while main house was fumigated. Electrical conduit from main house to the guest house was not sealed.

*Co-exposed to chloropicrin was assumed due to residential structure fumigation practice. NA-not available
Severe cases are defined as those with reported hospitalization, disability days or death.
Disability Days: The number of days in which an individual missed at least one full day (24-hour period) of work or other normal activity, such as school.
Hospitalization: The number of days in which an individual was hospitalized as an in-patient status at least one full day (24-hour period).

II.A.2. National Incidence

In 2016, US EPA's Office of Inspector General (OIG) reported that at least 11 deaths and two serious injuries occurred since 2002 during residential fumigations in the two US states with the most fumigations treatments, California and Florida. The main factors contributing to these severe adverse impacts include: (1) no requirement to secure tenting around structures undergoing fumigation, (2) ineffective devices used to detect pesticide levels inside of structures, and (3) failure to attend mandatory training for residential pesticide applicators who conduct fumigations (U.S. EPA, 2016).

An open literature search yielded 8 human case reports (Table 3). Reported symptoms are similar to those non-lethal cases (see above), while the primary autopsy finding was pulmonary edema and congestion. One specific human poisoning case involved a 9-year-old boy in Florida who survived exposed to sulfuryl fluoride after entering a fumigated house that had been cleared for re-entry (Mulay *et al.*, 2016). The boy initially experienced nausea and vomiting, and later showed signs of dysarthria, dystonia, rigidity and hyperreflexia, and choreoathetosis[9] that progressed to involve both arms, legs, and both sides of his face (Mulay *et al.*, 2016). The boy was treated with calcium gluconate to correct hypocalcemia and two runs of hemodialysis in an attempt to remove excess fluoride in his body. He continued to have expressive aphasia and choreoathetoid movements of the face, trunk, and extremities after transfer to a rehabilitation facility (Mulay *et al.*, 2016). Documentation of serum fluoride concentration and pulmonary effects was not apparent in his medical record. Magnetic resonance imaging showed basal ganglia injury. The treating physicians attributed his neurologic findings to sulfuryl fluoride poisoning, manifested by basal ganglia necrosis (Mulay *et al.*, 2016). Three other family members (grandmother, mother, and daughter) also showed initial symptoms of nausea and vomiting, but they were discharged on the same day after visiting the emergency room (ER), presuming no further adverse symptoms would develop. It is not known whether the boy's vulnerability was because he spent more time in less ventilated parts of the house or higher susceptibility (Mulay *et al.*, 2016). The finding of basal ganglia necrosis was of significance, as sulfuryl fluoride is known to cause brain lesions in the basal ganglia region across all tested animal species (rat, mouse, rabbit, dog).

Table 3. Nationwide sulfuryl fluoride human case reports from open literature

Age/Sex	Exposure	Fluoride level	Clinical signs	Pathological signs	Autopsy findings	Notes	Ref.
Male (30)	*4 hrs at > 5 ppm	Positive in blood	**Initial:** GI, irritant **Later:** persistent throat scratching, flatulence, difficulty reading	Not examined	NA (patient survived)	Patient discharged 4 days after admission	Taxay (1966)

[9] The occurrence of involuntary movements in a combination of chorea (irregular migrating contractions) and athetosis (twisting and writhing) or a movement disorder that causes involuntary twitching or writhing.

Table 3. Nationwide sulfuryl fluoride human case reports from open literature

Age/Sex	Exposure	Fluoride level	Clinical signs	Pathological signs	Autopsy findings	Notes	Ref.
Male (29)	*~16 hrs in fumigated apartment	**40-52 hpe:** 50.42 mg/L (postmortem blood)	NA (patient found dead)	NA (patient found dead)	Pulmonary congestion	Suicide; died 16 hpe; benzos, propoxyphene found	Scheuer man (1986)
Male (22)	Unknown	Not measured	NA (patient found dead)	NA (patient found dead)	Pulmonary congestion, edematous brain	Suicide	Scheuer man (1986)
Female (19)	*< 6 hrs inside a residence duplex undergoing fumigation	**12 hpe:** 20 mg/L (antemortem blood)	**Initial:** Irritant, coughing, chest discomfort, hypotension **Later:** hyperexcitable, hyperventilated, supraventricular tachycardia, cough, drooling, tetany, dysrhythmias	Pulmonary edema	Pulmonary edema and congestion	Died 12 hrs after exposure	Scheuer man (1986)
Female (Elderly)	*~24 hrs in fumigated home after clearance for reentry (ran fans for 2.5 hrs, air SF not measured)	**6 dpe:** 0.5 mg/L (postmortem blood) <LOQ (1 mg/kg) (kidneys, liver and lungs)	**Initial:** weakness, nausea, repeated vomiting, **Later:** severe weakness, dyspnea, intermittent chills, anorexia	Severe hypoxemia, diffuse pulmonary infiltrates, ventricular fibrillation	Pulmonary edema	Died 6 days after reentry of fumigated home	CDC (1987)
Male (Elderly)	*~24 hrs in fumigated home after clearance for reentry (ran fans for 2.5 hrs, air SF not measured)	Not measured	**Initial:** dyspnea, restlessness **Later:** severe dyspnea and cough, generalized seizure followed by cardiopulmonary arrest	Not examined	Pulmonary edema	Died 2 days after reentry of fumigated home	CDC (1987)
Female (37)	*Underneath a tarpaulin	**3 hpe:** 24 mg/L (antemortem blood) >100 mg/L (postmortem urine)	**Initial:** abdominal pain, irritant, nausea, shortness of breath **Later:** tetany	Torsade de pointes, ventricular fibrillation, asystole	Pulmonary edema and congestion	Died 3 hours after EMS arrival; Methamphetamine present	Schneir *et al.* (2008)
Male (9)	*~14 hrs in fumigated home after clearance for reentry	Not measured	**Initial:** nausea, vomiting **Later:** dysarthria, dystonia, rigidity, hyperreflexia, aphasia, choreoathetosis	Basal ganglia necrosis	NA (patient survived)	Other family members showed initial signs of nausea and vomiting, but was released the same day of ER visit	Mulay *et al.* (2016)

*Co-exposed to chloropicrin was assumed due to residential structure fumigation practice. dpe, days post exposure; hpe, hours post exposure; hrs, hours; LOQ, limit of quantitation. GI, gastrointestinal symptoms: nausea, vomiting, abdominal pain. Table based on data from Schneir *et al.* (2008) and Mulay *et al.* (2016).

II.B. Genotoxicity

The DPR 2006 RCD concluded that sulfuryl fluoride was not genotoxic based on negative results in the *Salmonella* reverse mutation assay, the mouse bone marrow micronucleus test, and the rat hepatocyte unscheduled DNA synthesis assay. However, in two studies not reviewed in the 2006 DPR risk assessment, sulfuryl fluoride was positive *in vitro* for mutagenicity (mouse lymphoma forward mutation assay) (Gollapudi *et al.*, 2002) and chromosomal damage (rat lymphocyte chromosomal aberration assay, see below) (Gollapudi *et al.*, 2005). The potential genotoxicity was likely from fluoride, the presumptive toxic species and one of the main hydrolysis products of sulfuryl fluoride. Despite this, sulfuryl fluoride was not carcinogenic in the chronic rat and mouse oncogenicity inhalation studies (Quast *et al.*, 1993c; Quast *et al.*, 1993b).

Gollapudi *et al.* (2002): Mouse lymphoma L5178Y cells (clone 3.7.2 ($TK^{+/-}$)) were exposed to sulfuryl fluoride (purity 99.8%; 0, 100, 500, 1000, 2000, 2500, 3000, 3500, 4000, 5000, 6000, and 7000 ppm) for 4 hours at 37°C with and without a rat liver S9 fraction metabolic activation. The exposure period was followed by 48 hours of incubation to allow for the expression of possible mutants and ~12 days for selection and viability. 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. Analytical chemistry indicated that sulfuryl fluoride was hydrolyzed in the culture medium to yield free fluoride and fluorosulfate, with less than 10% of the parent compound remaining at the end of the 4 hour treatment. Based upon this observation and the extensive literature on the genotoxicity of fluoride, the authors concluded that the weak mutagenic response observed in this assay was likely mediated through fluoride released into the culture medium.

Gollapudi *et al.* (2005): Primary lymphocyte cultures derived from blood of male Sprague-Dawley rats that had been treated with PHA for 48 hours were exposed to sulfuryl fluoride for 4 hours in the absence and presence of rat liver S9 fractions. Concentrations ranged between 100 and 50000 ppm. The cultures were harvested 20 hours later. Significant increases in the frequencies of cells with chromosomal aberrations were detected at 15000, 20000, and 25000 ppm. This response was independent of the exogenously supplied rat liver S9 microsomal and cytosolic enzymes. The NOEL for clastogenicity at the nominal exposure concentration was 2500 ppm. Analytical chemistry results indicated that sulfuryl fluoride hydrolyzes in tissue culture medium to yield free fluoride. The authors concluded that the clastogenic activity observed in sulfuryl fluoride treated cultures was mediated through fluoride released into the culture medium.

II.C. Developmental Neurotoxicity

A special non-guideline postnatal DNT/toxicokinetic study to address gaps in the toxicity

database concerning neurotoxicity was submitted to both US EPA and DPR in 2015 (Marty *et al.*, 2015). This study exposed rats between postnatal day (PND) 11 and PND 21. A standard DNT study would consist of gestational and postnatal exposures. The authors designed the study not to include exposure to dams/pups during the period of gestational day (GD) 6 to PND 10 based on results from previous developmental and reproductive toxicity studies that did not show increased sensitivity to the development of brain lesions resulting from in utero exposure. In addition, inhalation exposures between GD 20 and PND 10 are difficult to conduct because the stress from daily separation of pups from dams can confound any observed treatment effect (DPR, 2017a).

In this DNT study, three cohorts of Crl:CD (SD) rat pups of both sexes were whole body exposed to 0, 5, 20, and 150 ppm sulfuryl fluoride for 6 hours/day between PND 11 and 21 (Marty *et al.*, 2015). One cohort was used to evaluate the potential neurobehavioral and neuropathological effects (neurotoxicity cohort). The other two cohorts were used to characterize the toxicokinetics of sulfuryl fluoride degradates. The study reported a lowest-observed-effect level (LOEL) of 150 ppm for all three cohorts based on reduced body weight gain in male/female pups between PND 17 and PND 21. The investigators did not consider increases in the motor activity observed at 20 ppm to be treatment related due to lack of dose responsiveness. DPR concluded that the elevated motor activity at 20 ppm was treatment related (see Appendix C). US EPA arrived at the same conclusion (US EPA, 2016). 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). No treatment related lesions were found in the brain at PND 78, but brain lesions were not evaluated immediately after exposure at PND 21. Based on findings from this study, pharmacokinetic studies reviewed in this Addendum, and the developmental and reproductive toxicity studies reviewed in the 2006 RCD, DPR concludes that there is no evidence for increased pharmacokinetic susceptibility to the young, but uncertainties in pharmacodynamics remain (see Appendix C).

II.D. Oncogenicity

The conclusion that sulfuryl fluoride is not carcinogenic remains the same as that expressed in DPR's 2006 RCD. Because fluoride is considered as the principal toxicant of sulfuryl fluoride, DPR reviewed literature updates on fluoride carcinogenicity. It is acknowledged that the fluoride exposure and osteosarcoma connection is biologically possible since fluoride is known to stimulate osteoblasts (bone forming cells) and may increase the risk of bone cancer. There are epidemiological evidence showing that fluoride may be associated with osteosarcoma in young males, but several confounding factors limit the interpretation of these studies (Bassin *et al.*, 2006; Cohn, 1992; Maurer *et al.*, 1993). An updated review by the National Research Council (NRC, 2006) failed to reach an unequivocal conclusion on this issue in light of mixed findings from animal and human studies, genotoxicity assays, and mechanistic studies. The 2006 NRC

report concluded that "The combined literature does not clearly indicate that fluoride either is or not carcinogenic in humans (pg. 284)." An epidemiological study found a positive association between estimated fluoride exposure in the drinking water and incidence of osteosarcoma in 6 to 8 year old boys (Bassin *et al.*, 2006). This study examined data from a hospital-based case-control study collected between 1989 and 1992. However, a separate study that examined another set of osteosarcoma cases collected between 1993 and 2000 at the same hospitals did not demonstrate a significant association between bone fluoride levels and osteosarcoma risk (Kim *et al.*, 2011). The major advantage of the latter study is the use of bone fluoride concentrations as the measure of fluoride exposure, rather than estimating fluoride exposure in drinking water (ATSDR, 2003; Bassin *et al.*, 2006). However, bone fluoride levels were only measured at a single time point (total accumulated dose). As such, it is difficult to evaluate the exposure-effect relationship and what the critical window of time for fluoride exposures may be. In addition, epidemiological studies of inhalation exposure to hydrogen fluoride and fluoride dust also concluded that carcinogenicity via inhalation of fluoride is unlikely (ATSDR, 2003). 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.

II.E. Pharmacokinetics

The absorption, distribution, metabolism and excretion of sulfuryl fluoride was examined in rats and rabbits. In both species, sulfuryl fluoride was rapidly hydrolyzed to fluorosulfate and fluoride at the portal of entry in the respiratory tract. Further hydrolysis of fluorosulfate to sulfate and fluoride occurred over a longer time period (> 20 hours). Elimination half-lives for both fluorosulfate and fluoride in the plasma were on the order of 1-2 hours and 2-3 hours, respectively. While urine was the primary route of excretion, clearance of fluoride from plasma was also influenced by its adsorption to bone. Sulfuryl fluoride pharmacokinetics have been extensively evaluated in rats at different life stages, including adult, pregnant and lactating dam, fetus, and pup under acute and repeat dosing regimens. No significant difference in the pharmacokinetic handling of fluorosulfate or fluoride was identified among different life stages or between acute vs. repeated exposure regimens. Brain deposition of fluorosulfate was low compared to that observed for fluoride, implying that the latter was the active principle in the brain vacuolation observed upon repeated exposure to sulfuryl fluoride. 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). Data from the pharmacokinetic studies reviewed in the following paragraphs were used by Dow AgroSciences in the development of a physiologically based pharmacokinetic (PBPK) model of sulfuryl fluoride in rats, rabbits, and humans.

II.E.1. Pharmacokinetics in Rat

Hotchkiss *et al.* (2008): This study assessed the nasal and pulmonary absorption and metabolism of sulfuryl fluoride. Adult male F344/DuCrl rats (age, 8-12 weeks) were exposed to 300 ppm sulfuryl fluoride, nose-only, for 4 hours. Local and systemic absorption and metabolism were assessed in both surgically modified (upper respiratory tract exposure only) and non-modified (combined upper and lower respiratory tract exposure) anesthetized rats. Sulfuryl fluoride and its hydrolysis products fluorosulfate and fluoride were measured in tissue samples.

No parent compound was detected in blood or lavage samples, thus the authors concluded that sulfuryl fluoride is rapidly hydrolyzed at the portal of entry and is not present systemically. 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). Surgically modified rats had markedly lower levels of fluorosulfate in plasma (0.92 ± 0.37 µg/mL or 9.2 ± 3.7 nmol/mL), nasal tissue (0.54 ± 0.07 µg/g or 5.4 ± 0.7 nmol/g) and olfactory bulbs (0.79 µg/g or 7.9 nmol/g). Furthermore, nasal lavage and cerebral tissue levels were below their respective LLQs (0.05 µg/mL and 0.5 µg/g). Fluoride[10] levels above the LLQ (1.36 µg/mL or 71.6 nmol/mL) were only detectable in the plasma of non-modified rats (2.95 ± 0.96 µg/mL or 155.3 ± 50.5 nmol/mL). 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). An earlier study showed that approximately 12.5% of inhaled sulfuryl fluoride was absorbed by the combined effects of the upper (nasal) and lower (pulmonary) respiratory tract based on the amount of ^{35}S-sulfuryl fluoride-derived radioactivity recovered in the urine, feces and tissues (Mendrala *et al.*, 2002). As such, the authors concluded that the sulfuryl fluoride absorbed by the lower respiratory tract and lung was 7.6%. However, DPR's evaluation indicates that the value of 4.9% may underestimate the upper respiratory tract absorption for rats under normal physiological condition because nasal tissue fluorosulfate levels were significantly different between surgically modified and non-modified rats (5 vs. 63 nmol/g). DPR's conclusion was also supported by higher fluoride concentrations in nasal mucosa (340 nmol/g) than that in the lungs (165 nmol/g) when measured immediately after 6 hours of exposure of rabbits to 600 ppm sulfuryl fluoride (Hotchkiss *et al.*, 2011b). Thus based on these recent findings, DPR suggests there may be substantial absorption of sulfuryl fluoride in the upper respiratory tract region.

[10] Unless specified otherwise, fluoride concentration refers to net free fluoride concentration (see Appendix E for details).

The kinetics of urinary excretion of fluorosulfate and fluoride were assessed in rats exposed to 0, 3, 30 or 300 ppm sulfuryl fluoride for 4 hours. Urine was collected during post-exposure hours 0-6, 6-12, and 12-24. The amount of fluorosulfate excreted in urine during the 24 hours following exposure was roughly proportional to the sulfuryl fluoride exposure concentration. An average of 2.9, 43.7, and 863 µg fluorosulfate as recovered in the urine of rats exposed to 3, 30, and 300 ppm sulfuryl fluoride, respectively. However, quantifiable fluorosulfate levels in all three post-exposure intervals were only found at the high dose, which revealed that approximately 92% fluorosulfate excretion happened during the first 6 hours after exposure, suggesting rapid excretion in the urine. Urinary fluoride levels could not be determined for many of the rats exposed to 3 or 30 ppm sulfuryl fluoride because the analytical method required sample volumes greater than those available. At 300 ppm, 8.62 µmol fluoride was excreted over 24 hours, of which 68% was excreted within the first 6 hours after exposure. These data confirm that absorbed sulfuryl fluoride is rapidly hydrolyzed to fluorosulfate and fluoride and excreted in the urine with a clearance half-life ($t_{1/2}$) of 2.0 and 4.1 hours, respectively, in rats exposed to 300 ppm sulfuryl fluoride.

The contribution of systemic fluorosulfate metabolism to the urinary excretion of fluoride was examined by injecting rats with an intravenous (i.v.) dose of potassium fluorosulfate (25 mg/kg bw[11]) and measuring fluorosulfate and fluoride concentrations in urine collected 0-12, 12-24, and 24-48 hours after injection. The overall recovery of fluorosulfate and fluoride at the end of 48 hours averaged 96.9%, indicating a good mass balance. Fluoride accounted for 38-44% of the total amount of fluorosulfate and fluoride excreted in the urine. These values also represent the amount of fluorosulfate hydrolyzed to fluoride and sulfate. An earlier toxicokinetic study with inhalation exposure at 300 ppm for 4 hours showed sulfate accounted roughly 37% of the total amount of sulfate and fluorosulfate recovered in the urine collected 24 hours after exposure (Mendrala *et al.*, 2002). This suggest that roughly 40% fluorosulfate was further hydrolyzed to fluoride and sulfate during this inhalation study. Based on these data, DPR concludes that once in circulation, approximately 60% fluorosulfate would be excreted via urine in its original form and 40% undergoes further hydrolysis to fluoride and sulfate before excreted in urine.

In addition to fluorosulfate and fluoride, sulfuryl fluoride can form stable covalent linkages with endogenous proteins, such as fluorosulfonated albumin (FSO_2-albumin) adducts. Nasal lavage fluid (NLF), bronchoalveolar lavage fluid (BALF) and plasma samples were collected from rats one day following 4-hour inhalation exposures to 0, 3, 30, or 300 ppm sulfuryl fluoride in order to quantify FSO_2-albumin adducts. Only rats exposed to 30 or 300 ppm sulfuryl fluoride had FSO_2-albumin adducts detected in BALF and plasma at levels higher than the LLQ. The authors compared µg fluorosulfate equivalents for free fluorosulfate and FSO_2-albumin adduct concentrations and concluded that formation of these adducts represents a minor degradation pathway (0.5-2.7%). However, based on the same findings, DPR suggests that sulfuryl fluoride,

[11] This i.v. dose of potassium fluorosulfate is equivalent to an inhalation exposure of 200 ppm sulfuryl fluoride

although not detected in the blood, may not undergo complete hydrolysis.

Rick and Filary (2009): This study determined hydrolysis sites and rates of sulfuryl fluoride and fluorosulfate in rats and humans. Specifically, sulfuryl fluoride was incubated *in vitro* with preparations of rat liver and lung S9 fraction, rat whole blood, rat plasma, rat red blood cells (RBC), rat liver S3 subcellular fraction, rat lung S3 cell fraction, rat nasal tissue S3 cell fraction, rat lung lavage fluid, and whole blood from PND10 rat pups. The incubation was first done in a headspace-type[12] configuration. Minimal hydrolysis of sulfuryl fluoride occurred (< 10%) in subcellular fractions from portal of entry tissues (nasal and lung lavage) and classically metabolizing tissues (liver and lung). Blood components (blood, plasma, RBCs) were shown to be most effective in hydrolyzing sulfuryl fluoride (Table 4). Essentially no hydrolysis occurred in blank buffers (water or saline); sulfuryl fluoride displayed selective partitioning to the gas phase due to its high vapor pressure.

Table 4. Sulfuryl fluoride hydrolysis using the headspace-type *in vitro* incubation

Test System	Percent of sulfuryl fluoride hydrolyzed (%)	
	87 ppm	870 ppm
S3-Rat Liver	25%	--
S3-Rat Lung	16%	--
S3-Rat Nasal Tissue	0%	--
S9-Rat Liver	0%	0%
S9-Rat Lung	0%	--
S9-Human Liver	15%	0%
S9-Human Lung	--	0%
Lung Lavage-Rat	0%	--
Plasma-Rat (1/10 dilution)	34%	46%
Plasma-Rat (undiluted)	43%	--
RBC-Rat (undiluted)	84%	--
Whole Blood-Rat (undiluted)	84%, 79%	53%, 78%
Whole Blood-Rat (undiluted)-PND10	41%, 62%	51%, 63%
Whole Blood-Human	80%	67%

"--"not available
Rat samples were collected from F344/DuCrl rats; Human blood was purchased from Bioreclamation IVT Inc. (now BioIVT, https://www.bioivt.com/; samples presumably from adults).

In order to eliminate the latency of air-to-liquid phase transfer and to better estimate the biologically mediated rates of sulfuryl fluoride hydrolysis, a "no-headspace" incubation method

[12] Headspace configuration: 2 mL of biological matrix in 24-mL glass vial with 22 mL of sulfuryl fluoride in headspace; incubate for 1 hour at 37°C

was utilized. Small quantities of pure sulfuryl fluoride gas were injected into 5-mL vials completely filled with medium and subsequently added to blood from adult Crl:CD (SD) female rats, PND10 rats (both sexes), and human donors. In contrast to the headspace-type incubation, sulfuryl fluoride was rapidly hydrolyzed in Milli-Q water and blank saline in this no-headspace configuration. This finding suggests that once dissolved in a simple aqueous solution, sulfuryl fluoride will be rapidly hydrolyzed. The high background hydrolysis rate of control medium was subtracted from the overall rate to provide an estimate of hydrolysis due to the blood component. The resulting net rate of sulfuryl fluoride hydrolysis in blood was similar between PND10 and adult rats both at low (42 µg) or high (208 µg) sulfuryl fluoride loads. The net hydrolysis rate in adult human blood was approximately twice that of rat blood (Table 5), suggesting faster relative hydrolysis in humans than in rats. Unlike the *in vivo* findings (Hotchkiss *et al.*, 2008), secondary hydrolysis of fluorosulfate to fluoride and sulfate did not occur *in vitro* with blood, liver or lung subcellular fractions from adult rats or humans when fluorosulfate was added directly to preparations of these tissues. The reason for such difference is unclear.

Table 5. Sulfuryl fluoride hydrolysis using the no-headspace *in vitro* incubation

Test System	**Rate of hydrolysis in 1st min interval (µg/min/mL of blood)**	
	42 µg	**208 µg**
Adult Rat Blood	133	737
PND10 Rat Blood	154	750
Human Blood	301	1322

Adult and PND10 rat samples were collected from Crl:CD (SD) rats

Hotchkiss *et al.* (2011c): This study assessed the pharmacokinetics of sulfuryl fluoride in adult rats. Specifically, adult male F344/DuCrl rats (age, 12 weeks) were exposed to 30 and 300 ppm sulfuryl fluoride, nose-only, for 2 or 4 hours. The animals were sacrificed immediately upon completion of the exposures, with additional animals from the 4-hour exposure group sacrificed at 2, 4 or 8 hours post exposure. Fluorosulfate and fluoride were measured in plasma, kidney and brain tissues.

Rats exposed to 30 ppm sulfuryl fluoride exhibited peak fluorosulfate levels of 12 ± 1.6 nmol/mL in plasma (LLQ = 0.5 nmol/mL); levels in kidney and brain were nonquantifiable (LLQ = 5 nmol/g tissue). At the completion of 4-hour exposure to 300 ppm sulfuryl fluoride, mean peak fluorosulfate levels in plasma, kidney and brain were approximately 194 ± 58 nmol/mL, 78 ± 16 nmol/g and 6.4 nmol/g (data from one rat only), respectively. Fluoride was initially analyzed using ion chromatography with suppressed conductivity detection and negative ion electrospray ionization mass spectrometry (IC/SCD/NESI/MS). However, the authors mentioned that there were large interference peaks in the plasma and brain samples, thus these data were not included. Less complication from interfering peaks was found for the kidney,

which showed similar fluoride levels between the control and exposed groups. Subsequently, fluoride concentration was assessed with an indirect method: First total fluoride (free fluoride + acid-ionizable fluoride[13]) was measured using the microdiffusion with ion-selective electrode detection (MD/ISE) method, fluorosulfate was measured with IC/SCD/NESI/MS method, and then fluoride concentration was calculated by the difference between total fluoride and fluorosulfate. This indirect method was used to determine fluoride levels in the remaining plasma samples. Exposure to 30 ppm sulfuryl fluoride resulted in nonquantifiable plasma fluoride levels at all sample times (LLQ for total fluoride is 10.5 nmol/mL). Exposure to 300 ppm sulfuryl fluoride resulted in mean plasma fluoride levels of 77.7 ± 53.1 nmol/L immediately after 2 hours of exposure and 105.5 ± 63.6 nmol/L immediately after 4 hours of exposure. Furthermore, for the 4-hour exposure group, 33.5 ± 15.1 and 9.1 ± 9.7 nmol/mL were detected at 2 and 4 hours post-exposure, respectively. Fluoride level in brain was not measured due to the loss of all samples used for analysis with the initial method. Overall, plasma fluorosulfate and fluoride levels were greatest at the end of 4 hours of exposure, and decreased rapidly post-exposure. These data were used by Poet and Hinderliter (2011) for PBPK model calibration (see below).

Hotchkiss *et al.* (2011d): This study also assessed the pharmacokinetics of sulfuryl fluoride in adult rats. Specifically, adult male F344/DuCrl rats (age, 8 weeks) were exposed nose only to 0, 3, 30, or 300 ppm sulfuryl fluoride for 4 hours. Plasma, kidney, cerebrum, olfactory bulb, nasal and pulmonary tissues (pooled sample from 10 animals) were analyzed for fluorosulfate and fluoride levels immediately after exposure as well as at 2, 4, and 8 hours post exposure. Fluorosulfate was measured directly using IC/NESI/MS and fluoride was measured indirectly (= total fluoride by MD/ISE –fluorosulfate).

The authors indicated that fluorosulfate and fluoride are rapidly eliminated from adult male rats exposed to 3-300 ppm sulfuryl fluoride. Immediately after exposure, peak fluorosulfate and fluoride levels in plasma, lung, and kidney were roughly proportional to the sulfuryl fluoride exposure concentration. Fluorosulfate levels immediately after exposure to 300 ppm sulfuryl fluoride were highest in kidney (191 nmol/mL), followed by plasma (129 nmol/mL), lung (69.4 nmol/mL), and nasal tissue (16.1 nmol/mL). However, fluorosulfate above the LLQ (3.73 nmol/g) was not detected in cerebrum at any time in any exposure group. The investigators suggested that this finding could be due either to limited transfer of fluorosulfate across the blood-brain barrier (BBB) or a high rate of fluorosulfate elimination through hydrolysis to free fluoride and sulfate in the cerebrum. Unfortunately, fluorosulfate levels in the olfactory bulb, and the adjacent brain region, were not measured due to insufficient amounts of tissue sample. The net total fluoride levels in the cerebrum (145 nmol/g) immediately after exposure to 300 ppm were comparable to net total fluoride levels in the lungs (122 nmol/g) and plasma (179 nmol/mL), but slightly lower than the net total fluoride levels in the kidney (218 nmol/g) and olfactory bulb (213 nmol/g). The lack of detectable fluorosulfate in the cerebrum rendered the

[13] Mainly refers to fluorosulfate in samples exposed to sulfuryl fluoride.

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. High background fluoride levels were detected in nasal tissue samples. The investigators suggested that these may reflect the presence of bone in nasal tissue. Based on the 300 ppm exposure data, the elimination half-lives of fluoride were 2.61 hours for plasma, 2.71 hours for lung and 2.14 hours for cerebrum. The elimination half-lives of fluorosulfate were 1.50 hours for plasma, 1.07 hours for kidney, 1.8 hours for lung and 1.50 hours for nasal tissue. Calculated half-lives for fluorosulfate in the kidney and lungs based on 30 ppm exposure data (5.6 hours for kidney and 11.3 hours for lung) were much longer than those calculated from 300 ppm exposure data. However, the investigators suggested that the 30 ppm exposures may have been either compromised or more variable due to the fact that fluorosulfate levels in these tissues were close to the LLQ. These data were used by Poet and Hinderliter (2011) for the PBPK model calibration.

Hotchkiss *et al.* (2011a): This study assessed the pharmacokinetics of sulfuryl fluoride with repeated exposure in adult rats. Specifically, adult male F344/DuCrl rats (age, 9-10 weeks) were subjected to whole-body exposure to 0, 3, 30, or 300 ppm sulfuryl fluoride, 6 hours/day, 5 days/week for two weeks. Animals were sacrificed immediately after exposure on study days 1 (1^{st} exposure) and 12 (10^{th} exposure) to determine whether repeated exposures to sulfuryl fluoride will alter the concentrations and clearance rates of sulfuryl fluoride hydrolysis products, fluorosulfate and fluoride, from plasma, kidney, and cerebrum. Fluorosulfate concentrations were measured using the IC/NESI/MS method. Fluoride concentrations in plasma and tissues were determined directly using ion-selective electrodes (ISE). Net free fluoride was measured directly using ISE after validation using a method that discerned the difference between total fluoride (measured by MD/ISE) and fluorosulfate.

Fluorosulfate was present at detectable levels in plasma from all exposure groups with similar plasma concentrations found between rats exposed for one or 10 days. Similarly, time-course profiles of plasma fluorosulfate were comparable over the 18-hour post-exposure monitoring period between single and repeated exposure. The highest mean values were found in rats exposed to 300 ppm (143 ± 12.3 vs. 110 ± 9.2 nmol/mL for 1^{st} and 10^{th} exposure, respectively). Fluorosulfate was not detected in the cerebrum in either single or repeat dose rats. Fluorosulfate was present in the kidneys at levels above the LLQ (4.85-4.99 nmol/g) only in rats exposed to 300 ppm sulfuryl fluoride. Mean fluorosulfate concentrations were 14.2 ± 4.4 nmol/g for single exposures, and 6.80 nmol/g for repeated dosing. Fluoride in plasma was only detected following the highest concentration for single exposure, but was detected at all concentrations upon repeated exposure. The mean plasma fluoride concentrations at the termination of single or repeated exposures were 39.0 ± 4.6 and 46.0 ± 4.2 nmol/mL, respectively. Time course profiles of plasma fluoride were comparable over the 18-hour post-exposure monitoring period between single and repeated exposures. Fluoride concentrations in cerebrum were similar in single and

repeat dose rats, with measured mean values of 117.0 ± 11.5 (single) and 98.1 ± 3.3 nmol/g (repeated) reported at 300 ppm sulfuryl fluoride. Fluoride concentrations in kidney were also similar for single and repeat dose animals at all exposure levels. Plasma elimination half-lives for fluorosulfate from rats with single and repeated exposure to 300 ppm sulfuryl fluoride were 1.7 and 1.6 hours, respectively. Plasma elimination half-lives for fluoride from rats with single and repeated exposures to 300 ppm were 2.3 and 2.6 hours, respectively. Mean concentrations of fluorosulfate and fluoride in urine from the 0-6 hour collection interval were higher in repeated exposed rats than single exposed rats. Mean concentrations of these two hydrolysis products in urine at the 6-12 hour and 12-18 hour collection intervals were similar in single and repeat dose animals. Based on the similar tissue concentrations of fluorosulfate and fluoride and their rapid elimination rates in plasma, the investigators suggested that neither metabolite would accumulate in plasma or cerebrum following repeated exposures to sulfuryl fluoride. These data were used by Poet and Hinderliter (2011) for PBPK model validation.

Marty *et al.* (2011a): This study assessed the pharmacokinetics of sulfuryl fluoride in dams with gestational and lactational exposure, fetuses with in utero exposure, and neonatal pups with oral exposure. Specifically, pregnant CD rats (age, 9-10 weeks) were exposed whole-body to 0, 5, 30 or 150 ppm sulfuryl fluoride, 6 hours/day from GD 6 to GD 20 and lactation day (LD) 5 to LD 10 (without litters). In addition, PND 10 pups were exposed by gavage to 0, 4, 20 or 40 μg of an equivalent mass of potassium fluorosulfate and fluoride in the milk. Tissue samples were collected on GD 20, LD10 (dams), and PND 10 for sulfuryl fluoride, fluorosulfate, and fluoride measurements.

For GD 20 rats, plasma from dams and fetuses (pooled by litter) was assayed for sulfuryl fluoride, fluorosulfate and fluoride. Fetal brain and kidney tissues were also analyzed for fluorosulfate and net free (brain) or net total fluoride (kidneys). Maternal urine samples were collected overnight immediately after exposure on GD18 and until the animals were returned to the inhalation chambers on GD19. Urinary elimination of fluorosulfate and fluoride exhibited a half-life of 1.8 hours for fluorosulfate and 4.48 hours for fluoride at 150 ppm sulfuryl fluoride. No parent sulfuryl fluoride was detected in plasma from dams or fetuses. Dam plasma showed a dose-proportional increase in fluorosulfate levels. Fetal plasma levels of fluorosulfate were non-detectable at 5 ppm and had a mean value of 1.8 ± 0.7 and 7.9 ± 2.0 nmol/mL (about 12% of dam plasma levels) at 30 and 150 ppm. Fetal brain levels of fluorosulfate were not detectable in the 5 or 30 ppm litters, and had a mean value of 2.7 ± 1.5 nmol/g in the 150 ppm litters. Fetal kidneys had nondetectable fluorosulfate at 5 ppm, but had 2.2 – 2.6 times of fetal plasma levels at 30 and 150 ppm. Fluoride in dam plasma exhibited a mean value of 1.8 ± 0.5, 5.9 ± 2.1, and 24.7 ± 4.8 nmol/mL in the 5, 30, and 150 ppm, respectively. The corresponding fetal plasma fluoride were 0.28 ± 0.08, 1.8 ± 2.0, and 11.2 ± 7.3 nmol/mL (15-45% of dam plasma level). Fetal brain fluoride were all below the LLQ except one sample (0.327 nmol/mL) in the 150 ppm group. Net total fluoride (fluorosulfate + free fluoride) in fetal kidneys was 1.48 and 6.63 ± 1.69

nmol/g for the 30 and 150 ppm dose groups, but these values were lower than the corresponding fluorosulfate levels (4.6 ± 0.04 and 17.1 ± 1.4 nmol/g, respectively). The investigators suggested that this may be due to the sequestration of fluoride into fetal bone during development as reported by others (Whitford, 1996).

To determine lactational exposure, dam plasma and milk, and pup plasma were sampled at 0 and 2 hours post-exposure. All samples were assayed for fluorosulfate and total fluoride, with net free fluoride calculated by subtracting fluorosulfate from net total fluoride. A preliminary trial of 0 and 150 ppm was used to assess parent sulfuryl fluoride; no sulfuryl fluoride was found in any tissue samples. Dam plasma fluorosulfate increased with exposure dose at 0 hours, but decreased by 56 – 77% at 2 hours post-exposure. The highest mean fluorosulfate level of 38.4 ± 8.0 nmol/mL was found at 150 ppm. Dam milk fluorosulfate was higher at 0 hours than at 2 hours, DPR suggests the decrease at 2 hours is likely resulted from milk consumed by pups when dams were returned to cage after the exposure. On average, fluorosulfate was 3 – 4 fold greater in milk than in plasma at all timepoints and all sulfuryl fluoride exposure concentrations. Pup plasma fluorosulfate was only detectable at 2 hours post-exposure at 30 ppm (0.7 ± 0.2 nmol/mL) and 150 ppm (2.7 ± 1.1 nmol/mL) treatment groups, similarly, DPR suggests this may due to delayed feeding in pups. The fluorosulfate pup plasma/dam plasma ratio was 0.15 and 0.16 for the 30 and 150 ppm doses, respectively. Fluoride in dam plasma, milk and pup plasma exhibited similar pharmacokinetics as that of fluorosulfate, with the exception of the dam milk/dam plasma ratio (2 – 14x variation) and the pup plasma/dam plasma ratio (0.12 for 30 ppm and 0.04 for 150 ppm). The highest mean fluoride concentration in dam plasma (22.2 ± 12.3 nmol/mL) was reported at 0 hours in the 150 ppm group.

For rat pups (PND 10) who were orally administered fluorosulfate and fluoride, blood, brain, and kidney samples were collected at 1, 3, or 6 hours post-dose for fluorosulfate and fluoride analysis. Fluorosulfate was not detected in any pup brains at any dose. The elimination half-lives of fluorosulfate in plasma were 2.25 and 5.05 hours for the 20 and 40 µg/pup treatment levels, respectively. Fluorosulfate levels in kidney and plasma were similar in their corresponding treatment groups. Fluoride was consistently present in the brain only at the 40 µg/pup group, however, DPR noticed an increasing rather than decreasing trend with increasing post-dose time intervals. This trend was different at the two lower dose treatment levels: decrease in 4 µg/pup and unchanged in 20 µg/pup. The elimination half-lives of fluoride in plasma were 1.94 and 3.12 hours for the 20 and 40 µg/pup treatment levels, respectively. Net total fluoride levels were consistently present in the kidneys from the 20 and 40 µg/pup treatment groups and were higher than their corresponding fluorosulfate or free fluoride levels.

The investigators conclude that these pharmacokinetic data demonstrate a relatively rapid elimination of fluorosulfate and fluoride from the plasma of gestational/lactating dams and PND 10 pups similar as in adult male rats (Hotchkiss *et al.*, 2011d; Hotchkiss *et al.*, 2011c). In addition, the authors state that these data showed lower concentrations of fluorosulfate and

fluoride in fetal/pup plasma than in dam plasma, despite higher concentrations of fluorosulfate and fluoride in dam milk than in dam plasma. Based on these findings, the study authors suggested that there was a low degree of concern for potential pre- and postnatal effects in human infants and children. These data were used by Poet and Hinderliter (2011) for PBPK model validation.

Marty *et al.* (2011b): This study assessed the pharmacokinetics of sulfuryl fluoride in rat weanlings. Specifically, male weanling Crl:CD rats (PND 22) were whole-body exposed to 0, 3, 30 or 300 ppm sulfuryl fluoride for 4 hours. Plasma, brain and kidneys were assayed for fluorosulfate and fluoride at 0, 2, 4, and 8 hours post-exposure. Fluorosulfate concentrations were measured by the IC/NESI/MS method and fluoride concentrations were determined indirectly (= total fluoride by MD/ISE –fluorosulfate).

The results indicate rapid elimination of fluorosulfate and fluoride from rat pups. At 300 ppm, mean fluorosulfate levels were highest in plasma (104.6 ± 13.4 nmol/mL), followed by kidney (66.2 ± 34.4 nmol/g) and brain (3.6 ± 1.5 nmol/g). The minimal presence of fluorosulfate in the brain suggests the general impermeability of the weanling blood-brain barrier to fluorosulfate. The elimination half-lives for fluorosulfate from the plasma were 2.01 and 2.33 hours for the 30 and 300 ppm exposure levels, respectively. Fluoride levels in plasma were either not detectable (< LLQ) or undetermined (higher fluorosulfate than total fluoride[14]) immediately after exposure in all treatment groups, but a small amount of 3.3 ± 0.6 nmol/mL was reported at 8 hour post-exposure in 300 ppm. Free fluoride in kidney was not measured due the rapid hydrolysis of fluorosulfate during the analytical preparations. Instead, total fluoride (fluorosulfate + free fluoride) was measured, and their levels were comparable to fluorosulfate levels in this organ. DPR indicates that this data implied only a minimal presence of free fluoride in the kidney, and this is likely due to rapid adsorption of fluoride by the developing bone in rat pups (Whitford *et al.*, 1991). Fluoride levels in brain were undetectable at 3 ppm, but were relatively high at 30 ppm (5.69 ± 0.81 nmol/g) and 300 ppm (73.2 ± 5.2 nmol/g) when compared to plasma fluoride. The study authors state that the reason for these high brain fluoride levels is unknown. These data were used by Poet and Hinderliter (2011) for the PBPK model validation.

Marty *et al.* (2015): This study was part of a special non-guideline DNT study that examined the pharmacokinetics of sulfuryl fluoride in postnatal pups after a short-term exposure of 11 days. Two cohorts of Crl:CD (SD) rat pups of both sexes were exposed to 0, 5, 20, and 150 ppm sulfuryl fluoride 6 hours/day from PND 11 through PND 21 by whole body inhalation. One cohort was maintained on low fluoride diet and ultrapure water to minimize background fluoride contamination and the other was maintained on regular rodent diets and municipal drinking water. Blood and brain samples were collected from these two cohorts on PND 21 immediately after the final exposure.

[14] Measurement errors for values at low exposed doses or measurements close to LLQ.

Plasma fluorosulfate was detected at all sulfuryl fluoride concentrations, while both brain fluorosulfate and plasma/brain fluoride were quantifiable only at the 150 ppm exposure level. The levels of fluorosulfate in plasma were comparable between these two cohorts (low F vs. regular): 1.7 ± 0.3 vs. 1.5 ± 0.2 nmol/g for 5 ppm, 5.7 ± 1.1 vs. 4.7 ± 1.0 nmol/g for 20 ppm, and 97.4 ± 15.2 vs. 75 ± 8.5 nmol/g for 150 ppm, respectively. The investigators indicated a supralinear (situated above the linear curve derived from the two low doses) pharmacokinetics of plasma fluorosulfate at 150 ppm. The levels of free fluoride at 150 ppm in plasma and brain were also comparable between these two cohorts (low F vs. regular): 28.8 ± 2.8 vs. 27.7 ± 2.2 nmol/g in the plasma and 68.2 ± 4.8 vs. 69.5 ± 4.5 nmol/g in the brain. These data were not used for PBPK model calibration or validation because this special DNT study was conducted after PBPK model was built in 2011 (Poet and Hinderliter, 2011). However, when compared to adult rats with similar exposure duration (whole-body exposure, repeated dosing for 6 hours/day, 5 days/week x 2 weeks, a total of 10 doses), DPR found plasma fluoride in these PND 21 pups exposed to 150 ppm sulfuryl fluoride was 60% of that in adults exposed to 300 ppm. Brain fluoride was equal to 70% of that in adults, suggesting a slightly higher brain accumulation of fluoride in pups than adults with repeated exposure.

II.E.2. Pharmacokinetics in Rabbit

Hotchkiss *et al.* (2011b): New Zealand White rabbits (male, ~3 kg) were exposed nose-only to 600 ppm of sulfuryl fluoride for 6 hours. Blood samples were collected during exposure at 2, 4 and 6 hours, and post-exposure at 2, 4, 8, 12, and 18 hours. Kidney, lung, brain, olfactory bulb, nasal tissue (mucosa plus underlying turbinate bone), and nasal mucosa (bone removed) were collected immediately after exposure and at 18 hour post-exposure. Urine samples were collected both during the exposure and through 18 hours post-exposure. Because no sulfuryl fluoride above the LLQ (0.156 μg/g) was detected in any sample, only those collected during the 6-hour exposure period were used for sulfuryl fluoride analysis. All other samples were analyzed for fluorosulfate and fluoride. Fluorosulfate concentrations were measured using the IC/NESI/MS method and fluoride concentrations were determined indirectly (= total fluoride by MD/ISE – fluorosulfate).

Plasma fluorosulfate and fluoride increased during exposure, peaking at 392 ± 68 and 361 ± 137 nmol/mL, respectively, at the end of exposure. During the 18-hour post-exposure period, the study authors indicated that both analytes followed first-order elimination kinetics, exhibiting half-lives of 2.06 and 3.39 hours for fluorosulfate and fluoride, respectively. Mean fluorosulfate levels in kidney, lung, cerebrum, nasal tissue and mucosa were 274 ± 37, 201 ± 54, 23 ± 2, 116 and 89.7 nmol/g, respectively, at the conclusion of the exposure and non-detectable (< LLQ) 18 hours post-exposure. Similar fluorosulfate concentration in nasal tissue and mucosa (with vs. without bone) led DPR to conclude that minimal turbinate bone accumulation of fluorosulfate during exposure. Fluoride in lung and cerebrum were 165 ± 17 and 254 ± 3 nmol/g at the

conclusion of the exposure, declining to 24.5 ± 10.4 and 2.97 ± 0.49 nmol/g, respectively, at 18 hours post-exposure. DPR found that data for fluoride in olfactory bulb, nasal tissue and mucosa were difficult to interpret due to high background levels detected in untreated controls. Urinary elimination of fluorosulfate and fluoride occurred rapidly as indicated by the significant amounts of total fluoride detected during the 6-hour exposure period. DPR noticed that half-lives for urine excretion could not be determined because sample collection was inconsistent.

II.E.3. Pharmacokinetics in Rat vs. Rabbit

Rick *et al.* (2011): This study compared the pharmacokinetics of sulfuryl fluoride in adult rats and rabbits in order to assess possible species differences. Adult male F344/DuCrl rats (age, 9-10 weeks) and adult female New Zealand White rabbits (age, 20 months) were subjected to whole body exposure to sulfuryl fluoride at 0, 3, 30 or 300 ppm for 6 hours. Immediately following exposure, plasma, cerebrum and kidney samples were collected and analyzed for fluorosulfate and fluoride. Fluoride was measured directly using the ISE method.

Rat and rabbit plasma fluorosulfate were found to be similar and both increased proportionally with exposure concentration between 3 and 300 ppm. For example, mean plasma fluorosulfate levels were 103 ± 9 vs. 112 ± 48 nmol/mL for rats and rabbits, respectively, at 300 ppm. Fluorosulfate in rat kidney was only detectable at 300 ppm, exhibiting a mean value of 7.45 ± 1.18 nmol/g, much lower than the rabbit kidney value of 127 ± 41 nmol/g at the same exposure dose. DPR suggests that this may be due to spontaneous fluorosulfate hydrolysis during sample preparation in rat kidneys. Fluorosulfate was not detectable in any rat cerebral samples and was nearly at the LLQ (5.93 vs. LLQ = 5.02 nmol/g) in rabbit cerebrum at the highest exposure dose. Plasma fluoride in rats and rabbits increased non-proportionally with exposure levels. DPR suggests that this could be due to the dynamic equilibration of fluoride between plasma and bone at different exposure levels (Whitford, 1996). Mean plasma fluoride peaked at 46.8 ± 6.5 nmol/mL in rats and 120 ± 25 nmol/mL in rabbits at 300 ppm. DPR's analysis indicates that the nearly 3-fold difference in plasma fluoride concentration was likely due to differences in fluoride renal clearance (1.14 vs. 3.61 mL/min/kg, in rabbits and rats, respectively) (Whitford *et al.*, 1991). Intravenous dosing of sodium fluoride in rats and rabbits also showed higher plasma fluoride in rabbits than rats due to slower renal clearance in rabbits (Monsour *et al.*, 1985). DPR suggests that the same reason may also explain the lower total kidney fluoride concentrations found in rats (137 ± 18 nmol/g) than in rabbits (253 ± 70 nmol/g) at 300 ppm. Fluoride levels in cerebrum were detectable at 30 and 300 ppm in rats and only at 300 ppm in rabbits, where the levels were comparable (rats, 76.2 ± 4.4 nmol/g; rabbits, 86.4 ± 2.2 nmol/g). Ultimately, it was only in plasma and kidney that fluoride levels diverged markedly between rats and rabbits. These data were used by Poet and Hinderliter (2011) for the PBPK model validation.

II.E.4. Pharmacokinetics in Human

No sulfuryl fluoride pharmacokinetics studies in humans were available for analysis. *In vitro* findings summarized above suggest more rapid sulfuryl fluoride hydrolysis in human blood than in rat blood (Rick and Filary, 2009). However, earlier studies reported slower plasma fluoride clearance in humans than rats (2.7 vs. 7.2 mL/min/kg) (Whitford, 1996).

II.E.5. Summary of Pharmacokinetics

Sulfuryl fluoride: Sulfuryl fluoride was not detected above the LLQ in samples of nasal and bronchoalveolar lavage, milk and blood collected immediately upon termination of exposures at the highest tested doses (Table 6).

Fluorosulfate: Measurable fluorosulfate levels in rat and rabbit tissues following exposure to high concentrations of sulfuryl fluoride were detected in plasma, kidney, lung and lung lavage (rats only), and nasal and nasal lavage. Generally, low fluorosulfate levels were detected in cerebral tissues. These results are summarized in Table 7.

Fluoride: Measurable fluoride levels in rat and rabbit tissues following exposure to high concentrations of sulfuryl fluoride were detected in plasma, cerebrum, kidney, olfactory bulb, lung and nasal mucosa. Generally, high fluoride levels were detected in cerebral tissues. These results are summarized in Table 8.

Table 6. Tissue samples collected for sulfuryl fluoride measurement

Study	Species (dose)	Tissue sample
Hotchkiss *et al.* (2008)	Rat, adult (300 ppm)	Whole blood (< LLQ = 84 ng/mL) Nasal lavage fluid (< LLQ = 42 ng/mL) Bronchoalveolar lavage fluid (< LLQ = 42 ng/mL)
Marty *et al.* (2011a)	Rat, adult/fetus (150 ppm)	Dam blood at gestational day 20 Fetal blood Dam blood at lactation day 10 Dam milk at lactation day 10
Hotchkiss *et al.* (2011b)	Rabbit, adult (600 ppm)	Whole blood (< LLQ = 156 ng/g)

Table 7. Peak mean tissue fluorosulfate concentration in rats and rabbits exposed by inhalation to 150 or 300 ppm of sulfuryl fluoride

Study	Species-strain	Stage (sex)	Route	Duration	Dose (ppm)	Plasma (nmol/mL)	Cerebrum (nmol/g)	Olfactory bulb (nmol/g)	Lung (nmol/g)	Lung lavage (nmol/mL)	Nasal tissue (nmol/g)	Nasal lavage (nmol/mL)	Kidney (nmol/g)
Mendrala *et al.* (2002)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	134.5[a]	--	--	--	--	--	--	--
Hotchkiss *et al.* (2008)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	169	<LLQ=5.05	11	18.8	2.3	63.5	1.1	40
Hotchkiss *et al.* (2011c)	Rat-F344	Adult (M)	Nose only	Single, 2 hrs	300	118	5.5[b]	--	--	--	--	--	48
Hotchkiss *et al.* (2011c)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	194	6.4[b]	--	--	--	--	--	77
Hotchkiss *et al.* (2011d)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	129	<LLQ=3.73	--	69.4	--	16.1	--	191
Hotchkiss *et al.* (2011a)	Rat-F344	Adult (M)	Whole body	Single, 6 hrs	300	143	<LLQ=4.83	--	--	--	--	--	14.2
Hotchkiss *et al.* (2011a)	Rat-F344	Adult (M)	Whole body	6 hrs/d, 5 d/wk, 2 wks	300	110	<LLQ=4.84	--	--	--	--	--	6.8
Rick *et al.* (2011)	Rat-F344	Adult (M)	Whole body	Single, 6 hrs	300	103	<LLQ=4.94	--	--	--	--	--	7.45
Marty *et al.* (2011b)	Rat-SD	PND22 (M)	Whole body	Single, 4 hrs	300	104	3.6	--	--	--	--	--	66.2
Marty *et al.* (2015)	Rat-SD	PND21 (M/F)	Whole body	6 hrs/d, PND11-21, Regular diet	150	75.1	1.88	--	--	--	--	--	--
Marty *et al.* (2015)	Rat-SD	PND21 (M/F)	Whole body	6 hrs/d, PND11-21, Low fluoride diet	150	97.4	2.39	--	--	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	Dam (F)	Whole body	6 hrs/d, GD6-20	150	63.6	--	--	--	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	Fetus (M/F)	In utero	6 hrs/d, GD6-20	150	7.87	2.74	--	--	--	--	--	17.1
Marty *et al.* (2011a)	Rat-SD	Dam (F)	Whole body	6 hrs/d, GD6-20, LD5-10	150	38.4	--	--	--	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	PND10 (M/F)	In utero, milk	6 hrs/d, GD6-20, LD5-10	150	2.73[c]	--	--	--	--	--	--	--
Rick *et al.* (2011)	Rabbit-NZW	Adult (F)	Whose body	Single, 6 hrs	300	112	5.93	--	--	--	--	--	127
Hotchkiss *et al.* (2011b)	Rabbit-NZW	Adult (M)	Nose only	Single, 6 hrs	600	392	23	--	201	--	116	89.7[d]	274

All values were measured immediately after completion of exposure unless otherwise specified. F344, F344/DuCrl rat; SD, Crl:CD (SD) rat; PND, postnatal development day; GD, gestation day; LD, lactation day; NZW, New Zealand white rabbit; LLQ, lower limit of quantitation. [a]Whole blood; [b]Single value from one animal; [c]Value measured at 2 hrs post exposure; [d]Nasal mucosa.

Table 8. Peak mean tissue free fluoride concentration in rats and rabbits exposed by inhalation to 150 or 300 ppm of sulfuryl fluoride

Study	Species-strain	Stage (sex)	Route	Duration	Dose (ppm)	Plasma (nmol/mL)	Cerebrum (nmol/g)	Olfactory bulb (nmol/g)	Lung (nmol/g)	Nasal mucosa (nmol/g)	Kidney (nmol/g)[b]
Mendrala *et al.* (2002)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	132[a]	119	--	--	--	292
Hotchkiss *et al.* (2008)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	155.4	--	--	--	--	--
Hotchkiss *et al.* (2011c)	Rat-F344	Adult (M)	Nose only	Single, 2 hrs	300	77.7	--	--	--	--	--
Hotchkiss *et al.* (2011c)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	105.4	--	--	--	--	--
Hotchkiss *et al.* (2011d)	Rat-F344	Adult (M)	Nose only	Single, 4 hrs	300	49.8	145	213[b]	53.1	--	218
Hotchkiss *et al.* (2011a)	Rat-F344	Adult (M)	Whole body	Single, 6 hrs	300	39	117	--	--	--	146
Hotchkiss *et al.* (2011a)	Rat-F344	Adult (M)	Whole body	6 hrs/d, 5 d/wk, 2 wks	300	46	98.1	--	--	--	146
Rick *et al.* (2011)	Rat-F344	Adult (M)	Whole body	Single, 6 hrs	300	46.8	76.2	--	--	--	139
Marty *et al.* (2011b)	Rat-SD	PND22 (M)	Whole body	Single, 4 hrs	300	3.30[c]	73.2	--	--	--	60
Marty *et al.* (2015)	Rat-SD	PND21 (M/F)	Whole body	6 hrs/d, PND11-21 Regular diet	150	27.7	69.5	--	--	--	--
Marty *et al.* (2015)	Rat-SD	PND21 (M/F)	Whole body	6 hrs/d, PND11-21 Low fluoride diet	150	28.8	68.4	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	Dam (F)	Whole body	6 hrs/d, GD6-20	150	24.7	--	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	Fetus (M/F)	In utero	6 hrs/d, GD6-20	150	11.2	0.327	--	--	--	6.63
Marty *et al.* (2011a)	Rat-SD	Dam (F)	Whole body	6 hrs/d, GD6-20, LD5-10	150	22.2	--	--	--	--	--
Marty *et al.* (2011a)	Rat-SD	PND10 (M/F)	In utero, milk	6 hrs/d, GD6-20, LD5-10	150	0.68[d]	--	--	--	--	--
Rick *et al.* (2011)	Rabbit-NZW	Adult (F)	Whole body	Single, 6 hrs	300	120	86.4	--	--	--	160
Hotchkiss *et al.* (2011b)	Rabbit-NZW	Adult (M)	Nose only	Single, 6 hrs	600	361	254	337	165	340	649

All values were measured immediately after completion of exposure unless otherwise specified. F344, F344/DuCrl rat; SD, Crl:CD (SD) rat; PND, postnatal development day; GD, gestation day; LD, lactation day; NZW, New Zealand white rabbit. [a]Whole blood; [b]Measured as net total fluoride not net free fluoride; [c]Value measured 8 hrs post exposure; [d]Value measured 2 hrs post exposure.

II.F. Sulfuryl Fluoride PBPK Model

In 2011, Dow AgroSciences constructed a PBPK model based on all available pharmacokinetic data to determine the dosimetry of sulfuryl fluoride and its hydrolysis products, fluorosulfate and fluoride, in rats, rabbits and humans (Poet and Hinderliter, 2011). The model for sulfuryl fluoride and fluorosulfate includes five compartments: blood, brain, kidney, slowly perfused tissues (fat, muscle, skin) and rapidly perfused tissues (liver, spleen, lung, intestines, pancreas). A bone compartment was added to the model for fluoride to accommodate bone adsorption and desorption kinetics (Rao *et al.*, 1995). A separate lung compartment was not included due to rapid hydrolysis of sulfuryl fluoride at the portal of entry. Two versions of this sulfuryl fluoride PBPK model were developed:

- An adult model that can be parameterized for humans, rats and rabbits
- An ontogenic model to account for growth from birth to adulthood in rats and humans.

Model assumptions: The model assumes that sulfuryl fluoride is rapidly hydrolyzed to form fluorosulfate and fluoride at the portal of entry in the respiratory system, with the hydrolysis products distributed to all other tissues via systemic circulation. The model also assumes that no parent compound is present in the systemic circulation and that both hydrolysis products are completely unbound to plasma protein. 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, 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 ($BW^{0.3}$). The fractional inhalation absorption is assumed to be 15% in humans and 45% in rabbits.

Model calibration: Two pharmacokinetic datasets on adult male rats (Hotchkiss *et al.*, 2011c; Hotchkiss *et al.*, 2011d) were used for initial model calibration. A separate pharmacokinetic dataset for adult male rats was used for parameter optimization (Hotchkiss *et al.*, 2008). The resulting adult male rat model was used as the basic structure for further modifications such as pregnancy and repeated dosing or development of rabbit and human PBPK models. To model oral gavage via milk in neonatal rats (PND10), the oral absorption parameters were optimized based on plasma concentrations of fluorosulfate and fluoride; all other parameters were retained from the adult rat model. To model repeated inhalation exposure during gestational periods (dams at GD6 – 20 and fetal rats), the model was modified to include pregnancy compartments (mammary gland and placenta). Other parameters were retained from the adult male rat model. The urinary elimination rates were optimized to fit this data. Placental transfer rates for the metabolites in dams and fetuses were fit to fetal plasma fluoride and fluorosulfate concentrations. To model exposure in adult rabbits, the model was updated with rabbit-specific physiology. All

other parameters were retained from the adult male rat model except for the fractional inhalation absorption (15% in rats vs. 45% in rabbits) and urinary elimination (0.7 L/hr/kg$^{-0.3}$ in rats vs. 3 L/hr/kg$^{-0.3}$ in rabbits). In order to model exposure in humans, the model was first updated with human-specific physiology. Because no data exist for the pharmacokinetics of sulfuryl fluoride or fluorosulfate in humans, data on sodium fluoride kinetics from a human study by Whitford *et al.* (2008) were used to calibrate the human oral absorption parameters to verify model fit. DPR recognized that calibration of sulfuryl fluoride, fluorosulfate, and fluoride via inhalation exposure in humans was not possible due to the lack of data.

Model validation: To validate model parameterization, all parameters were held constant and model predictions were compared to additional data from separate studies submitted by the registrant (rats/rabbits) or open literature (humans). The rat PBPK model was validated with pharmacokinetic datasets from neonatal (Marty *et al.*, 2011a), weanling (Marty *et al.*, 2011b), and repeated dosing studies (Hotchkiss *et al.*, 2011a). The rabbit PBPK model was validated with the parallel pharmacokinetic comparison study between rats and rabbits (Rick *et al.*, 2011). The human PBPK model was validated with two additional human fluoride datasets (oral exposure) from open literature (Buzalaf *et al.*, 2008; Maguire *et al.*, 2005). No quantitative analysis was provided to indicate the model fit. However, in most cases DPR noted that overall model predictions appear to overlap with actual values using visual examination except for brain fluorosulfate concentration, which the model overpredicts for almost all exposure routes and durations in both rats (except in fetal brain) and rabbits.

Model application: Dow AgroSciences later used this PBPK model to predict plasma fluoride levels following hypothetical exposures starting at 1 ppm sulfuryl fluoride in residential (24 hours/day for 15 days) or occupational (8 hours/day, 5 days/wk, 48 wk/year) settings. According to the Vikane® label for structural fumigation, a treated structure is cleared for reentry when the concentration in breathing zones is 1 ppm or less (Douglas Products, 2015). In the residential setting, the exposure concentrations were assumed to start at 1 ppm and then follow an exponential decay. 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. Plasma fluoride levels were higher in infants and children than in adults. In the occupational scenario, the exposure concentrations were assumed to be constant at 1 ppm without any decay over time. Plasma fluoride levels peaked at 0.3 nmol/mL during the day, then falling to baseline levels overnight. Peak plasma levels increased to 0.35 nmol/mL at the end of a year after repeated daily exposure. The PBPK modeling results indicate that peak plasma fluoride levels will be lower in adults and children reentering a recently fumigated home than those expected from consuming fluoridated drinking water. Although fluoride in the brain (target tissue) in humans was not assessed in the original PBPK model submitted to DPR, the current registrant Douglas Products subsequently provided brain fluoride and fluorosulfate concentrations for exposures modeled from 1 to 100 ppm sulfuryl fluoride for 6 hours (Poet and Bartels, 2017). The model predicted

lower internal brain concentrations in humans than in rats. This indicates that humans require higher exposures than rats to reach the same internal dose in the brain, thereby obviating the need for the pharmacokinetic component of the animal to human extrapolation factor (UF_A). The default UF_A is 10x consists of a 3x pharmacokinetic component and a 3x pharmacodynamic component. Based on this model prediction, the registrant proposed that the total UF_A for sulfuryl fluoride to be reduced from 10x to 3x to account for only the differences in pharmacodynamics. However, DPR notes that the model's predictive ability to simulate internal dosimetry of sulfuryl fluoride was based on the assumption that fluoride formed at the portal of entry in the respiratory system is distributed to the brain only via the systemic circulation. However, if it is found that the penultimate toxicant enters the brain via a pathway other than systemic circulation, the underlying assumptions of the model may be invalid. Thus, the model could not be used to support reducing the uncertainty factor. 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).

There are several uncertainties associated with the sulfuryl fluoride PBPK model (see Appendix F for details). Importantly, DPR found that the human inhalation PBPK model was not validated with human inhalation data, which are currently unavailable. Rather, the human inhalation model was validated against plasma fluoride data from human oral sodium fluoride studies (Buzalaf *et al.*, 2008; Maguire *et al.*, 2005). In so doing, the model becomes analogous to that of orally administered fluoride. As such, DPR chose not to use the PBPK model to calculate human equivalent concentrations for interspecies extrapolation or to estimate the intraspecies variation resulting from inhalation exposures.

III. POINTS OF DEPARTURE ESTABLISHED IN ANIMAL STUDIES

III.A. Summary of Critical Toxicological Effects by the Inhalation Route

The available inhalation sulfuryl fluoride studies show that non-neurotoxic effects mainly occur in the respiratory system, kidney, and teeth. Respiratory tract effects were reported in humans after accidental or intentional acute exposures and generally included respiratory and lung congestion, pulmonary edema, and reduced olfactory function (DPR, 2006a). In laboratory animals, common respiratory effects included nasal tissue inflammation, lung inflammation, alveolar microphage aggregates, alveolar histiocytosis, and lung congestion. These effects were almost always observed at the same doses as those causing neurotoxic effects (discussed below). One 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[15] occurred at 200 ppm (Quast *et al.*, 1993a). Kidney effects were not reported with acute exposure, although renal papillary necrosis and degeneration / regeneration of collecting ducts and proximal tubules were noted in rats at 600 ppm in a 2-week study. Mild renal effects such as hyperplasia of the collecting ducts, basophilic epithelial cells in the proximal tubules, and increased relative kidney weights were reported in female rats exposed to 300 ppm for 2 weeks. No lesions in the brain and respiratory system were noted in this study (Eisenbrandt *et al.*, 1985). Dental fluorosis was reported in two 13-week rat studies (LOEL = 100 ppm), a 1-year dog study (LOEL = 80 ppm), and a 2-year rat study (LOEL = 20 ppm). Additional non-neurotoxic effects included thyroid epithelial hypertrophy in mice (Quast *et al.*, 1993b), reduced maternal and fetal/pup body weights in rats (Breslin *et al.*, 1992) and rabbits (Hanley *et al.*, 1981; Hanley *et al.*, 1989), and reduced adult body weight in dogs (Nitschke and Quast, 1992).

The brain is identified as the most sensitive target tissue for inhalation exposure to sulfuryl fluoride. Neurotoxicity has been observed in both humans and laboratory animals exposed to sulfuryl fluoride. The typical neurologic symptomology that follows acute exposure to high levels of sulfuryl fluoride in humans include headache, dizziness, lightheadedness, weakness, fatigue, lethargy, and tingling or numbness in various body parts (see Section II.A. Pesticide Illnesses and Human Exposure). Case reports of human poisoning and mortality have also documented specific neurotoxic sequelae including basal ganglia necrosis, convulsion, tetany, and edematous brain (Mulay *et al.*, 2016; Schneir *et al.*, 2008). In laboratory animals, convulsions, tremors, lethargy, and incapacitation were common effects noted in sub-lethal acute inhalation studies conducted in rats and mice. In short-term studies (1-2 weeks), brain vacuolation (mouse and rabbit), convulsions and hyperactivity (rabbit), and intermittent tremors and tetany (dog) were reported at air concentrations of 100 or 300 ppm. Increased motor activity was observed at 20 ppm for developing young rat pups. In subchronic studies, brain vacuolation

[15] Malacia: liquefaction necrosis with blood vessels and some neuropil persisting within the lesion

was the predominant lesion found in all species (rat, rabbit, mouse, dog). Other effects included gliosis[16] (dog) and electrophysiological anomalies (rat). In chronic studies, brain vacuolation was again the predominant lesion in all species (rat, mouse, dog), although it was not detected in a two-day rat acute study (6 hours/day) at the highest concentration (300 ppm) tested. No visible brain lesions have been detected in any animal species under acute exposure scenarios, even at lethal doses (Miller *et al.*, 1980). However, the incidence and severity of brain vacuolation increased with exposure level and with repeated dosing (DPR, 2006a).

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.

The pattern of neurotoxic effects observed following inhalation studies in laboratory animals is of particular importance because similar basal ganglia necrosis was found via MRI in a 9-year old boy exposed to sulfuryl fluoride after entering his fumigated home (Mulay *et al.*, 2016). Basal ganglia necrosis was accompanied with clinical signs of dysarthria, dystonia, rigidity, hyperreflexia, and choreoathetosis. Because the boy stayed in his fumigated house for approximately 14 hours (4 pm re-entry, 6 am the next day exit to ER), his exposure duration can be considered acute and the dose sub-lethal. While it appears that animals must be exposed more than once for brain lesions to form, based on this single case report, humans may develop brain lesions following a single, acute inhalation exposure.

III.B. Selection of Points of Departure

Points of departures (PODs) are the highest concentrations that produce no toxicologically significant effects. The PODs herein used were either experimentally-determined (i.e. NOELs) or data-derived. Data-derived POD values were used whenever effects were observed at the lowest treatment level in a study or when low-dose extrapolation could be used to provide a more accurate no effect level than relying on a study's predetermined treatment levels. DPR used a benchmark dose (BMD) approach to derive points of departure (PODs) for all data that were amenable for modeling. The US EPA's Benchmark Dose Software (BMDS; version 3.1.2) was used to estimate the threshold of toxicity for a corresponding endpoint. For this Addendum, all data that could be modeled were quantal. Quantal or dichotomous response data are reported as either the presence or absence of an effect (incidence). DPR's default threshold response level (the benchmark response or BMR) for quantal data is 10% (U.S. EPA, 2012b). Each model resulted in the generation of a corresponding BMD or benchmark concentration (BMC) value as well as a value representing a 95% lower bound of the BMD (BMDL) or BMC (BMCL) and the

[16] Gliosis: hypertrophy of glial cells

POD for the observed effect. The terms BMC/BMCL were used here because the endpoint data modeled had dose levels expressed as air concentrations (ppm).

In the BMD/BMC approach, the data for each endpoint were used to generate a family of models. The goodness-of-fit was then evaluated for each model over the full dose range to select a "best" model for each effect's dataset. The evaluation process was based on a hierarchical examination of (a) the results for statistical tests for goodness-of-fit, (b) the lowest Akaike Information Criteria (AIC) score for relative goodness-of-fit, (c) closeness of BMD and BMDL to each other and to nearest dose levels for goodness-of-fit and model dependence, (d) visual inspection of lines over data points for goodness-of-fit and toxicological plausibility, (e) the magnitude of residuals for goodness-of-fit, and (f) considerations of sample size, variability, and whether there is maximum response at high dose.

The "best" models for each endpoint were next evaluated as part of the hazard identification process for their fitness to provide PODs for risk assessment. This evaluation reconsidered factors that included the toxicological plausibility of the effect and the quality of the data, as well as the relative magnitude of the threshold of toxicity represented by the BMDL.

III.C. Points of Departure for Acute Exposure

A two day rat inhalation study (Albee *et al.*, 1993) was considered the most appropriate for evaluating acute inhalation risk. Investigators exposed rats to sulfuryl fluoride for six hours/day on two consecutive days following which functional observational batteries (FOB) and electrophysiological assessments were conducted. Neuropathology was not evaluated in this study. No effects were observed at the highest tested dose of 300 ppm. Other acute studies in rats and mice established NOELs ranging from 300-600 ppm as well as LOELs (Table 9). These studies mainly focused on establishing lethality (LC50), which occurred at air concentrations only slightly higher than 300 ppm. This emphasizes the narrow concentration range between which no observed effects and death from sulfuryl fluoride were observed. More recent studies investigating pharmacokinetic properties of sulfuryl fluoride did not provide any data which could be used to establish an acute POD. Therefore, the NOEL of 300 ppm is the most appropriate on which to establish the POD.

It is important to note that in the most recent human case report, the prominent clinical finding was basal ganglia necrosis (Mulay *et al.*, 2016). While the Mulay *et al.* (2016) findings suggest that sulfuryl fluoride may cause brain lesions in humans after acute inhalation exposure (~ 14 hours), DPR is unable to derive an acute POD from the human data without knowing the measured air concentration. None of the acute inhalation toxicity studies presented in Table 9 were suitable for BMD modeling. Ultimately, the careful examination of neurological parameters by Albee *et al.* (1993) resulted in its recognition as the critical study for acute toxicity.

Table 9. Summary of acute toxicity studies

Species/Duration	Effects at LOEL	LOEL (ppm)	NOEL (ppm)	References
Rat, 6 hrs/d for 2d	**No effects**	**--**	**300 HDT**	**Albee *et al.* (1993)**
Rat, up to 6 hrs	Slight tremors after 2-3 hours of exposure and weight loss, 1 death	1000	ND	Dow Chemical Company (1959)
Rat, 4 hrs	Lethargy (females)	750	450	Miller *et al.* (1980)
Rat, 20 min (head-only)	Transient increase of respiratory frequency, decreased mean tidal volume & mean minute volume	4000	ND	Landry and Streeter (1983)
Rat, 1 hr	Decreased body temperature and heart rate, increased blood pressure, death	4000	ND	Gorzinski and Streeter (1985)
Rat, 41 min	Incapacitation	4000	ND	Albee *et al.* (1983)
Rat, 6 hrs/d x 5d/w	Moribund and death between 2nd and 6th dose	600	300	Eisenbrandt *et al.* (1985)
Mouse, 4 hrs	Tremors, lethargy, death	700	600	Nitschke and Quast (1990)
Mouse, 4 hrs	Tremors, lethargy, death	600	400	Nitschke and Lomax (1989)

Abbreviations: d, day; HDT, highest dose tested; hr(s), hour(s); min, minutes; w, week; ND, not determined as LOEL was at the lowest dose tested. All studies evaluated effects in adult animals.

IV. DERIVATION OF REFERENCE CONCENTRATIONS

The purpose of this Addendum is to update the interim acute reference concentration for sulfuryl fluoride for residential bystanders. This Addendum establishes reference concentrations (RfCs) from inhalation toxicity studies of varying durations: acute (1 – 2 days), short-term (10 – 14 days), subchronic (13 weeks), and chronic (greater than one year). Since sulfuryl fluoride is a fumigant, inhalation exposure remains to be the most critical exposure scenario for residential bystanders, as described in detail in the Exposure Assessment of the 2006 Evaluation of Sulfuryl Fluoride as a Toxic Air Contaminant (DPR, 2006b).

IV.A. Methodologies for RfC Derivation

Reference concentrations (RfCs) are target air concentrations that are estimates of inhalation exposures that are likely to be without appreciable risk of deleterious effects. These values are calculated by dividing the critical endpoint concentrations (points of departure, POD) by the uncertainty factors appropriate to the exposure scenarios evaluated. Commonly used default UFs are 10x to account for interspecies variability (UF_A) and 10x to account for intraspecies (human) sensitivity (UF_H). Both UFs are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x. Additional UF may be applied to account for gaps in the database (UF_{DB}).

For inhalation risk assessments, DPR is currently using the US EPA's RfC methodology to derive human equivalent concentrations (HEC) for inhalation exposures (U.S. EPA, 1994; U.S. EPA, 2012a). The HEC is the external air concentration that produces the same internal target tissue dose in humans as that achieved in laboratory animals. Traditionally, HEC calculation involves two steps. First, the critical POD from the selected animal study is adjusted by the estimated human exposure duration (i.e., 24 hours/day and 7 days/week for residential bystanders). This results in a duration-adjusted POD (POD_{ADJ}). Then, the POD_{ADJ} is converted to a HEC or POD_{HEC} using a dosimetric adjustment factor (DAF). The choice of appropriate DAF depends on whether the effect is mediated through systemic distribution or represents a local or portal of entry effect.

Under the RfC methodology, the critical endpoint selection is based on the endpoints occurring at the lowest HECs, which may not necessarily be the lowest animal POD. In addition, different HECs could be calculated for the same animal POD depending on whether the effect is considered to be systemic or portal of entry, and on the duration adjustments that differ for non-occupational and occupational exposure scenarios.

Because DAF adjustments account for physiological and anatomical differences between humans and animals, the use of POD_{HEC} for RfC derivation typically reduces the pharmacokinetic

component in the UF_A from 3x to 1x. Alternatively, the HEC calculation can also be accomplished by using a PBPK model. A sulfuryl fluoride PBPK model is available for, rats, rabbits, and humans.

IV.A.1. Methodology

The traditional methodology for HEC calculation has only been developed for toxic endpoints classified as systemic effects through blood circulation or portal of entry effects at the respiratory tract. DPR determined in this assessment that the calculation of a HEC using the PBPK model can only applied to systemic effects based on the underlying assumptions in the design of the current sulfuryl fluoride PBPK model (Poet and Hinderliter, 2011).

For sulfuryl fluoride induced neurotoxic effect, both DPR's 2006 risk assessment (DPR, 2006a) and the registrant-developed PBPK model assumed that the presumptive neurotoxic species is fluoride, which enters the brain from the systemic circulation. However DPR's current analysis provides evidence that fluoride may enter the brain by alternative routes, such as a direct path originating in the olfactory region of the nasal cavity or local vascular pathway through the countercurrent exchange between the cavernous sinus and internal carotid artery (see Appendix E for details). These potential alternative entry routes are supported by the following observations:

1) Fluoride levels in the brain or fluoride brain-to-plasma (T/P[17]) ratio (standardized by the highest plasma level or across entire time course of 1 – 14 hours) were high in sulfuryl fluoride inhalation studies, but not in oral, intravenous, or intraperitoneal studies with sodium fluoride;
2) Sulfuryl fluoride-induced brain lesions that are confined to the basal ganglia after inhalation exposure, but not after oral sodium fluoride exposure;
3) Direct CNS access via intranasal absorption has been demonstrated for molecules of different sizes and charges (for example, manganese, insulin, albumin, oxytocin, dextran and interferon), as well as for living cells (microglia and mesenchymal stem cells); and
4) Local vascular access via countercurrent exchange between cavernous sinus and internal carotid artery has been demonstrated for many steroids (luteinizing hormone-releasing hormone, β-endorphin, progesterone, oxytocin, 5α-androstenol, testosterone) and chemicals such as diazepam, tyrosine, dopamine, and propanol.

According to DPR's analysis of the updated toxicology database, neurotoxicity induced by inhaled sulfuryl fluoride could be mediated by the following possible pathways:

[17] DPR abbreviated brain-to-plasma ratio as T/P (tissue-to-plasma) instead of B/P (brain-to-plasma) because T/P is conventionally used in the literature to refer to any tissue.

- Systemic circulation: Toxic metabolites are first absorbed into blood through the respiratory tract and enter the brain from the systemic circulation.
- Extrathoracic (nasal) portal of entry: Toxic metabolites enter the brain directly from the nasal cavity via the olfactory and/or trigeminal nerves, bypassing the systemic circulation.
- Local vascular: Toxic metabolites enter the brain following nasal absorption and subsequent uptake into local vascular networks, bypassing the lung and heart.
- Unknown mode of action: Toxic metabolites enter the brain through multiple routes (systemic, nasal portal of entry, or local vascular); neurotoxicity results from delivery of the toxicant to the brain that is dependent on air concentration, duration of exposure and route-specific pharmacokinetics.

The following paragraphs review several approaches and propose the most appropriate approach for deriving sulfuryl fluoride RfCs based on current understanding of the available database for each type of toxic effect.

For systemic effects:

In 1994, US EPA published its first guideline for HEC calculation entitled *Methods for Derivation of Inhalation Reference Concentrations and Applications of Inhalation Dosimetry* (1994 RfC Method, (U.S. EPA, 1994). The 1994 RfC Method recommended using the chemical-specific blood:gas (air) partition coefficient ($H_{b/g}$) to determine the partitioning of the agent from inhaled air into blood at the alveolar endothelial interface at equilibrium (U.S. EPA, 1994). The main assumptions for this approach include:

- Toxicity is directly related to the concentration of the agent at the target site;
- The concentration of the agent at the target site is related both to the concentration of the agent in the arterial blood at equilibrium and the duration of exposure;
- Arterial blood concentration at equilibrium is related to concentration in inspired air; and
- DAF defaults to 1 when chemical-specific $H_{b/g}$ values are unavailable.

In September 2012, US EPA published an updated guideline entitled "Advances in Inhalation Gas Dosimetry for Derivation of a Reference Concentration (RfC) and Use in Risk Assessment" based on recent advances in inhalation dosimetry (U.S. EPA, 2012a). The 2012 RfC Update showed that PBPK model-derived DAFs are ≥ 1 for most chemicals when detailed data relevant to partitioning were available, thus generally supporting a DAF default of 1. Therefore, DPR adopted the 1994 RfC Method with a default DAF = 1 for systemic effects induced by sulfuryl fluoride. The pharmacokinetic portion (3x) of the 10x uncertainty factor for interspecies variability (e.g., extrapolation from animal studies to humans, UF_A) is thus eliminated.

Alternatively, HEC can be derived by reverse dosimetry using the sulfuryl fluoride PBPK model.

For portal of entry effects:

The 1994 RfC Method uses animal:human ratios of species-specific overall minute ventilation (V_E) and the overall surface area for the affected respiratory tract region (U.S. EPA, 1994). This approach relates the gas dose per respiratory tract surface area to observed toxicity at the site of entry, and is referred to as the regional gas dose ratio (RGDR) method. The application of the default approach typically results in DAFs of 0.1 to 0.3 for the extrathoracic region and 2 to 3 for the tracheobronchial and pulmonary regions. Assumptions underlying this approach are:

- uniformity of airflow
- uniformity of surface area
- uniformity of deposition on respiratory tract surfaces.

According to the 2012 RfC Update, these assumptions were not supported when applied to the extrathoracic region based on studies that took into account airflow patterns, airflow and lesion correlation, and computational fluid dynamic modeling. Consequently, the 2012 RfC Update recommends the use of a DAF of 1 for effects occurring in the extrathoracic region (U.S. EPA, 2012a). For effects occurring in the tracheobronchial and pulmonary regions, the available information from airflow modeling suggests that uniformity of airflow, surface area, and deposition are reasonable assumptions. Therefore, in these regions, the recommendations given in the 2012 RfC Update support the 1994 RfC Method. For this Addendum, DPR used the DAF of 1 for effects occurring in the extrathoracic region (nasal cavity) and species-specific DAFs for effects occurring in the tracheobronchial or pulmonary regions.

As with systemic non-neurotoxic effects, the 3x pharmacokinetic portion of the 10x interspecies variability uncertainty factor (UF_A) is eliminated. For sulfuryl fluoride, respiratory tract lesions are considered to be portal of entry effects with nasal effects occurring at the extrathoracic region and lung effects occurring at the pulmonary region.

For effects with unknown mode of action:

When toxic effects could not be readily classified by either a systemic or portal of entry mode of action, DPR applied no dosimetric adjustments and subsequently no HEC calculation. Instead, the RfC derivation was based on a duration adjusted POD from the animal study combined with uncertainty factors for intraspecies variability and interspecies sensitivity (UF_A and UF_H, 10x each). The full 10x of UF_A was retained because there was no HEC adjustment for the pharmacokinetic component.

Regardless of which mode of action, an additional database uncertainty factor (UF_{DB}, 3x) was applied when the study NOELs were derived from adult animals. A UF_{DB} was reduced to 1x when critical endpoints were based on effects observed in developing organisms (see Appendix C and Section V.E. for UF_{DB} details). The various dosimetric adjustment approaches for HEC calculation and associated uncertainty factors for RfC derivation of sulfuryl fluoride are summarized in Table 10.

Table 10. Dosimetric adjustment approaches and associated uncertainty factors (UF) used for sulfuryl fluoride induced toxic effects

Toxic Effect	Dosimetric adjustment	UF (A x H x DB[a])
Systemic effects	Default $DAF_{SYS} = (H_{b/g})_A/(H_{b/g})_H = 1$ Reverse dosimetry using PBPK model	3 x 10 x 3 = 100 3 x 10 x 3 = 100
Portal of entry effects at extrathoracic region	Default DAF = $RGDR_{ET}$ = 1	3 x 10 x 3 = 100
Portal of entry effects at pulmonary region	Default DAF = $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H$	3 x 10 x 3 = 100
Effects with unknown mode of action	No dosimetric adjustment	10 x 10 x 3 = 300

UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies (human) sensitivity; UF_{DB}, database uncertainty factor. DAF, dosimetric adjustment factor; $H_{b/g}$, blood/gas partition coefficient; $RGDR_{ET}$, regional gas dose ratio at extrathoracic region; $RGDR_{PU}$, regional gas dose ratio at pulmonary region; V_E, minute ventilation (L/min); SA_{PU}, surface area at pulmonary region. UF of 3 is a rounded number of 3.16. [a]UF_{DB} = 1 when data were derived from young animals.

IV.A.2. Example RfC Calculations for Residential Bystanders

A 13-week rat study (Nitschke *et al.*, 1987a) was used as an example to demonstrate these different methods of RfC derivation for a residential bystander exposure scenario (24 hours per day and 7 days per week). This study had NOELs of 100 ppm for kidney hyperplasia (systemic effect), nasal inflammation (portal of entry effect at extrathoracic region), alveolar histiocytosis (portal of entry effect at pulmonary region), and brain vacuolization (effect with unknown mode of action). Rats in this study were exposed to sulfuryl fluoride for 6 hours/day and 5 days per week for 13 weeks. The animal NOEL of 100 ppm yielded an adjusted POD of 18 ppm for residential bystanders (see calculation formula below). RfCs were calculated using different approaches based on whether the observed effects are systemic, portal of entry or with unknown mode of action.

$$Animal\ NOEL = 100\ ppm$$

$$POD_{ADJ} = NOEL \times \frac{hrs/day\ (animals)}{hrs/day\ (humans)} \times \frac{days/wk\ (animals)}{days/wk\ (humans)} = 100 \times \frac{6}{24} \times \frac{5}{7} = 18\ \text{ppm}$$

RfC derivation for systemic effect (kidney hyperplasia):

- $DAF_{SYS} = \frac{(H_{b/g})_A}{(H_{b/g})_H} = 1$
- $POD_{HEC} = POD_{ADJ} \times DAF_{SYS} = 18 \times 1 = 18$ ppm
- $RfC = \frac{POD_{HEC}}{UF_A \times UF_H \times UF_{DB}} = \frac{18}{3 \times 10 \times 3} = \mathbf{0.18\ ppm}$

RfC derivation for portal of entry effect at extrathoracic region (nasal inflammation):

- $DAF = RGDR_{ET} = 1$
- $POD_{HEC} = POD_{ADJ} \times RGDR_{ET} = 18 \times 1 = 18$ ppm
- $RfC = \frac{POD_{HEC}}{UF_A \times UF_H \times UF_{DB}} = \frac{18}{3 \times 10 \times 3} = \mathbf{0.18\ ppm}$

RfC derivation for portal of entry effect at pulmonary region (alveolar histiocytosis):

- $DAF = RGDR_{PU} = \frac{(V_E/SA_{PU})_A}{(V_E/SA_{PU})_H} = \frac{0.1/0.34}{13.8/54} = 1.2$
- $POD_{HEC} = POD_{ADJ} \times RGDR_{PU} = 18 \times 1.2 = 21.6$ ppm
- $RfC = \frac{POD_{HEC}}{UF_A \times UF_H \times UF_{DB}} = \frac{21.6}{3 \times 10 \times 3} = \mathbf{0.22\ ppm}$

A default body weight of 124 g (female, subchronic) for Fisher 344 rats was used for ventilation calculation. Based on the allometric equation for rats, $V_E = e^{-0.578+0.821\ln(BW)} = 0.10$ 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, DAF = $(V_E/SA_{pu})_A/(V_E/SA_{pu})_H = 1.2$.

RfC derivation for effects with unknown mode of action (brain vacuolization):

- $RfC = \frac{POD_{ADJ}}{UF_A \times UF_H \times UF_{DB}} = \frac{18}{10 \times 10 \times 3} = \mathbf{0.06\ ppm}$

IV.A.3. Example RfC Calculations from the Sulfuryl Fluoride PBPK Model

The critical acute NOEL of 300 ppm from the inhalation neurotoxicity study in rats (Albee *et al.*, 1993) was used as an example to illustrate how RfCs can be derived using the sulfuryl fluoride

PBPK model. In Albee *et al*. (1993), adult rats were exposed to sulfuryl fluoride for six hours/day on two consecutive days. The PBPK model assumed that the fluoride is the toxic species and that neurotoxicity is mediated through a systemic mode of action (Poet and Hinderliter, 2011). The acute HEC and the resulting acute RfC for a residential bystander (child) exposed to sulfuryl fluoride for 24 hours can be calculated as follow:

- Run the rat PBPK model at 300 ppm for 6 hours, and obtain the peak brain free fluoride concentration in rat (73.84 µmol/g)
- Run human PBPK model for 24 hours using a fixed body weight of 7.6 kg (for infants) by varying the external dose to see which dose yields similar internal peak brain free fluoride concentration of 73.84 µmol/g
- HEC = human external dose resulting same internal rat dose = 326 ppm
- $RfC = \frac{HEC}{UF_A \times UF_H \times UF_{DB}} = \frac{326}{3 \times 10 \times 3} = 3.26\ ppm$

IV.B. RfCs for Acute Toxicity

The purpose of this Addendum is to propose regulatory targets (acute RfCs) for residential bystanders. As mentioned in Section III.C, the NOEL of 300 ppm from a two-day inhalation neurotoxicity study in adult rats (Albee *et al.*, 1993) was considered the most appropriate for evaluation of acute inhalation risk.

The current database revealed that the sulfuryl fluoride induced neurotoxicity cannot be clearly defined as either a systemic or portal of entry MOA at the extrathoracic (nasal) region, largely because of the ambiguity of how the toxicant enters the mammalian brain (see Appendix E). Thus, DPR elected to derive RfCs based on the following three assumptions: 1) systemic, 2) portal of entry at nasal cavity (extrathoracic region), or alternatively 3) unknown routes of entry (Table 11). If it is assumed that the neurotoxic effects are mediated by a systemic MOA, the acute RfC for bystanders would be 3.26 ppm based on the PBPK model (see calculation in Section IV.A.3) and 0.75 ppm based on the $H_{b/g}$ ratio. If instead, it is assumed that sulfuryl fluoride-induced neurotoxic effects are mediated via a portal of entry MOA, the acute RfC for bystanders would be 0.75 ppm based on application of the DAF from U.S. EPA's 2012 position document (U.S. EPA, 2012a). Finally, if neither a systemic nor a portal of entry MOA is assumed, the HECs for pharmacokinetic adjustment between animals and humans would not be calculated. Rather, the default UF of 10 for interspecies variability is retained, resulting in an acute RfC for bystanders of 0.25 ppm. DPR did not consider the sulfuryl fluoride PBPK model as an alternative approach for derivation of the acute RfC due to model uncertainties (Appendix F) and the lack of validation of the human model with inhalation data.

Table 11. Reference concentrations (RfCs) for acute exposure of residential bystanders

Parameters	Mode of Action		
	Systemic	Portal of Entry (Extrathoracic region)	Unknown
NOEL (ppm)	300	300	300
POD_{ADJ} (ppm)	75	75	75
Dosimetric adjustment method	Default DAF = 1[a]	Default DAF = 1[b]	None
POD_{HEC} (ppm)	75	75	--
UF_A	3	3	10
UF_H	10	10	10
UF_{DB}	3	3	3
UF-total	100	100	300
RfC =HEC/UF (ppm)	0.75	0.75	--
RfC = POD_{ADJ}/UF (ppm)	--	--	0.25

Abbreviations: DAF, dosimetric adjustment factor; $H_{b/g}$, blood:gas (air) partition coefficient; NOEL, no observed effect level; POD, point of departure; POD_{ADJ}, POD adjusted by duration. POD_{HEC}, human equivalent concentration; ppm, parts per million; RfC, reference concentration; RGDR, regional gas dose ratio; UF_A, uncertainty factor to account for interspecies variability; UF_H, uncertainty factor to account for intraspecies (human) sensitivity; UF_{DB}, database uncertainty factor. References: [a]U.S. EPA (1994); [b]U.S. EPA (2012a).

For residential bystanders, the exposure duration assumed to be 24 hrs/day, 7 days/week, thus duration adjusted POD is calculated as below:

$$POD_{ADJ} = NOEL\ (ppm) \times \frac{6\ hrs/day\ (animals)}{24\ hrs/day\ (humans)} = 300 \times 0.25 = 75\ ppm$$

RfC for neurological effects in adults: Dosimetric adjustment based on either a systemic or portal of entry MOA, UF_A=3, UF_H =10, UF_{DB} =3, total UF =100; No dosimetric adjustment, UF_A =10, UF_H =10, UF_{DB} =3, total UF = 300.

$H_{b/g}$ ratio:

- $DAF_{SYS} = \frac{(H_{b/g})_A}{(H_{b/g})_H} = 1$
- $HEC = POD_{ADJ} \times DAF_{SYS} = 75 \times 1 = 75\ ppm$
- $RfC = \frac{HEC}{UF} = \frac{75}{100} = 0.75\ ppm$

2012 US EPA RGDR approach:

- $DAF = RGDR_{ET} = 1$
- $HEC = POD_{ADJ} \times DAF = 75 \times 1 = 75\ ppm$
- $RfC = \frac{HEC}{UF} = \frac{75}{100} = 0.75\ ppm$

No dosimetric adjustment:

- $RfC = \frac{POD_{ADJ}}{UF} = \frac{75}{300} = 0.25\ ppm$

IV.C. RfCs for Short-Term (10-14 days) Toxicity

BMD analysis was performed on the data from all available short-term toxicity studies with sulfuryl fluoride (Table 12). The incidences of cerebral vacuolation in males and females in the 2 week-mouse study (Nitschke and Quast, 2002), and the brain lesions in the 2-week rabbit study (Eisenbrandt *et al.*, 1985) were both amenable for BMD modeling. However, the small sample sizes used in both studies (n = 5 for mouse; n = 3 for rabbit) resulted in model estimates of variance that DPR considered to be unacceptably large per "best practices," excluding them from consideration as potential PODs (also see Appendix D for details).

The lowest NOEL was 5 ppm, established in a non-guideline postnatal DNT study (Marty *et al.*, 2015). This determination was based on elevated motor activity in PND 22 rat pups following 11 consecutive days (6 hours/day) of exposure to 20 ppm. The study yielded the most sensitive endpoint (motor activity) and the lowest POD (1.25 ppm) and RfC (0.013 ppm; Table 12). The apparent absence of dose responsivity at 150 ppm was plausible due to supralinear toxicokinetics, which may induce sufficient systemic toxicity to override the brain stimulation underlying the elevated motor activity (see Appendix C for details). A similar RfC, 0.018 ppm, was derived from a two-week inhalation study in adult male mice, where the LOEL was based on brain lesions occurring at 100 ppm. Other effects following short-term exposure included decreased body weight, kidney and liver effects, and respiratory tract lesions in adult rats, rabbits, and dogs with NOELs ranging from 100 to 225 ppm. Therefore, the critical NOEL of 5 ppm based on increased motor activity in immature organisms will be protective against brain and kidney histopathology. The critical short-term RfC was 0.013 ppm was calculated from the critical NOEL adjusted for study exposure length and a total UF of 100.

Table 12. Summary of short-term (10-14 days) inhalation studies

Species/ Exposure (ppm)	Effects	LOEL (ppm)	NOEL (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Rat 6 hrs/d x 5d/w x 2w 0, 100, 300, 600	Kidney lesions	300	100	18	18 DAF=1	0.18 UF=100	Eisenbrandt *et al.* (1985)
Rat 6 hrs/d x GD 6-15[a] 0, 30, 100, 300	Decreased body weight; liver, kidney effects	300	100	25	25 DAF=1	0.25 UF=100	Hanley *et al.* (1980)
Rat 6 hrs/d x GD 6-15[a] 0, 25, 75, 225	No effects observed at HDT	--	225	56	--	0.19 UF=300	Hanley *et al.* (1981)

Table 12. Summary of short-term (10-14 days) inhalation studies

Species/ Exposure (ppm)	Effects	LOEL (ppm)	NOEL (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Rat (pups) 6 hrs/d x PND 11-21[a] 0, 5, 20, 150	**Elevated motor activity in PND22 males**	**20**	**5**	**1.25**	1.25 DAF=1 --	0.042 [b]UF=30 **(0.013) [b]UF=100**	**Marty *et al.* (2015);**
	Decreased body weight gain between PND11 and PND21 in male pups	20	5	1.25	1.25 DAF=1	0.042 [b]UF=30	
Mouse 6 hrs/d x 5d/w x 2w 0, 30, 100, 300	Cerebral vacuolation	100	30	5.4	5.4 DAF=1 --	0.054 UF=100 (0.018) UF=300	Nitschke and Quast (2002)
Rabbit 6 hrs/d x 5d/w x 2w 0, 100, 300, 600	Brain lesion (males/females)	300	100	18	18 DAF=1 --	0.18 UF=100 (0.06) UF=300	Eisenbrandt *et al.* (1985)
	Respiratory tract lesions	300	100	18	18 DAF=1	0.18 UF=100	
Rabbit 6 hrs/d x GD 6-18[a] 0, 30, 100, 300	Maternal: Decreased body weight and liver weight	300	100	25	25 DAF=1	0.25 UF=100	Hanley *et al.* (1980)
Rabbit 6 hrs/d x GD 6-18[a] 0, 25, 75, 225	Maternal and fetal: Decreased body weight	225	75	19	19 DAF=1	0.19 UF=100	Hanley *et al.* (1981)
Dog 6 hrs/d x 5d/w x 2w 0, 30, 100, 300	Intermittent tremors and tetany (day 5 onward)	300	100	18	18 DAF=1 --	0.18 UF=100 (0.06) UF=300	Nitschke and Quast (1991)
	Nasal tissue inflammation (slight)	300	100	18	18 DAF=1	0.18 UF=100	

Abbreviations: d, day; DAF, dosimetric adjustment factor; GD, gestation day; HD, highest dose tested; hrs, hours; min, minutes; w, week. For RfCs based on neurotoxic effects, the first value assumed that neurotoxic effects were mediated by either a systemic (DAF = $(H_{b/g})_A/(H_{b/g})_H$=1) or portal of entry mode of action at extrathoracic region (DAF = $RGDR_{ET}$ = 1), and the second value (in parenthesis) assumed unknown MOA and did not use any dosimetric adjustment. Bolded study is selected for the derivation of a short-term RfC.

$$POD_{ADJ} = NOEL\ or\ BMCL_{10}\ (ppm) \times \frac{hrs/day\ (animals)}{24\ hrs/day\ (humans)} \times \frac{5\ days/wk\ (animals)}{7\ days/wk\ (humans)}$$

$$POD_{HEC} = POD_{ADJ} \times DAF$$

$$RfC = \frac{POD_{HEC}\ or\ POD_{ADJ}}{UF_A \times UF_H \times UF_{DB}}$$

[a]No adjustment of 5 days/week factor due to continuous exposure to sulfuryl fluoride during testing periods.
[b]UF_{DB} = 1 when data were derived from young animals.

RfC for systemic effects: DAF = $(H_{b/g})_A/(H_{b/g})_H$ =1; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for neurological effects in adults: Dosimetric adjustment based on either a systemic or portal of entry MOA, UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100; No dosimetric adjustment, UF_A = 10, UF_H = 10, UF_{DB} = 3, total UF = 300.
For the POD_{ADJ} calculation, when both NOEL and $BMCL_{10}$ are available, $BMCL_{10}$ is used.

IV.D. RfCs for Subchronic Toxicity

BMD analysis was performed on the data from all available subchronic toxicity studies with sulfuryl fluoride. The only data that were amenable to modeling were the incidence of brain vacuoles and nasal inflammation in males and females in the 13 week-rabbit study (Nitschke *et al.*, 1987a). The calculated BMC_{10} and $BMCL_{10}$ values are shown in Table 13 (also see Appendix D for modeling details).

With subchronic inhalation exposure (13-weeks) to sulfuryl fluoride, the critical NOEL/$BMDL_{10}$ was 30 ppm based on neurological effects reported in three separate studies. These effects included electrophysiological changes in rats and brain vacuoles in mice and rabbit at a LOEL of 100 ppm. The combined POD of 30 ppm yielded the lowest RfC (0.018 ppm; Table 13) among all effects observed in the subchronic inhalation studies. Other effects occurring after subchronic exposure included nasal inflammation; kidney, lung, and thyroid lesions; and dental fluorosis in rats and dogs at LOELs of 200-300 ppm and NOELs of 100 ppm (Table 13). Because the subchronic RfC of 0.018 ppm was very close to the lowest short-term RfC of 0.013 ppm, 0.013 ppm was used as the RfC for both durations.

Table 13. Summary of subchronic inhalation studies

Species/ Exposure (ppm)	Effects	LOEL/ BMC_{10} (ppm)	NOEL/ $BMCL_{10}$ (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Rat 6 hrs/d x 5 d/w x 13 w 0, 30, 100, 300	Mottled incisors	100	30	5.4	5.4 DAF=1	0.054 UF=100	Nitschke *et al.* (1987a)
	Brain vacuoles	300	100	18	18 DAF=1 --	0.18 UF=100 (0.06) UF=300	
	Reduced body weight, kidney (hyperplasia)	300	100	18	18 DAF=1	0.18 UF=100	
	Alveolar histiocytosis, subpleural, multifocal	300	100	18	21.6 [a]DAF=1.2	0.22 UF=100	
	Nasal inflammation	300	100	18	18 DAF=1	0.18 UF=100	

Table 13. Summary of subchronic inhalation studies

Species/ Exposure (ppm)	Effects	LOEL/ BMC_{10} (ppm)	NOEL/ $BMCL_{10}$ (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Rat **6 hrs/d x 5 d/w x 13 w** **0, 30, 100, 300**	Mottled incisors	100	30	5.4	5.4 DAF=1	0.054 UF=100	**Mattsson *et al.* (1986)**
	Electrophysiological effects	**100**	**30**	**5.4**	5.4 DAF=1 --	0.054 UF=100 **(0.018)** **UF=300**	
Mouse **6 hrs/d x 5 d/w x 13 w** **0, 10, 30, 100**	**Brain (cerebrum and thalamus/ hypothalamus) vacuoles**	**100**	**30**	**5.4**	5.4 DAF=1 --	0.054 UF=100 **(0.018)** **UF=300**	**Nitschke and Quast (1993)**
	Thyroid hypertrophy	100	30	5.4	5.4 DAF=1	0.054 UF=100	
Rabbit **6 hrs/d x 5 d/w x 13 w** **0, 30, 100, 600/300***	**Brain vacuoles (females)**	**100/89**	**30/30**	**5.4**	5.4 DAF=1 --	0.054 UF=100 **(0.018)** **UF=300**	**Nitschke *et al.* (1987b)**
	Nasal inflammation lesions (males)	100/93	30/41	7.3	7.3 DAF=1	0.073 UF=100	
Dog 6 hrs/d x 5 d/w x 13 w 0, 30, 100, 200	Brain lesion (gliosis and vacuoles)	200	100	18	5.4 DAF=1 --	0.054 UF=100 (0.06) UF=300	Nitschke and Quast (1992)
	Reduced body weight gain	200	100	18	18 DAF=1	0.18 UF=100	

Abbreviations: d, day; DAF, dosimetric adjustment factor; hrs, hours; w, week. For RfCs based on neurotoxic effects, the first value assumed that neurotoxic effects were mediated by either a systemic (DAF = $(H_{b/g})_A/(H_{b/g})_H$=1) or portal of entry mode of action at extrathoracic region (DAF = $RGDR_{ET}$ = 1), and the second value (in parenthesis) assumed unknown MOA and did not use any dosimetric adjustment. Bolded study is selected for the derivation of a subchronic RfC. Benchmark Concentration Lower Confidence Limit (BMCL): a value representing a 95% lower bound of the benchmark concentration (BMC) for the observed effect; subscripts indicates an effect threshold based on data for concurrent controls (10 = 10% extra risk).

$$POD_{ADJ} = NOEL\ or\ BMCL_{10}(ppm) \times \frac{hrs/day\ (animals)}{24\ hrs/day\ (humans)} \times \frac{5\ days/wk\ (animals)}{7\ days/wk\ (humans)}$$

$$POD_{HEC} = POD_{ADJ} \times DAF$$

$$RfC = \frac{POD_{HEC}\ or\ POD_{ADJ}}{UF_A \times UF_H \times UF_{DB}}$$

RfC for systemic effects: DAF = $(H_{b/g})_A/(H_{b/g})_H$=1; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for portal of entry effects (extrathoracic): DAF = $RGDR_{ET}$ = 1; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for portal of entry effects (pulmonary): DAF = $RGDR_{PU}$; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for neurological effect in adults: Dosimetric adjustment based on either a systemic or portal of entry MOA, UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100; No dosimetric adjustment, UF_A = 10, UF_H = 10, UF_{DB} = 3, total UF = 300.
For POD_{ADJ} calculation, when both NOEL and $BMCL_{10}$ are available, $BMCL_{10}$ is used.

[a]For the 13-week rat (Fischer 344) inhalation study that exhibited portal of entry effects at pulmonary region (lung), a default body weight of 124 g (female, subchronic) for Fisher 344 rats was used for ventilation calculation. Based on the allometric equation for rats, $V_E = e^{-0.578+0.821\ln(BW)} = 0.10$ 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, $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H = 1.2$

IV.E. RfCs for Chronic Toxicity

BMD analysis was performed on the data from all available chronic toxicity studies with sulfuryl fluoride (Table 14). The incidence data amenable for BMD modeling included the following: dental fluorosis in males and females in the 1-year study in dogs (Quast *et al.*, 1993a); dental fluorosis at 12 and 24 months in males and females in the 2-year study with rats (Quast *et al.*, 1993c); lung inflammation in females in the 1-year study in dogs (Quast *et al.*, 1993a), and lung alveolar macrophage aggregates in parental animals in the 2-generation reproductive toxicity study in rats (Breslin *et al.*, 1992). However, only the incidence of lung alveolar macrophage aggregates in rats from the 2-generation reproductive toxicity study (Breslin *et al.*, 1992) produced models that were deemed usable for derivation of PODs. The calculated BMC_{10} and $BMCL_{10}$ values are shown in Table 14 (also see Appendix D for modeling details).

The lowest NOEL from the chronic studies was 20 ppm from rats and mice (POD_{ADJ} = 3.57 in both cases) (Table 14). This determination was based on an increased incidence of brain vacuoles at 80 ppm in both the 2-year rat and 18-month mouse studies (Quast *et al.*, 1993c; Quast *et al.*, 1993b) and at 150 ppm for the F1 parents in the 2-generation rat study (Breslin *et al.*, 1992). A lower NOEL/LOEL of 5 ppm / 20 ppm based on dental fluorosis (POD_{ADJ} = 0.89 ppm) was established in rats (Quast *et al.*, 1993c). However, because the fluorosis was graded as "very slight," this effect was regarded as cosmetic rather than toxic, which would have required a grade of "severe" (NRC, 2006). A $BMCL_{10}$ of 3.2 ppm based on maternal lung inflammation and alveolar macrophage aggregation in females (POD_{ADJ} = 0.57 ppm) was established in a rat 2-generation reproductive study (Breslin *et al.*, 1992). While the lung inflammation $BMCL_{10}$ of 3.2 ppm was lower than the NOEL of 20 ppm based on brain vacuoles, the RfC for brain vacuolation (0.012 ppm, calculated with a total uncertainty factor of 300) was similar to that for lung effects (0.015 ppm, calculated with a DAF of 2.7 for portal of entry action and a total uncertainty factor of 100) (Table 14).

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).

Table 14. Summary of chronic inhalation studies

Species/ Exposure (ppm)	Effects	LOEL/ BMC_{10} (ppm)	NOEL/ $BMCL_{10}$ (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Rat 6 hrs/d x 5 d/w x 1 y 0, 5, 20, 80	No neurotoxicity	--	80	14	14 DAF=1 --	0.14 UF=100 (0.05) UF=300	Spencer *et al.* (1994)
Rat **6 hrs/d x 5 d/w x 2 y** **0, 5, 20, 80**	Dental fluorosis (males)	20	5	0.89	0.89 DAF=1	0.009 UF=100	**Quast *et al.* (1993c)**
	Brain vacuoles	**80**	**20**	**3.57**	3.57 DAF=1 --	0.036 UF=100 **(0.012)** **UF=300**	
	Glomerulonephropathy, reactive hyperplasia (nasal)	80	20	3.57	3.57 DAF=1	0.036 UF=100	
	Aggregates of alveolar macrophages, multifocal	80	20	3.57	6.79 [a]DAF=1.9	0.068 UF=100	
Rat **6 hrs/d x 5 d/w x 2-generation study** **0, 5, 20, 150**	Lung alveolar macrophage aggregates (maternal-females)	20/4.3	5/3.2	0.57	1.54 [b]DAF=2.7	0.015 UF=100	**Breslin *et al.* (1992)**
	Brain vacuoles (maternal)	**150**	**20**	**3.57**	3.57 DAF=1 --	0.036 UF=100 **(0.012)** **UF=300**	
	Dental fluorosis (maternal)	150	20	3.57	3.57 DAF=1	0.036 UF=100	
	Reduced body weight (pups)	150	20	3.57	3.57 DAF=1	0.12 [c]UF=30	
Mouse **6 hrs/d x 5 d/w x 18 m** **0, 5, 20, 80**	**Brain vacuoles**	**80**	**20**	**3.57**	3.57 DAF=1 --	0.036 UF=100 **(0.012)** **UF=300**	**Quast *et al.* (1993b)**
	Lung congestion	80	20	3.57	11.1 [c]DAF=3.1	0.11 UF=100	
	Decreased body weight, decreased survival, systemic amyloidosis, thyroid hypertrophy, heart thrombus	80	20	3.57	3.57 DAF=1	0.036 UF=100	

Table 14. Summary of chronic inhalation studies

Species/ Exposure (ppm)	Effects	LOEL/ BMC_{10} (ppm)	NOEL/ $BMCL_{10}$ (ppm)	POD_{ADJ} (ppm)	POD_{HEC} (ppm)	RfC (ppm)	Ref.
Dog 6 hrs/d x 5 d/w x 1 y 0, 20, 80, 200	Dental fluorosis	80	20	3.57	3.57 DAF=1	0.036 UF=100	Quast *et al.* (1993a)
	Lung inflammation (females)	80	20	3.57	1.25 [d]DAF=0.35	0.013 UF=100	
	Brain malacia	200	80	14.3	14.3 DAF=1 --	0.14 UF=100 (0.06) UF=300	

Abbreviations: d, day; DAF, dosimetric adjustment factor; hrs, hours; ND, not determined; w, week; y, year. For RfCs based on neurotoxic effects, the first value assumed that neurotoxic effects were mediated by either a systemic (DAF = $(H_{b/g})_A/(H_{b/g})_H$ =1) or portal of entry mode of action at extrathoracic region (DAF = $RGDR_{ET}$ = 1), and the second value (in parenthesis) assumed unknown MOA and did not use any dosimetric adjustment. Bolded study is selected for the derivation of a chronic RfC. Benchmark Concentration Lower Confidence Limit (BMCL): a value representing a 95% lower bound of the benchmark concentration (BMC) for the observed effect; subscripts indicates an effect threshold based on data for concurrent controls (10 = 10% extra risk).

$$POD_{ADJ} = NOEL\ or\ BMCL_{10}(ppm) \times \frac{hrs/day\ (animals)}{24\ hrs/day\ (humans)} \times \frac{5\ days/wk\ (animals)}{7\ days/wk\ (humans)}$$

$$POD_{HEC} = POD_{ADJ} \times DAF$$

$$RfC = \frac{POD_{HEC}\ or\ POD_{ADJ}}{UF_A \times UF_H \times UF_{DB}}$$

RfC for systemic effects: DAF = $(H_{b/g})_A/(H_{b/g})_H$ =1; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for portal of entry effects (extrathoracic): DAF = $RGDR_{ET}$ = 1; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for portal of entry effects (pulmonary): DAF = $RGDR_{PU}$; UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100
RfC for neurological effect in adults: Dosimetric adjustment based on either a systemic or portal of entry MOA, UF_A = 3, UF_H = 10, UF_{DB} = 3, total UF = 100; No dosimetric adjustment, UF_A = 10, UF_H = 10, UF_{DB} = 3, total UF = 300.
For POD_{ADJ} calculation, when both NOEL and $BMCL_{10}$ are available, $BMCL_{10}$ is used.

[a]For the 2-year rat (Fisher 344) study that exhibited portal of entry effects at pulmonary region (lung), the mean body weight ranged from 100 g to 265 g in females and 120 g to 436 g in males. A default body weight of 229 g (female, chronic) for Fisher rats was used for ventilation calculation. Based on the allometric equation for rats, $V_E = e^{-0.578+0.821\ln(BW)}$ = 0.17 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, $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H$ = 1.9

[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

[c]For the 18-month mouse (CD-1) study that exhibited portal of entry effects at pulmonary region (lung), the mean body weight ranged from 21 g to 35 g in females and 27 g to 42 g in males. A default body weight of 35.3 g (female, chronic) for B6C3F1 mice was used for ventilation calculation. Based on the allometric equation for rats, $V_E = e^{-}$

$^{0.326+1.050\ln(BW)}$ = 0.04 L/min. The default pulmonary region surface area for rats is 0.05 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, $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H = 3.1$

[d]For the 1-year dog (Beagle) inhalation study that exhibited portal of entry effects at pulmonary region (lung), the body weight ranged from 10 kg to 16 kg in males, and 8 kg to 12 kg in females. The pulmonary region surface area for dogs with a mean body weight of 11.6 kg (range, 10.0-14.5 kg; N = 9) is 43.2 m^2 (Gehr *et al.*, 1981). We thus assume a default bw of 11.6 kg to match the surface area parameter. Based on allometric equation, $V_E = e^{-0.391+0.709\ln(BW)} = 3.84$ L/min. 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, $RGDR_{PU} = (V_E/SA_{pu})_A/(V_E/SA_{pu})_H = 0.35$

[e]UF_{DB} = 1 when data were derived from young animals.

IV.F. Summary of Critical NOELs, adjusted PODs, and RfCs

The critical NOELs and the range of RfCs for acute, short-term, subchronic, and chronic toxicity are presented in Table 15. The acute RfC ranged from 0.25 to 0.75 ppm based on a two-day rat acute inhalation study where no effects were observed in FOB and electro-physiological tests at the highest tested dose. The RfCs for the short-term exposure was 0.013 ppm based on elevated motor activity in rat pups. The RfC for subchronic exposure was 0.018 ppm based on brain lesions in rats, rabbits, and mice. The RfC for chronic exposure was 0.012 ppm based on brain lesions in rats and mice. Because the uncertainty was lower for the critical short-term study (total UF = 100, instead of 300 for the other critical studies), we recommend using the short-term values to assess risk under all repeated exposure durations.

All critical endpoints selected for various exposure durations were based on sulfuryl fluoride induced neurological effects. The acute RfCs for residential bystander inhalation exposures (24 hours/day, 7 days/week) were calculated by dividing the POD by the total UF, the magnitude of which depended on the RfC derivation approach. If dosimetric adjustment was used, the total UF was 100x, comprised of 3x for intraspecies sensitivity, 10x for interspecies variability, and 3x for incomplete database relating to developmental neurotoxicity. If no dosimetric adjustment was used, then the total UF was 300, comprised of 10x each for interspecies variability and intraspecies sensitivity, and 3x for incomplete database relating to developmental neurotoxicity. Table 15 shows the entire range of PODs and RfCs for acute exposure.

While the focus of this document is to propose acute RfCs for residential bystanders, the short-term, subchronic, and chronic values are also shown in Table 15. The short-term RfCs assume a total UF of 100 (10x intraspecies, 10x interspecies) when no dosimetric adjustment was employed. The additional 3x database factor was not required because the critical NOEL was obtained from a postnatal DNT study on PND21 pups. The subchronic and chronic RfCs were calculated using a total UF of 300 (10x intraspecies, 10x interspecies, and 3x for incomplete database relating to developmental neurotoxicity). The short-term RfC of 0.013 ppm was similar to the subchronic and chronic RfCs values (0.018 ppm and 0.012 ppm, respectively), but incorporated the lowest total uncertainty factor. Therefore, DPR selected the short-term RfC of

0.013 ppm as the target air concentration for all three exposure durations (short-term, subchronic and chronic).

Table 15. No-observed-effect levels (NOELs), points of departure (PODs), and reference concentrations (RfCs) for residential bystanders (infants) exposed to sulfuryl fluoride

Duration	NOEL (ppm)	POD_{ADJ} or POD_{HEC} (ppm)	RfC[a] (ppm)	Critical Endpoint
Acute (1 day)	300	75	0.25 – 0.75 [b] UF 100 – 300	No effect in FOB, motor activity, and electrophysiological tests in rats
Short-term (10-14 days)	5	1.25	0.013 UF = 100	Elevated motor activity in rat pups
Sub-chronic (13-weeks)	30	5.4	0.018 UF = 300	Brain lesions (vacuoles) in rats, rabbits, and mice
Chronic	20	3.57	0.012 UF = 300	Brain lesions (vacuoles) in rats and mice

[a]For residential bystanders, exposure duration assumed to be 24 hrs/day, 7 days/wk:

$$RfC_{acute} = NOEL\ (ppm) \times \frac{6\ hrs/day\ (animals)}{24\ hrs/day\ (humans)} \div 300\ (UF)$$

$$RfC_{1-2wk} = NOEL\ (ppm) \times \frac{6\ hrs/day\ (animals)}{24\ hrs/day\ (humans)} \times \frac{7\ days/wk\ (animals)}{7\ days/wk\ (humans)} \div 100(UF)$$

$$RfC_{sub/chronic} = NOEL\ (ppm) \times \frac{6\ hrs/day\ (animals)}{24\ hrs/day\ (humans)} \times \frac{5\ days/wk\ (animals)}{7\ days/wk\ (humans)} \div 300\ (UF)$$

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

V. UNCERTAINTIES IN THE REFERENCE CONCENTRATION DETERMINATION

Every risk assessment has inherent limitations with respect to the application of existing data to estimate potential risk to human health. Qualitatively, human health risk assessments carry multiple uncertainties, the magnitude of which can vary depending on the availability and quality of the data being assessed. Specific areas of uncertainty are delineated in the following sections.

V.A. Appraisal of Uncertainties Associated with the Toxicology and Hazard Identification

Uncertainties in risk estimates originate from limitations in pharmacokinetic and toxicological study designs, particularly with respect to the adequacy of the specific exposure routes, durations, and resultant data to address the expected human exposure. With respect to sulfuryl fluoride, the most prominent question is whether an accurate neurotoxicokinetic model can be formed from the available data. In the present document, reasonable assumptions were made in the development of critical NOELs relevant to potential acute, short-term, subchronic, and chronic exposures of human populations to sulfuryl fluoride.

V.A.1. Acute Toxicity

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.

V.A.2. Short-term Toxicity

The short-term POD of 5 ppm was based on increased motor activity observed at a LOEL of 20 ppm in a special non-guideline DNT study (Marty *et al.*, 2015). This effect was only seen in

males and was not detected at the next-higher dose level of 150 ppm. The LOEL was 5-fold lower than the LOEL of 100 ppm for brain vacuolization in adult mice after 2 weeks of exposure (Nitschke and Quast, 2002). In addition, the duration adjusted PODs were 1.25 ppm for increased motor activity in pups and 5.4 ppm for neuropathological findings (brain vacuoles) in the 2 week study in adult mice (Nitschke and Quast, 2002), reinforcing the use of motor activity in rat pups as the critical short-term effect. Finally, it is worth noting that the motor activity effects occurred 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 with young animals.

V.A.3. Subchronic Toxicity

The subchronic POD was based on neurological effects observed at a LOEL of 100 ppm in mice, rats, and rabbits in three separate 13-week studies (Mattsson *et al.*, 1986; Nitschke *et al.*, 1987b; Nitschke and Quast, 1993). The results from the rabbit study (Nitschke *et al.*, 1987b) was used in 2006 by DPR to establish the subchronic POD. The NOEL/$BMCL_{10}$ of 30 ppm for all studies was based on electrophysiological effects (rats) and the presence of brain vacuoles (mice and rabbits). The common LOEL of 100 ppm across all three studies attests to the robustness of this POD. Furthermore, dogs and rats showed brain vacuole-based LOELs of 200 and 300 ppm, respectively, suggesting that the mouse and rabbit NOELs were conservative. The selection of neurological effects as the critical endpoints for subchronic exposure was also based on the brain being the common target for sulfuryl fluoride across all tested species (Table 13).

V.A.4. Chronic Toxicity

The chronic POD was based on increased incidence of brain vacuoles at the LOEL of 80 ppm in both the 2-year rat and 18-month mouse studies (Quast *et al.*, 1993b; Quast *et al.*, 1993c) and at the LOEL of 150 ppm in the 2-generation reproductive study in rats (Quast *et al.*, 1993c). The NOEL was 20 ppm (adjusted POD = 3.57 ppm) for all three studies. Because of the dose selection, this NOEL was 4 to 8 fold lower than the LOEL. Thus, it is possible that the NOEL for brain vacuolization could be closer to the LOEL of 80 ppm in mice and rats.

V.B. Uncertainties in the Toxic Metabolites of Sulfuryl Fluoride

It is postulated that fluoride is the ultimate toxic species derived from parental sulfuryl fluoride, though lack of mechanistic data makes this uncertain. The current database does not exclude the possibility that toxic effects may result from fluorosulfate or sulfonated adducts (see Appendix G). Sulfuryl fluoride itself reacts with endogenous nucleophiles such as ammonium and amino moieties on proteins to form sulfonated adducts. However, sulfonated adduct formation in brain tissues was not measured either in young or adult animals. Both fluorosulfate and sulfonated

adducts may function as carbonic anhydrase inhibitors, which may affect neuronal functions through pH disregulation in the brain (Ruusuvuori and Kaila, 2014; Sapirstein *et al.*, 1984). Thus, fluorosulfate and sulfonated adducts may also contribute to the neurotoxicity observed with sulfuryl fluoride exposure (see Appendix G for details).

V.C. Uncertainties in the PBPK Model

The PBPK model is based on pharmacokinetic studies in laboratory animals and is designed as a cross-species model to inform animal-to-human (interspecies) extrapolation. The current sulfuryl fluoride PBPK is a multi-compartment model (blood, brain, kidney, slowly perfused tissues, rapidly perfused tissues) based on animal and human data for systemic circulation of two hydrolysis products, fluorosulfate and fluoride. The human PBPK model parameters were validated with two human fluoride ingestion datasets to optimize human specific metabolism and physiological processes.

DPR reviewed the model development and the data used for the input parameters, as well as model calibration and validation. Visual examination of the outputs suggest that the model predictions provide a reasonable estimate of actual values. The only exception to this was the brain fluorosulfate concentration, which the model overpredicts for most exposure routes and durations in both rats (except in fetal brain) and rabbits, thereby resulting in a more conservative outcome. The model adequately predicts the kinetics of sulfuryl fluoride hydrolysis, although its predictive ability regarding internal dosimetry of sulfuryl fluoride was based on the assumptions that (1) fluoride is the ultimate toxicant and (2) fluoride formed at the portal of entry in the nasal cavity is distributed to the brain via the systemic circulation. This model does not account for other possible distribution mechanisms, such as direct intranasal transport of fluoride and/or possibly sulfuryl fluoride to the brain. If the model was revised to include the direct nasal-to-brain route, as has been done for several other pharmacokinetic models (Ramoju *et al.*, 2017; Stevens *et al.*, 2011), it may be possible to use it to generate a PBPK-derived POD.

The model also assumes that the fractional inhalation absorption of sulfuryl fluoride is 45% in rabbits and 15% in humans (Poet and Hinderliter, 2011). The measured fractional inhalation absorption in rats was 12.5 – 14% (Mendrala *et al.*, 2002). Due to a 3-fold difference in plasma fluoride level detected between rats and rabbits for the same exposure (Rick *et al.*, 2011), a fractional inhalation absorption of 45% was assumed for the rabbit. The investigators attributed this to differences in airway geometry (surface area) and airflow (minute ventilation) between rats and rabbits (Corley *et al.*, 2009; Minard *et al.*, 2006), and used the same logic to assume a conservative estimation of 15% for humans (Poet and Hinderliter, 2011). DPR's analysis indicates that higher plasma fluoride in rabbits may be due to slower elimination, not higher absorption. Thus, the assumed values of fractional inhalation absorption in rabbits and humans are uncertain.

In addition to the uncertainties discussed above, there are inherent limitations associated with the model. These include designation of partition coefficients derived from QSAR predictions, parameters optimized to fit the experimental data, and the need for a more complete model validation (see Appendix F for details).

V.D. Uncertainties in the Interspecies and Intraspecies UF

Default uncertainty factors for deriving RfCs are conventionally set at 10x to account for interspecies variability (UF_A) and 10x to account for intraspecies (human) sensitivity (UF_H). Both UFs are themselves products of two separate components, a pharmacokinetic uncertainty factor of 3x and a pharmacodynamic uncertainty factor of 3x.

For RfCs derived from HECs, the conventional interspecies uncertainty factor of 10x was reduced to 3x because the interspecies pharmacokinetic differences were considered resolved by the HEC conversion, regardless of whether the MOA was portal of entry or systemic. The remaining default interspecies pharmacodynamic UF of 3x was retained because data relating to tissue level interactions were insufficient to quantitatively resolve potential animal-to-human differences (U.S. EPA, 1994). The full 10-fold intraspecies factor was also retained to reflect the range of sensitivity within the human population.

For RfCs derived from PODs that represented duration-adjusted animal values without dosimetric adjustment (thus requiring no HEC calculation), the full default interspecies and intraspecies UFs of 10x were retained. DPR recognizes the inherent uncertainty in this default approach, as neither UF was based on actual data specific to sulfuryl fluoride.

V.E. Additional Database Uncertainties

Additional UFs may be applied to account for gaps in the database (UF_{DB}) or for other deficiencies or uncertainties. As such, DPR maintains a practice of applying an additional database uncertainty factor (UF_{DB}) to account for the possibility of increased pre- and post-natal sensitivity when data on young animals are not available. The sulfuryl fluoride toxicity database includes one reproductive toxicity study, two developmental toxicity studies, a special non-guideline DNT study, and a series of pharmacokinetic studies.

V.E.1. UF_{DB} Pharmacokinetic Uncertainty

The available data did not indicate that exposure during gestation and developmental periods yields higher brain fluoride concentration than exposure during adulthood (see Appendix C for details). As such, the data supported the reduction of the 3x pharmacokinetic portion of the UF_{DB} to 1x.

V.E.2. UF_{DB} Pharmacodynamic Uncertainty

There was no evidence of reproductive or developmental toxicity in the evaluated studies. However, certain sensitive neurotoxicity parameters (e.g., electrophysiology or motor activity) were not measured in these studies. The special DNT study evaluated sensitive neurological effects such as FOB parameters, motor activity, and neuropathology. While elevated motor activity was detected at a LOEL of 20 ppm in PND 22 pups, similar evaluations of motor activity in adults under similar exposures were lacking. Additionally, neuropathological examinations were not conducted until PND 78 (56 days after the end of exposure), leaving some question as to whether PND 22 animals might have expressed some type of neurohistopathology (*e.g.*, vacuoles) if captured at an earlier time point. The special DNT study only covered a limited postnatal developmental period (PND10 – PND21). Furthermore, motor activity was not measured immediately after sulfuryl fluoride exposure when fluoride (the putative toxic species) concentrations were shown to peak in the brain. Finally, although exposure during gestation and lactation did not necessarily increase the sensitivity to development of brain lesions in the reproductive toxicity study (see Appendix C), F_1 fetuses and pups in this study were not exposed to sulfuryl fluoride directly through inhalation, which is admittedly difficult. DPR acknowledged the difficulty in conducting any inhalation exposure study on such young animals (DPR, 2017a). Even so, the sensitivity of that age group remains unknown, and therefore represents a database gap that should be accounted for. Considering all these uncertainties, the 3x pharmacodynamic component was retained, resulting in a final UF_{DB} of 3x.

V.F. Uncertainties in the Derivation of Acute Reference Concentrations for Sulfuryl Fluoride

The development of RfCs was challenging because the relevant neurotoxic effects do not fit typical systemic or portal of entry MOAs. The available data suggest that the induced neurotoxicity following inhalation exposures may, instead, be mediated through alternative pathways that permit direct access of sulfuryl fluoride to the CNS from the point of contact in the nasal cavity. One potential pathway is that the toxicant migrates via the olfactory region through intracellular or extracellular pathways or through the perivascular space. Another potential pathway is that sulfuryl fluoride enters the CNS through countercurrent exchange between the cavernous sinus and internal carotid artery. Either pathway would result in sulfuryl fluoride exposure to critical tissues in anatomical proximity to the basal ganglia (caudate nucleus and putamen; see Appendix E).

To account for these uncertainties, this Addendum calculated acute RfCs using three different assumptions in order to propose regulatory targets:

1) A systemic MOA using a default dosimetric adjustment factor of 1;

2) A portal of entry MOA at extrathoracic region using a default dosimetric adjustment factor of 1; or,

3) An unknown MOA employing no dosimetric adjustment using a default uncertainty factor of 10x for animal to human extrapolation.

- Systemic MOA: If it is assumed that sulfuryl fluoride causes neurotoxicity via a systemic MOA, a default DAF (= $H_{b/g}$ ratio) of 1 is used to calculate the HEC. Both the US EPA 1994 RfC Method and the US EPA 2012 RfC Update showed that the $H_{b/g}$ in animals is generally greater than $H_{b/g}$ in humans. However, without corresponding data, the use of a DAF of 1 creates uncertainty.
- Portal of Entry MOA: Likewise, assuming that sulfuryl fluoride-induced neurotoxicity is mediated via a portal of entry MOA through the nasal cavity, a default DAF of 1 (for the extrathoracic region) was also applied to calculate the HEC. This method is supported by US EPA's 2012 RfC Update, which states that published studies and advance kinetic models consistently demonstrate that the ratio of target tissue doses in animals versus humans in the extrathoracic region relative to external concentrations are close to or greater than 1 (U.S. EPA, 2012a). However, without specific data to support this default value, the use of a DAF of 1 creates uncertainty.
- Unknown MOA: If sulfuryl fluoride-induced neurotoxic effects are not mediated by either a systemic or a portal of entry MOA, but by an unknown pathway, then no dosimetric adjustments can be applied to calculate HECs. Rather, the animal POD is used for RfC calculation and the uncertainty is addressed by retaining the full 10x to account for interspecies variability.

DPR asserts that the main uncertainties accompanying the RfC derivation reside with the mode of action assumptions, which can only be resolved by further experimentation. Until then, DPR has selected three methodologies to derive sulfuryl fluoride inhalation RfCs that carry the least uncertainty. This analysis has resulted in a proposed **range of acute inhalation RfCs** of **0.25 – 0.75 ppm**.

VI. CONCLUSION

The purpose of this Addendum is to propose regulatory targets (acute reference concentrations, RfCs) for residential bystanders for potential exposure to the fumigant sulfuryl fluoride. Determination of these values is supported by a comprehensive analysis of all available data, including studies submitted prior to the Department of Pesticide Regulation's (DPR) 2006 Risk Characterization Document (RCD) and those after completion of the 2006 RCD. The database now includes 14 additional toxicology studies, updated human illness reports, additional neurotoxicity data, and new approaches to modeling internal doses and impacts to target tissues. In addition, the analysis incorporates results from an external scientific review and comments from the registrant and US EPA.

Acute, subchronic and chronic inhalation exposures to sulfuryl fluoride result in impacts to the nervous and respiratory systems, teeth, and kidney in both humans and in laboratory animals. In addition to DPR and US EPA, multiple international bodies have conducted toxicological evaluations and/or human health risk assessments on sulfuryl fluoride. These include the European Food Safety Authority (EFSA), the Australian Pesticides and Veterinary Medicines Authority (APVMA), Health Canada's Pest Management Regulatory Agency (PRMA), and the Food and Agriculture Organization / World Health Organization (FAO/WHO). In all of the available assessments, fluoride was considered the active principal in the toxicity of sulfuryl fluoride, exerting its effects through a systemic mode of action (MOA). The systemic MOA assumes that fluoride is absorbed through the respiratory system into the blood, and then distributed to target tissues such as brain, teeth, and kidney, with resultant toxic effects (brain vacuoles, dental fluorosis, kidney lesions). All assessments concluded that the most sensitive endpoint was neurotoxicity manifested as vacuoles in the basal ganglia region in rats, mice, rabbits, and dogs. The recent PBPK model for sulfuryl fluoride assumes systemic access of fluoride to the brain in order to predict brain fluoride concentrations in animals and humans. All regulatory agencies and international bodies calculated RfCs for humans based on neurotoxicity observed in animals. Dosimetric adjustments for systemic effects were based on the differences in body weight and inhalation rates between animals and humans.

This Addendum also considered fluoride as the principal toxicant, but concluded that the available data may not support a conventional systemic MOA for neurotoxicity that results following inhalation exposure. Rather, indirect comparison to fluoride levels in tissues after non-inhalation exposures (e.g., oral, i.v., i.p.), as well as inhalation studies with other chemicals, are suggestive of a direct entry path for fluoride into the brain through the intranasal cavity. However, without definitive proof for the direct route, uncertainty remains in the designation of an MOA for neurotoxicity.

As such, this Addendum calculated human RfCs using three different assumptions:

1) A systemic MOA using a default dosimetric adjustment factor of 1;
2) A portal of entry MOA at the nasal cavity using a default dosimetric adjustment factor of 1; or,
3) An unknown MOA employing no dosimetric adjustment by applying a default uncertainty factor of 10x for animal to human extrapolation.

DPR's assessment of the best available science resulted in a refinement of the acute RfCs to a range of 0.25 ppm – 0.75 ppm. An RfC of 0.25 ppm relies on the "No Dosimetric Adjustment" approach and an RfC of 0.75 ppm was derived for both systemic and portal of entry modes of action using default dosimetric adjustments. The main uncertainties accompanying the RfC derivation reside with the mode of action assumptions, which can only be resolved by further experimentation. Until then, DPR is proposing that the RfCs derived from these three methodologies carry the least uncertainty relative to the defaults used and, therefore, are appropriate for proposed regulatory targets.

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APPENDIX A.

PHYSICAL AND CHEMICAL PROPERTIES OF

SULFURYL FLUORIDE

APPENDIX A.

Physical and Chemical Properties of Sulfuryl Fluoride

Chemical name, IUPAC name, Chemical Abstracts Service (CAS) registry number	Sulfuryl fluoride, sulfuryl difluoride, 2699-79-8
Common names	Sulfuryl fluoride, sulfuric oxyfluoride
Trade names	Vikane®, ProFume®
Physical appearance	Colorless, odorless gas, non-flammable, non-corrosive
Molecular formula Molecular weight	SO_2F_2 102.1 g/mole
Specific gravity (25 °C) Compared to water at 4 °C Compared to air	 1.34 (Kenaga, 1957) 3.52 (Kenaga, 1957)
Vapor density (20 °C)	4.3 g/L (Dow AgroSciences, 2001)
Vapor pressure	15.2 atm at 20 °C (Dow AgroSciences, 2001) 17.7 atm at 25 °C (Kenaga, 1957)
Henry's law constant (atm m^3/mol)	0.11 (Cady and Misra, 1974)[a] 1.57 (Krieger, 2001) 0.0328 (Dow AgroSciences, 2001)[b]
Boiling point (1 atm)	-55 °C (Kenaga, 1957)
Melting point (1 atm)	-136 °C (McDonald and Hildenbrand, 1957)
Octanol-water partition coefficient (K_{ow}), with log K_{ow} in brackets	2.57 [0.41] (Dow AgroSciences, 2001)[c] 1.38 [0.14] at pH 7, 20 °C (Comb, 2001)[d]
Solubility (g/L) In water (25 °C) In water (20 °C) In n-Octanol (20 °C)	 0.75 (Kenaga, 1957) 1.04 (Comb, 2001) 14 (Comb, 2001)
Hydrolysis: Half-life @25 °C	5.3 days (pH 2), 3.1 days (pH 5.9), 7.0 hours (pH 7), 10 min (pH 8.3) (Cady and Misra, 1974)
Mass spectrum	102 M^+, 83 M^+-F, 67 m/z 83 -O
Conversion factor (25 °C and 760 mmHg)	1 ppm = 4.17 mg/m^3 1 oz/1,000 ft^3 = 1 g/m^3 = 241 ppm

[a]Calculated based on the average water solubility measured at 1 atm, 23 °C: 100 mL water dissolved 23.3 cm^3 sulfuryl fluoride gas (2 cm^3 gas contains 8 x 10^{-5} moles of sulfuryl fluoride).
[b]Estimated
[c]Estimated using a structural fragment method
[d]Calculated

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APPENDIX B.

ADDITIONAL STUDIES AND INFORMATION

REVIEWED FOLLOWING COMPLETION OF THE 2006 RISK

CHARACTERIZATION DOCUMENT FOR SULFURYL FLUORIDE

APPENDIX B.

Additional Studies and Information Reviewed following Completion of the 2006 Risk Characterization Document for Sulfuryl Fluoride

Study	Title (Study No.)	EPA MRID	DPR Vol (Record No)
Gollapudi *et al.*, 2002	Evaluation of sulfuryl Fluoride in the Mouse Lymphoma (L5178Y $TK^{+/-}$) Forward Mutation Assay (001144)	48549201	50223-0101 (264589)
Gollapudi *et al.*, 2005	Revised report for: Evaluation of sulfuryl fluoride in an in vitro chromosomal aberration assay utilizing rat lymphocytes (001133)	48549202	50223-0102 (264590)
Rick *et al.*, 2009	In vitro determination of the sites and rates of hydrolysis of sulfuryl fluoride and fluorosulfate in the rat and human (061145)	48549203	50223-0103 (264591)
Hotchkiss *et al.*, 2011	Sulfuryl fluoride: limited pharmaocokinetics and metabolism in F344/DuCrl Rats (061180)	48549205	50223-0105 (264593)
Hotchkiss *et al.*, 2008	Nasal and pulmonary uptake and metabolism of inhaled sulfuryl fluoride in male F344/DuCrl rats (061056)	48549206	50223-0106 (264594)
Hotchkiss *et al.*, 2011	Quantitation of fluorosulfate and fluoride in selected tissues following inhalation exposure to sulfuryl fluoride in male F344/DuCrl Rats (071193)	48549207	50223-0107 (264595)
Hotchkiss *et al.*, 2011	Sulfuryl fluoride: limited pharmacokinetics of repeated, 6-hour/day, inhalation exposures conducted for two weeks in F344/DuCrl rats (101159)	48549208	50223-0108 (264596)
Marty *et al.*, 2011	Sulfuryl fluoride: pharmacokinetics in CRL:CD(SD) rat dams, fetuses, and pups following vapor inhalation or gavage exposure during gestation and lactation (081144)	48549209	50223-0109 (264597)
Marty *et al.*, 2011	Sulfuryl fluoride: pharmacokinetics in CRL:CD(SD) rat weanlings following inhalation exposure on postnatal day (PND) 22 (081145)	48549210	50223-0110 (264598)
Hotchkiss *et al.*, 2011	Sulfuryl fluoride: probe study to evaluate absorption and limited pharmacokinetics following a single, 6-hour, 600 ppm exposure in New Zealand white rabbits (091139)	48549211	50223-0111 (264599)
Rick *et al.*, 2011	Sulfuryl fluoride: species comparison of limited pharmacokinetics following single, 6-hour inhalation, exposures of F344/DuCrl rats and New Zealand White Rabbits (101161)	48549212	50223-0112 (264600)
Poet and Hinderliter, 2011	Sulfuryl fluoride cross-species PBPK model: age- and species-related pharmacokinetics of sulfuryl fluoride and its metabolites (NS000031)	48549213	50223-0113 (264601)
Eisenbrandt *et al.*, 2011	Sulfuryl fluoride: scientific basis for removal of the database uncertainty factor as well as a waiver for a developmental neurotoxicity study (NS000035)	48549214	50223-0114 (264602)
Marty *et al.*, 2015	Sulfuryl fluoride: neurotoxicity and toxicokinetics assessment in Crl:CD(SD) rats following inhalation exposure from postnatal days 11-21 (141074)	49609101	50223-0130 (284097)

Study	Title (Study No.)	EPA MRID	DPR Vol (Record No)
Schneir *et al.*, 2008	Systemic fluoride poisoning and death from inhalational exposure to sulfuryl fluoride. *Clin. Toxicol.* 46:850-854.	--	--
Stevens *et al.*, 2011	Systemic and direct nose-to-brain transport pharmacokinetic model for remoxipride after intravenous and intranasal administration. *Drug Metab. Dispos.* 39:2275-2282.	--	--
DPR, 2016	Results from Post-Fumigation Monitoring of a Structure Associated with a Pesticide Illness Report from Orange County	--	--
Mulay *et al.*, 2016	Notes from the field: acute sulfuryl fluoride poisoning in a family - Florida, August 2015. *MMWR Morb. Mortal. Wkly. Rep.* 65:698-699.	--	--
US EPA, 2016	Chemical Safety: Additional Measures Can Be Taken to Prevent Deaths and Serious Injuries From Residential Fumigations. EPA Office of Inspector General. Report No. 17-P-0053	--	--
Poet and Bartels, 2017	Sulfuryl fluoride cross-species PBPK model: pharmacokinetics of sulfuryl fluoride and its metabolites. A slide presentation to California Department of Pesticide Regulation on June 1, 2017.	--	50223-0145
Ramoju *et al.*, 2017	The application of PBPK models in estimating human brain tissue manganese concentrations. *Neurotoxicology* 58:226-237.	--	--
Barreau *et al.*, 2019	Sulfuryl Fluoride Poisonings in Structural Fumigation, a Highly Regulated Industry-Potential Causes and Solutions. *International journal of environmental research and public health* 16.	--	--

APPENDIX C.

ESTABLISHING SULFURYL FLUORIDE UNCERTAINTY FACTORS FOR ACUTE AND SHORT-TERM EXPOSURE

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SUBJECT: Establishing Sulfuryl Fluoride Uncertainty Factors for Acute and Short-term Exposures

Background

Sulfuryl fluoride is a fumigant registered for use in California for both structural and food commodity fumigation. In 2001, US EPA required the completion of an inhalation developmental neurotoxicity study (DNT) for sulfuryl fluoride because of concerns of neurotoxicity and disturbances in electrophysiological responses in rats, mice, dogs and rabbits. The clinical signs included the neurotoxic lesions of malacia (necrosis) and vacuolation of white fiber tracts in the basal ganglia of the caudate-putamen. US EPA's DNT study requirement was later waived and replaced by a 10-fold uncertainty factor (Food Quality Safety Act [FQPA] safety factor[1] of 10X). In 2006, the California Department of Pesticide Regulation (DPR) completed a risk characterization document (RCD) for sulfuryl fluoride (Vikane®) for structural and non-food commodity fumigation in California, which was reviewed by the Scientific Review Panel that is charged with evaluating risk assessments of substances proposed for identification as toxic air contaminants. In the 2006 RCD, DPR established critical NOELs and reference concentrations based on the following uncertainty factors (UF):

Table 1. Sulfuryl Fluoride Uncertainty Factors from CDPR, 2006

Exposure Duration	**Total UF**	
	Workers (adults)	Residents/Bystanders (infants)
Acute (1 day)	100[a]	1000[b]
Short-term (1-2 weeks)	100[a]	1000[b]

[a] 10-fold for intraspecies variability; 10-fold for interspecies extrapolation

[b] 10-fold for intraspecies variability; 10-fold for interspecies extrapolation; 10-fold for database factor of lack of a developmental neurotoxicity study

[1] A primary consideration in implementation of the Food Quality Safety Act (FQPA) safety factor provision is assessing the degree of concern regarding the potential for pre- and postnatal effects. In many cases, concerns regarding pre- and postnatal toxicity can be addressed by calculating a reference dose or margin of exposure from the pre- or postnatal endpoints in the offspring and when traditional uncertainty factors are applied to account for deficiencies in the toxicity data. More information on the determination of the appropriate FQPA Safety Factors in assessing pesticide tolerances can be found at https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/determination-appropriate-fqpa-safety-factors

While waiving the requirement for a DNT study, US EPA, in consultation with DPR, required the registrant to conduct a special non-guideline postnatal DNT/toxicokinetic (TK) study to address inadequacies in the toxicity database concerning neurotoxicity. The final postnatal DNT/TK study "Sulfuryl fluoride: neurotoxicity and toxicokinetic assessment in Crl:CD(SD) rats following inhalation exposure from postnatal days 11-21" was submitted to US EPA and DPR in 2015 (Marty et al., 2015).

Sulfuryl Fluoride Uncertainty Factor Recommendations

Acute Exposure: The Risk Assessment Section reviewed the study findings from which it concluded that the postnatal DNT/TK study (Marty et al. 2015) did not establish an acute NOEL and did not address all the uncertainties for the potential of sulfuryl fluoride to adversely impact neural development of infants and juveniles. Therefore, we recommend that the 10X uncertainty factor for infants, children, and women of childbearing age only be reduced to 3X when using the acute NOEL of 300 ppm from the acute neurotoxicity study in adult rats, as set in the 2006 RCD.

Short-term Exposure: The Risk Assessment Section reviewed the study findings from which it established an inhalation No-Observed-Effect Level (NOEL) of 5 ppm for increased motor activity and reduced body weight gain in pups exposed to sulfuryl fluoride between postnatal day (PND) 11 and 21. Therefore, we recommend that this NOEL be used for short-term (1-2 week) to subchronic exposures of child bystanders and workers (women of childbearing age). Furthermore, it is recommended that the additional UF of 10X for lack of DNT study be reduced to 1X.

Table 2. Recommended Sulfuryl Fluoride Uncertainty Factors

Exposure Duration	Exposure Scenario	Original Total UF	Exposure Scenario	Recommended Total UF
Acute (1 day)	Workers (**adults**)	100[a]	Workers (**females**)	300[c]
	Residents/Bystanders (infants)	1000[b]	Residents/Bystanders (infants)	300[c]
Short-term (1-2 weeks)	Workers (adults)	100[a]	Workers (females)	100[a]
	Residents/Bystanders (infants)	1000[b]	Residents/Bystanders (infants)	100[a]

[a] 10-fold for intraspecies variability; 10-fold for interspecies extrapolation

[b] 10-fold for intraspecies variability; 10-fold for interspecies extrapolation; 10-fold for database factor of lack of a developmental neurotoxicity study

[c] 10-fold for intraspecies variability; 10-fold for interspecies extrapolation; 3-fold for database factor of lack of a developmental neurotoxicity study

Summary of the Non-Guideline Inhalation Postnatal DNT/TK Study

Three cohorts of Crl:CD (SD) rat pups of both sexes were exposed to 0, 5, 20, and 150 ppm sulfuryl fluoride 6 hours/day from postnatal day (PND) 11 through PND 21 by whole body inhalation. A standard DNT study would consist of gestational and post-natal exposures (e.g., gestation day (GD) 6- to PND 21). The rational for not exposing dams/pups during the period GD 6 to PND 10 was based on earlier developmental and reproductive toxicity studies that did not show increased sensitivity to the development of brain lesions resulting from in utero exposure. In addition, inhalation exposures between GD 20 and PND 10 are difficult to conduct because the stress from daily separation of pups from dams can confound the treatment effect.

One cohort was used to evaluate the potential neurobehavioral and neuropathological effects (neurotoxicity cohort). The other two cohorts were used to characterize the toxicokinetics (TK) of sulfuryl fluoride metabolites (fluorosulfate and fluoride), with one group maintained on low fluoride diet and ultrapure water (low F TK cohort) and the other maintained on regular rodent diets and municipal drinking water (weanling TK cohort). The study reported Lowest-Observed-Effect Levels (LOEL) of 150 ppm for all three cohorts based on reduced body weight gain in male/female pups between PND 17 and PND 21.

The study authors did not consider the increases in the motor activity at 20 ppm to be treatment related. However, US EPA questioned the statistical method used to analyze the motor activity responses. In their analysis, US EPA determined that the increased motor activity observed at 20 ppm was a treatment-related adverse effect consistent with excitatory effects observed in the literature for fluoride (U.S. EPA 2016). Our evaluation of the motor activity supported the US EPA findings. We compared individual motor activity data to the mean values of animals in the control group and scored the number of animals with elevated motor activity in each treatment group. Our analysis showed 8 out of 12 animals in the 20 ppm group had increased motor activity when compared to 2 out of 12 in the control group ($P = 0.0065$, Chi-squared test), but no significant difference between the 150 ppm dose group and the control group. Thus, the elevated motor activity in the 20 ppm dose group appears to be treatment related and the lack of dose response at 150 ppm may be due to non-linear toxicokinetics differences as described by Marty et al. 2015. It is possible that exposure to sulfuryl fluoride at 150 ppm may induce systemic toxicity which override the stimulatory responses in the brain.

The study authors concluded that decreases in body weight at the 20 ppm dose group in the low F TK cohort were not treatment related because this effect was only seen at 20 ppm in this cohort and did not follow a dose response. Although the reduced body weight gain in the low F TK cohort between PND 11 and PND 21 at 20 ppm was not replicated in the other two cohorts, we noted that pups in this cohort had a lower average starting body weight at PND 11 (~24.7 g) than the other two cohorts (~30 g). Therefore, the smaller male pups at the 20 ppm in low F TK cohort could be more sensitive to sulfuryl fluoride than the male pups in the other two cohorts. This was also evidenced by the greater reduction in body weight gain between PND 17 and PND 21 at the high-dose (150 ppm) group in the low F TK cohort (56% reduction in both males and

females) than that of the neurotoxicity cohort (33% reduction in males and 26% in females) and the weanling TK cohort (32% reduction in males and 33% in females). Similarly, the lack of clear dose response in body weight reduction between 20 and 150 ppm in the low F TK cohort could be due to non-linear TK at 150 ppm.

Due to the rapid hydrolysis of sulfuryl fluoride at the point of entry, free fluoride was implicated to be the active metabolite responsible for sulfuryl fluoride induced neurotoxicity based on:

1) Relatively high levels of free fluoride compared with absence or very low level of fluorosulfate in the cerebrum of rats and rabbits exposed to sulfuryl fluoride (Eisenbrandt et al. 2011);
2) Neurotoxicity was present at dose levels in rabbits and rats at which fluorosulfate was either not detected or detected only at low levels around the limit of quantification (LLQ) (Eisenbrandt et al. 2011); and
3) Neurotoxic response observed in rats exposed to sulfuryl fluoride was similar to that previously described for fluoride (Nitschke et al. 1986).

While free fluoride was not measured in the brain tissues for the 20 ppm group with repeated exposure of 11 days in the 2015 non-guideline postnatal DNT/TK study, an earlier inhalation pharmacokinetics study reported presence of net free fluoride in the rat brain at similar exposure concentration. In this study, male pups (PND 22) were exposed to 30 ppm sulfuryl fluoride for 4 hour and the measured net brain fluoride was 5.69 nmol/g (non-detectable in the control group) (Marty et al. 2011a). Overall, the levels of net free fluoride (minus background value) in the target tissue (brain) correlated with the observed toxicity.

NOEL for short-term exposure: Based on the effects seen in the 2015 postnatal DNT/TK study, the inhalation NOEL is 5 ppm for increased motor activity and reduced body weight gain in pups treated from PND 11 to PND 21. It is recommended that this NOEL be used for characterizing short-term (1-2 week) to subchronic exposure for child bystanders and workers (females of childbearing age).

NOEL for acute exposure: Pharmacokinetic studies of repeated sulfuryl fluoride exposure (6 hours/day, 5 consecutive days/week, for up to two weeks) showed similar levels of net free brain fluoride after the first day exposure to the last day of exposure (Hotchkiss et al. 2011b). The sulfuryl fluoride PBPK model also predicts constant free brain peak fluoride levels over a course of 12-day repeat exposure (Poet and Hinderliter 2011). If the level of fluoride in the brain correlates with neurotoxicity, then it may be expected that the changes in motor activity in pups following repeated exposures may also occur after a single day exposure. However, comparative studies of neurobehavioral functions between one day and repeated exposure are currently lacking and, therefore, the short-term NOEL of 5 ppm from the 2015 postnatal DNT/TK study could not be used to characterize acute exposures. It is thus recommended that the acute NOEL

remains at 300 ppm as set in the 2006 RCD for sulfuryl fluoride and established from the acute neurotoxicity study in adult rats (Albee et al. 1993a).

Establishing Sulfuryl Fluoride Uncertainty Factors

Based on the results from the 2015 postnatal DNT/TK study and data from other studies previously submitted, we recommend the reduction of the additional 10X uncertainty factor for infants, children, and women of childbearing age based on the following:

1) The neurotoxic effects of sulfuryl fluoride are presumably caused by free fluoride in the brain. Among studies with immature rats, three datasets with repeated exposures at 150 ppm (fetuses, PND 10 and PND 22 pups) were available for comparison of the fluoride levels in the brain. In these studies, the fetuses were from dams exposed between GD 6-20 (15 days exposure) (Marty et al. 2011b), PND 10 pups were from dams exposed to sulfuryl fluoride between GD 6-20 and lactation day (LD) 5-10 (15 days indirect in utero exposure and 5 days indirect exposure through milk) (Marty et al. 2011b) and PND 22 pups were exposed to sulfuryl fluoride between PND 11-21 (11 days direct exposure) (Marty et al. 2015). The PND 22 pups with 11 days of repeated exposure had the highest levels of net free fluoride in the brain. Two acute studies were available for comparison of the fluoride levels in pups and adults. An acute 4-hr inhalation exposure to 30 ppm sulfuryl fluoride showed similar brain fluoride levels in adult rats (Hotchkiss et al. 2011a) and PND 22 pups (Marty et al. 2011a). Altogether, these results do not indicate that exposure during gestation and developmental periods yields higher brain net free fluoride than exposure in adulthood.

2) In the reproductive toxicity study, the incidence of the brain vacuoles at 150 ppm in the F_0 generation (parents – adult only exposure) was higher (25/60) than in the F_1 generation (9/60), who were also exposed during gestation (GD 6-21) and lactation (milk only, no direct inhalation exposure) as well as into adulthood. Thus, exposure during gestation and lactation did not necessarily increase the sensitivity to development of brain lesions.

Despite these findings, some uncertainty still remains regarding the possible sensitivity of fetuses and neonates to sulfuryl fluoride.

Peak effect uncertainty: Pharmacokinetic studies in rats revealed that the serum half-life for fluorosulfate and fluoride was in the range of 2 to 4 hours (Hotchkiss et al. 2011a; Hotchkiss et al. 2011b). However, motor activity was evaluated approximately 18h after the last exposure in both the acute two-day study (Albee et al. 1993b) and the non-guideline DNT/TK study (Marty et al. 2015). This is when the concentrations of fluorosulfate and free fluoride in the brain or plasma are most likely below the limit of detection. Thus, additional effects may have been missed because motor activity evaluations were not measured concomitant to peak brain fluoride concentration.

Pharmacodynamic uncertainty: Although the pharmacokinetic findings showed no significant difference between pups and adults in their net free fluoride concentrations, the key event for the brain vacuoles formation has not yet been clearly elucidated and the potential toxicity of the metabolite fluorosulfate has not been evaluated. The vacuoles seen in the caudate-putamen of adult rats exposed to sulfuryl fluoride are correlated with the fluoride ion. However, fluorosulfate was detected above the lower limit of quantitation (LLQ) in the brain of fetuses at the high dose level (150 ppm), whereas the free fluoride ion was not detected (Marty et al., 2011).

Because of these uncertainties, we recommended that the 10X uncertainty factor for infants, children, and women of childbearing age be reduced to 3X when using the NOEL of 300 ppm from the acute neurotoxicity study. The proposed short-term/subchronic NOEL of 5 ppm should be protective for any effects seen in the postnatal DNT/TK study. Therefore, no additional uncertainty factor is recommended for short-term to subchronic exposure to sulfuryl fluoride in sensitive subpopulations such as infants, children, and women of childbearing age. The short-term/subchronic NOEL of 5 ppm is the same as the chronic NOEL established in DPR's 2006 RCD based on different endpoints.

The proposed critical endpoints and corresponding NOELs and reference concentrations for structural fumigation are shown in Table 3. The 2006 values are included for reference. These values will be re-evaluated during the risk characterization process, currently underway.

Table 3. Proposed Reference Concentrations for Sulfuryl Fluoride

Exposure Duration	2006 RCD NOEL (ppm)	NOEL (ppm)	LOEL (ppm)	Reference concentration		Critical Endpoint at LOEL
				Workers (Females)[a]	Residential Bystanders (Infants)[b]	
Acute 1 day	300	300	----	2.6 ppm 11 mg/m^3 UF= 300	0.41 ppm 1.7 mg/m^3 UF=300	No effect in FOB and electro-physiological tests in rats at 300 ppm
Short-term (1-2 wks)	100 (subchronic 13-wk = 30 ppm)	5	20	0.13 ppm 0.54 mg/m^3 UF= 100	0.015 ppm 0.06 mg/m^3 UF= 100	1) Elevated motor activity in PND 22 male rat pups at 20 ppm; 2) Reduced body weight gain between PND 11 and PND 21 in male rat pups at 20 ppm
Chronic	5	5	20	0.13 ppm 0.54 mg/m^3 UF=100	0.015 ppm 0.06 mg/m^3 UF=100	1) Dental fluorosis in 2-yr rat study at 20 ppm; 2) Lung inflammation & alveolar macrophage aggregate in rat repro study at 20 ppm

[a] For workers, the critical NOEL was converted to the RfC by multiplying it by the ratio of the breathing rate in rats (0.96 m^3/kg/day) to adult humans (0.28 m^3/kg/day), then multiplying by the ratio of exposure duration in animals to human (6 hrs/8 hrs) and finally dividing it by the appropriate uncertainty factor of 300 for acute or short-term.

[b] For residential bystanders, the breathing rate for children (0.59 m^3/kg/day) was used instead of adult breathing rate and the exposure duration in humans was assumed to be 24 hrs/day, 7 days/wk.

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APPENDIX D.

BENCHMARK DOSE MODELING

OF ENDPOINT DATA FOR SULFURYL FLUORIDE

Appendix D.

Benchmark Dose Modeling of Endpoint Data for Sulfuryl Fluoride

DPR used a benchmark dose (BMD) approach to derive points of departure (PODs) for all data that were amenable for modeling. The USEPA's Benchmark Dose Software (BMDS; version 3.1.2) was used to estimate the threshold of toxicity for a corresponding endpoint. For this addendum, all of the data that could be modeled were quantal. Quantal or dichotomous response data are reported as either the presence or absence of an effect (incidence). DPR's default threshold response level (the benchmark response or BMR) for quantal data is 10% (U.S. EPA, 2012). Each model resulted in the generation of a corresponding benchmark concentration (BMC) value as well as a value representing a 95% lower bound of the BMC (BMCL) and a POD for the observed effect. The terms BMC/BMCL were used here because the endpoint data modeled had dose levels expressed as air concentrations (ppm).

In the BMD/BMC approach, the data for each endpoint were used to generate a family of models. The goodness-of-fit was then evaluated for each model over the full dose range to select a "best" model for each effect's data set. The evaluation process was based on a hierarchical examination of (a) the results for statistical tests for goodness-of-fit, (b) the lowest Akaike Information Criteria (AIC) score for relative goodness-of-fit, (c) closeness of BMD and BMDL to each other and to nearest dose levels for goodness-of-fit and model dependence, (d) visual inspection of lines over data points for goodness-of-fit and toxicological plausibility, (e) the magnitude of residuals for goodness-of-fit, and (f) considerations of sample size, variability, and whether there is maximum response at high dose.

The "best" models for each end-point were next evaluated as part of the hazard identification process for their fitness to provide PODs for risk assessment. This evaluation re-considered factors that included the toxicological plausibility of the effect and the quality of the data, as well as the relative magnitude of the threshold of toxicity represented by the BMDL.

Acute inhalation studies

None of the acute inhalation toxicity studies presented in Table 9 in the Addendum with sulfuryl fluoride were suitable for BMD modeling.

Short-term (10-14 days) studies

BMD analysis was performed on the data from all available short-term toxicity studies with sulfuryl fluoride (see Table 12 in Addendum). The incidences of cerebral vacuolation in males and females in the 2 week-mouse study (Nitschke and Quast, 2002), and the brain lesions in the 2-week rabbit study (Eisenbrandt *et al.*, 1985) were both amenable for BMD modeling. However, the small sample sizes used in both studies (n = 5 for mouse; n = 3 for rabbit) resulted

in model estimates of variance that DPR considered to be unacceptably large per "best practices," excluding them from consideration as potential PODs.

Subchronic inhalation studies

BMD analysis was performed on the data from all available subchronic toxicity studies with sulfuryl fluoride (see Table 13 in Addendum). The only end-point data that were amenable to modeling were the incidence of brain vacuoles and nasal inflammation in males and females in the 13 week-rabbit study (Nitschke *et al.*, 1987). The models and the calculated BMC_{10} and $BMCL_{10}$ values are shown below (Table 1; Datasets 1 and 2).

Table 1. BMC_{10} and $BMCL_{10}$ for the best fit model for subchronic inhalation studies

Study	Endpoint	BMDS recommended model	DPR selected best model	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)	BMDS output
Rabbit, 13-week (Nitschke *et al.*, 1987) 0, 30, 100, 300 ppm	**Dataset 1**: Brain vacuolation (female)	Multistage degree 1 model (lowest BMDL)	Log-logistic (lowest AIC; BMD/BMDL < 3)	89	30	Attachment 1
	Dataset 2: Nasal inflammation (male)	Log-logistic model (lowest BMDL)	Multistage degree 1 (lowest AIC)	93	41	Attachment 2

Chronic inhalation studies

BMD analysis was performed on the data from all available chronic toxicity studies with sulfuryl fluoride (see Table 14 in Addendum). The incidence data amenable for BMD modeling included the following: dental fluorosis in males and females in the 1-year study in dogs (Quast *et al.*, 1993a); dental fluorosis at 12 and 24 months in males and females in the 2-year study with rats (Quast *et al.*, 1993b); lung inflammation in females in the 1-year study in dogs (Quast *et al.*, 1993a), and lung alveolar macrophage aggregates in parental animals in the 2-generation reproductive toxicity study in rats (Breslin *et al.*, 1992).

The models based on incidences of dental fluorosis in dogs and rats, and lung inflammation in dog passed the goodness-of-fit tests, however, they were not used for derivation of PODs due to (1) their small sample size, e.g., n = 4 in the dog study (Quast *et al.*, 1993a), (2) concerns with the visual inspection of lines over data points, and (3) datasets having only two response levels above control with large difference in the magnitudes of response, e.g., 25-fold (Quast *et al.*, 1993b).

The incidence of lung alveolar macrophage aggregates in rats from the 2-generation reproductive toxicity study (Breslin *et al.*, 1992) produced models that were deemed usable for derivation of PODs (Table 2). Modeling was performed on datasets for females, males, and for the combined incidences in both sexes (Datasets 3-5). Because a saturation of the response was observed at the highest tested concentration, we carried out sensitivity analysis by modeling the data without the high dose. The test BMD/BMDL were both within 20% indicating that the high dose data did not exert undue bias the overall model. When all above variables were considered together, the $BMCL_{10}$ of 3.2 ppm for the lungs-macrophage in females (dataset 4) was selected as the study POD based on its relative magnitude.

Table 2. BMC_{10} and $BMCL_{10}$ for the best fit model for chronic inhalation studies

Study	Endpoint	BMDS recommended model	DPR selected best model	BMC (ppm)	$BMCL_{10}$ (ppm)	BMDS output
Rat, 2-generation reproductive (Breslin *et al.*, 1992) 0, 5, 20, 150 ppm	**Dataset 3:** Lungs-aggregates of alveolar macrophage (male)	Probit (lowest AIC)	Probit (lowest AIC)	8.5	6.0	Attachment 3
	Dataset 4: Lungs-aggregates of alveolar macrophage (female)	Probit (lowest AIC)	Probit (lowest AIC)	4.3	3.2	Attachment 4
	Dataset 5: Lungs-aggregates of alveolar macrophage (male/female combined)	Probit (lowest AIC)	Probit (lowest AIC)	6.0	4.7	Attachment 5

References

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Attachment 1

BMDS 3.0 Analysis Report

Sulfuryl Fluoride

For

Dataset 1

4/21/2020 3:33:58 PM

Analysis Info

Analysis Name: Sulfuryl Fluoride

Analysis Description: C:\Users\qdong\Desktop\BMDS312

Model Type: Dichotomous

Selected Models:

- Frequentist Dichotomous Hill (restricted)
- Frequentist Gamma (restricted)
- Frequentist Log-Logistic (restricted)
- Frequentist Multistage (restricted)
- Frequentist Weibull (restricted)
- Frequentist Logistic (unrestricted)
- Frequentist Log-Probit (unrestricted)
- Frequentist Probit (unrestricted)

Option Sets:

- Option Set #1
  - Risk Type: Extra Risk
  - BMR: 0.1
  - Confidence Level: 0.95
  - Background: Estimated

Data

Dataset 1		
Rabbit-13wk-brain-female		
Dose	N	Incidence
ppm	[N]	[Incidence]
0	7	0
30	7	0
100	7	1
300	7	5

Frequentist Log-Logistic Restricted Option Set #1

User Input

Info	
Model	frequentist Log-Logistic v1.1
Dataset Name	Dataset 1
User notes	Rabbit-13wk-brain-female

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	ppm
Independent Variable	[Incidence]
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	89.43174297
BMDL	30.12215722
BMDU	155.7327121
AIC	18.22061194
P-value	0.971198565
D.O.F.	2
Chi^2	0.058448672

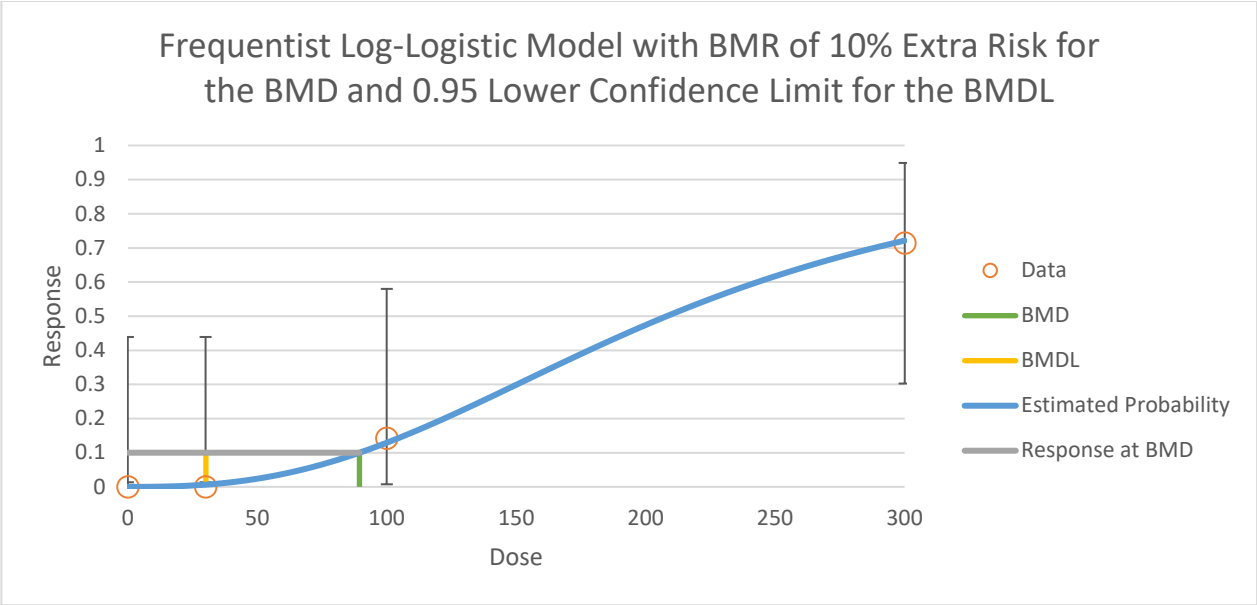
Model Parameters	
# of Parameters	3
Variable	Estimate
g	Bounded
a	-13.88612678
b	2.60130532

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.0661E-07	0	7	-0.0003265
30	0.006441244	0.045088705	0	7	-0.2130282
100	0.129355089	0.905485624	1	7	0.1064478
300	0.721346983	5.04942888	5	7	-0.0416704

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-7.058701348	4	-	-	-
Fitted Model	-7.11030597	2	0.10320924	2	0.9497043
Reduced Model	-14.5482355	1	14.9790683	3	0.0018346


Frequentist Log-Logistic Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
Response
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
Dose
Data
BMD
BMDL
Estimated Probability
Response at BMD

Attachment 2

BMDS 3.0 Analysis Report

Sulfuryl Fluoride

For

Dataset 2

4/21/2020 3:48:59 PM

Analysis Info

Analysis Name: Sulfuryl Fluoride

Analysis Description: C:\Users\qdong\Desktop\BMDS312

Model Type: Dichotomous

Selected Models:

- Frequentist Dichotomous Hill (restricted)
- Frequentist Gamma (restricted)
- Frequentist Log-Logistic (restricted)
- Frequentist Multistage (restricted)
- Frequentist Weibull (restricted)
- Frequentist Logistic (unrestricted)
- Frequentist Log-Probit (unrestricted)
- Frequentist Probit (unrestricted)

Option Sets:

- Option Set #1
  - Risk Type: Extra Risk
  - BMR: 0.1
  - Confidence Level: 0.95
  - Background: Estimated

Data

Dataset 2		
Rabbit-13wk-Nasal inflammation-male		
Dose	N	Incidence
ppm	[N]	[Incidence]
0	7	0
30	7	0
100	7	1
300	7	2

Frequentist Multistage Restricted Option Set #1

User Input

Info	
Model	frequentist Multistage degree 1 v1.1
Dataset Name	Dataset 2
User notes	Rabbit-13wk-Nasal inflammation-male

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	ppm
Independent Variable	[Incidence]
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	92.78941154
BMDL	40.6768409
BMDU	404.3683146
AIC	16.67908718
P-value	0.953312097
D.O.F.	3
Chi^2	0.335024867
Slope Factor	0.002458401

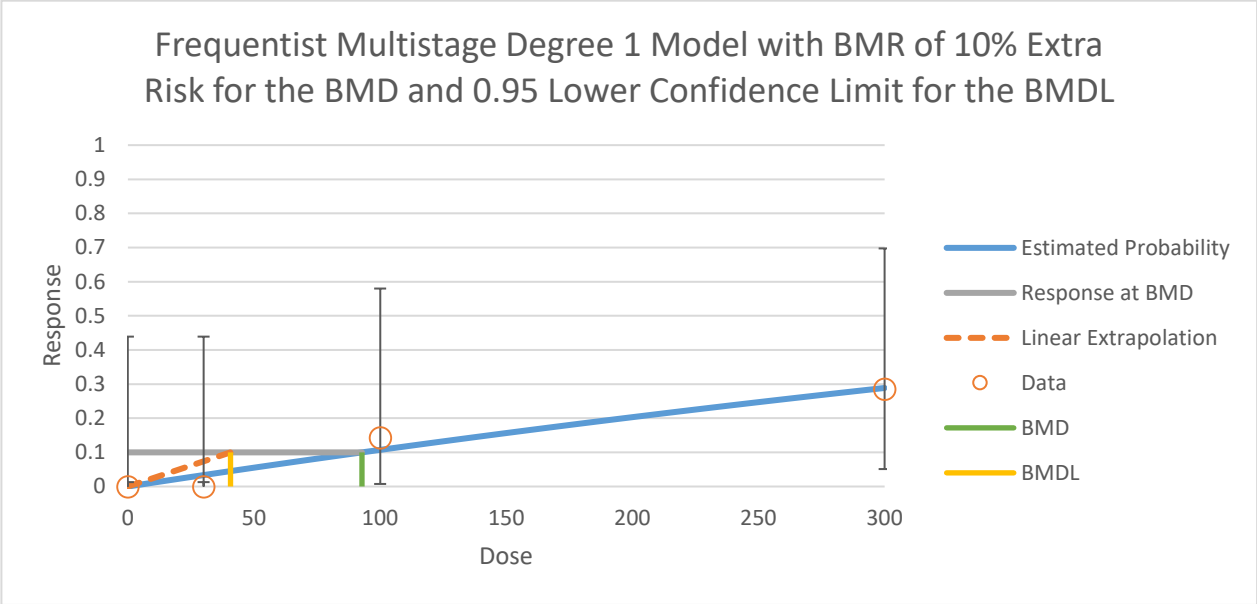
Model Parameters	
# of Parameters	2
Variable	Estimate
g	Bounded
b1	0.00113548

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	1.523E-08	1.0661E-07	0	7	-0.0003265
30	0.033490751	0.234435255	0	7	-0.4925025
100	0.107338652	0.751370562	1	7	0.3035862
300	0.288687887	2.020815207	2	7	-0.0173615

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-7.058701348	4	-	-	-
Fitted Model	-7.339543589	1	0.56168448	3	0.905145
Reduced Model	-9.533993797	1	4.9505849	3	0.1754515


Frequentist Multistage Degree 1 Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
Response
Dose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
50
100
150
200
250
300
Estimated Probability
Response at BMD
Linear Extrapolation
Data
BMD
BMDL

Attachment 3

BMDS 3.0 Analysis Report

Sulfuryl Fluoride

For

Dataset 3

4/21/2020 4:26:20 PM

Analysis Info

Analysis Name: Sulfuryl Fluoride

Analysis Description: C:\Users\qdong\Desktop\BMDS312

Model Type: Dichotomous

Selected Models:

- Frequentist Dichotomous Hill (restricted)
- Frequentist Gamma (restricted)
- Frequentist Log-Logistic (restricted)
- Frequentist Multistage (restricted)
- Frequentist Weibull (restricted)
- Frequentist Logistic (unrestricted)
- Frequentist Log-Probit (unrestricted)
- Frequentist Probit (unrestricted)

Option Sets:

- Option Set #1
  - Risk Type: Extra Risk
  - BMR: 0.1
  - Confidence Level: 0.95
  - Background: Estimated

Data

Dataset 3		
Rat-2gen-lung-male		
Dose	N	Incidence
ppm	[N]	[Incidence]
0	30	3
5	30	5
20	30	11
150	30	30

Frequentist Probit Unrestricted Option Set #1

User Input

Info	
Model	frequentist Probit v1.1
Dataset Name	Dataset 3
User notes	Rat-2gen-lung-male

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	ppm
Independent Variable	[Incidence]
Total # of Observations	4

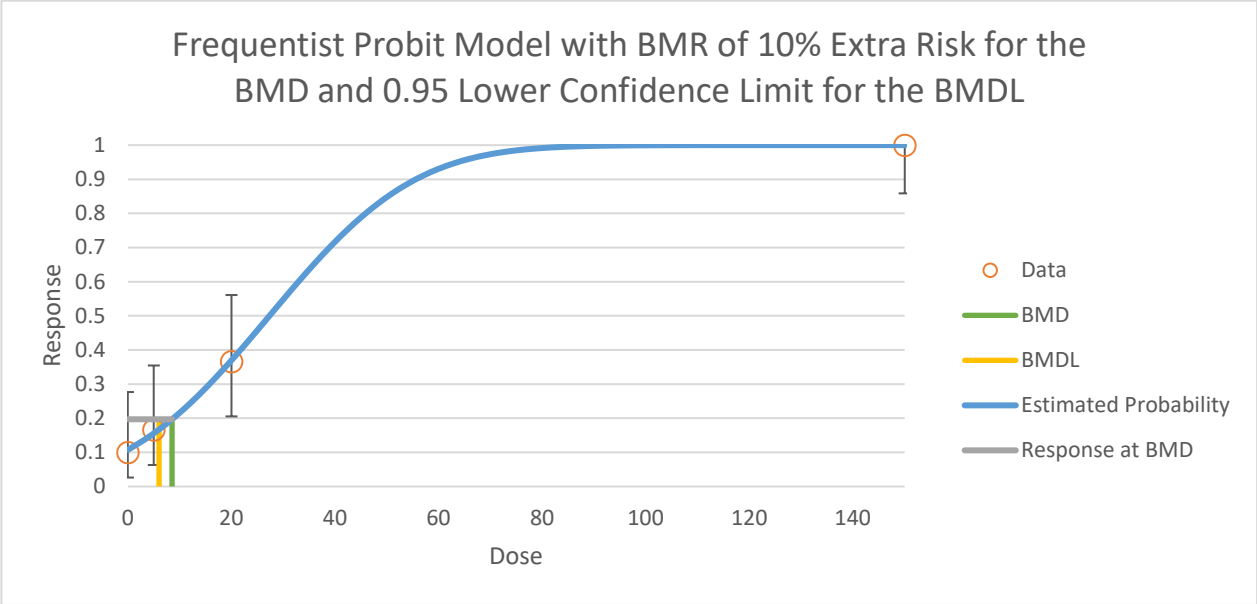
Model Results

Benchmark Dose	
BMD	8.523076921
BMDL	6.016324534
BMDU	14.89951122
AIC	90.01516853
P-value	0.976703588
D.O.F.	2
Chi^2	0.047144126

Model Parameters	
# of Parameters	2
Variable	Estimate
a	-1.23866432
b	0.045305173

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.107734919	3.232047561	3	30	-0.1366442
5	0.155735929	4.672077858	5	30	0.1651112
20	0.369732894	11.09198681	11	30	-0.0347903
150	0.999999986	29.99999959	30	30	0.0006414

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-42.98405832	4	-	-	-
Fitted Model	-43.00758427	2	0.0470519	2	0.9767486
Reduced Model	-81.14954326	1	76.3309699	3	<0.0001


Frequentist Probit Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
Response
Dose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
Data
BMD
BMDL
Estimated Probability
Response at BMD

Attachment 4

BMDS 3.0 Analysis Report

Sulfuryl Fluoride

For

Dataset 4

4/21/2020 4:35:07 PM

Analysis Info

Analysis Name: Sulfuryl Fluoride

Analysis Description: C:\Users\qdong\Desktop\BMDS312

Model Type: Dichotomous

Selected Models:

- Frequentist Dichotomous Hill (restricted)
- Frequentist Gamma (restricted)
- Frequentist Log-Logistic (restricted)
- Frequentist Multistage (restricted)
- Frequentist Weibull (restricted)
- Frequentist Logistic (unrestricted)
- Frequentist Log-Probit (unrestricted)
- Frequentist Probit (unrestricted)

Option Sets:

- Option Set #1
  - Risk Type: Extra Risk
  - BMR: 0.1
  - Confidence Level: 0.95
  - Background: Estimated

Data

Dataset 4		
Rat-2gen-lung-female		
Dose	N	Incidence
ppm	[N]	[Incidence]
0	30	7
5	30	10
20	30	19
150	30	30

Frequentist Probit Unrestricted Option Set #1

User Input

Info	
Model	frequentist Probit v1.1
Dataset Name	Dataset 4
User notes	Rat-2gen-lung-female

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	ppm
Independent Variable	[Incidence]
Total # of Observations	4

Model Results

Benchmark Dose	
BMD	4.325585783
BMDL	3.182077119
BMDU	7.195529865
AIC	114.2261532
P-value	0.995274489
D.O.F.	2
Chi^2	0.009473423

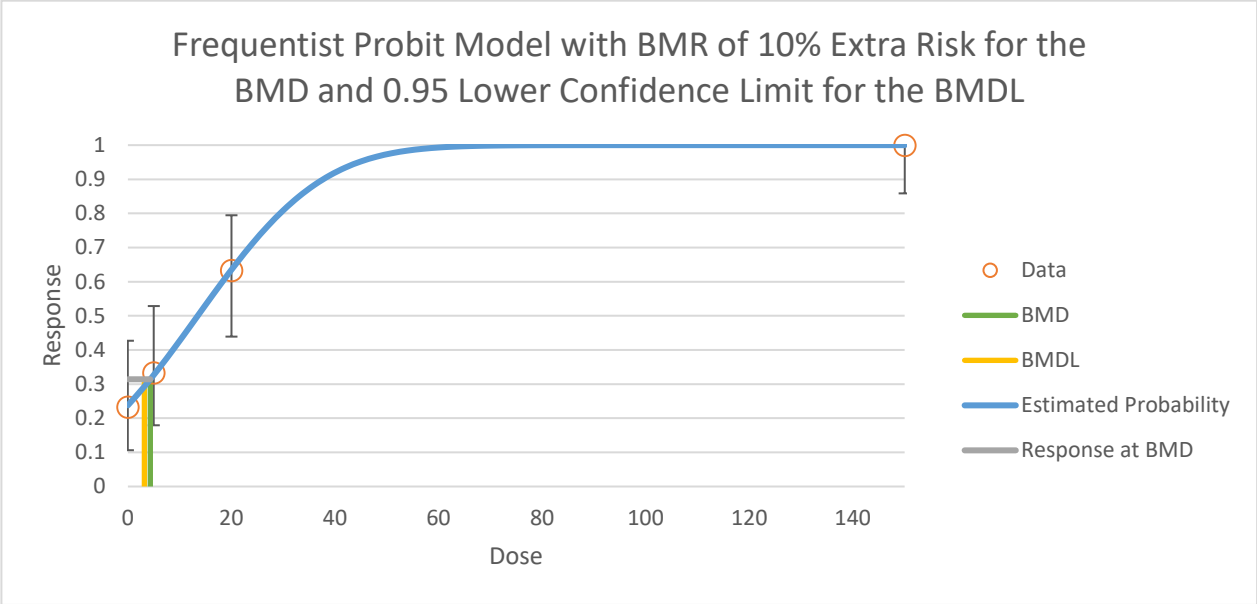
Model Parameters	
# of Parameters	2
Variable	Estimate
a	-0.712903636
b	0.052895394

Goodness of Fit

Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.237952694	7.138580832	7	30	-0.0594164
5	0.32692265	9.807679499	10	30	0.0748531
20	0.634954409	19.04863228	19	30	-0.0184425
150	1	30	30	30	2.778E-06

Analysis of Deviance

Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-55.10834133	4	-	-	-
Fitted Model	-55.1130766	2	0.00947053	2	0.9952759
Reduced Model	-82.57665765	1	54.9366326	3	<0.0001


Frequentist Probit Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
Response
Dose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
Data
BMD
BMDL
Estimated Probability
Response at BMD

Attachment 5

BMDS 3.0 Analysis Report

Sulfuryl Fluoride

For

Dataset 5

4/21/2020 4:39:07 PM

Analysis Info

Analysis Name: Sulfuryl Fluoride

Analysis Description: C:\Users\qdong\Desktop\BMDS312

Model Type: Dichotomous

Selected Models:

- Frequentist Dichotomous Hill (restricted)
- Frequentist Gamma (restricted)
- Frequentist Log-Logistic (restricted)
- Frequentist Multistage (restricted)
- Frequentist Weibull (restricted)
- Frequentist Logistic (unrestricted)
- Frequentist Log-Probit (unrestricted)
- Frequentist Probit (unrestricted)

Option Sets:

- Option Set #1
  - Risk Type: Extra Risk
  - BMR: 0.1
  - Confidence Level: 0.95
  - Background: Estimated

Data

Dataset 5		
Rat-2gen-lung-male/female		
Dose	N	Incidence
ppm	[N]	[Incidence]
0	60	10
5	60	15
20	60	30
150	60	60

Frequentist Probit Unrestricted Option Set #1

User Input

Info	
Model	frequentist Probit v1.1
Dataset Name	Dataset 5
User notes	Rat-2gen-lung-male/female

Model Options	
Risk Type	Extra Risk
BMR	0.1
Confidence Level	0.95
Background	Estimated

Model Data	
Dependent Variable	ppm
Independent Variable	[Incidence]
Total # of Observations	4

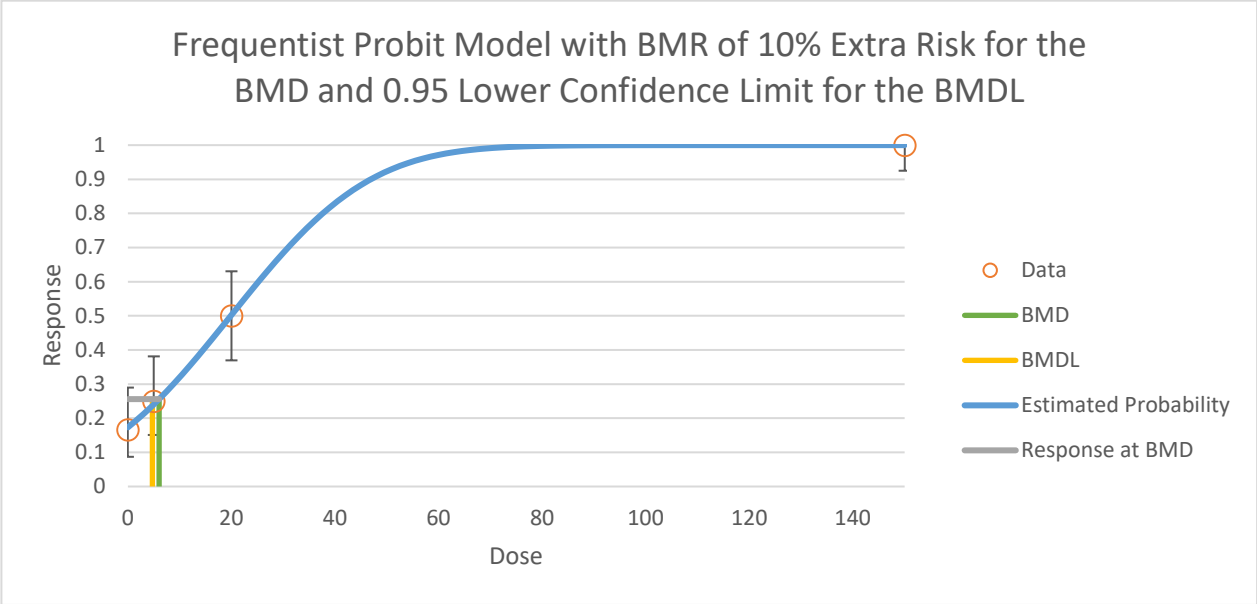
Model Results

Benchmark Dose	
BMD	6.02424757
BMDL	4.728259486
BMDU	8.684954068
AIC	208.7740325
P-value	0.975864421
D.O.F.	2
Chi^2	0.04886323

Model Parameters	
# of Parameters	2
Variable	Estimate
a	-0.941028188
b	0.047364375

Goodness of Fit					
Dose	Estimated Probability	Expected	Observed	Size	Scaled Residual
0	0.173345207	10.40071242	10	60	-0.1366593
5	0.24065215	14.43912899	15	60	0.1693839
20	0.502497087	30.14982522	30	60	-0.0386852
150	1	59.99999998	60	60	0.000146

Analysis of Deviance					
Model	Log Likelihood	# of Parameters	Deviance	Test d.f.	P Value
Full Model	-102.362612	4	-	-	-
Fitted Model	-102.3870163	2	0.04880846	2	0.9758911
Reduced Model	-166.1469297	1	127.568635	3	<0.0001


Frequentist Probit Model with BMR of 10% Extra Risk for the BMD and 0.95 Lower Confidence Limit for the BMDL
Response
Dose
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
20
40
60
80
100
120
140
Data
BMD
BMDL
Estimated Probability
Response at BMD

APPENDIX E.

ACCESS OF FLUORIDE TO THE BRAIN

BY THE INTRANASAL ROUTE: ALTERNATIVE ENTRY PATHWAYS

APPENDIX E.

Access of Fluoride to the Brain by the Intranasal Route: Alternative Entry Pathways

Introduction

Previous toxicological assessments assumed that the neurotoxicity occurring upon inhalation of sulfuryl fluoride gas resulted from hydrolysis and absorption of fluoride through the lungs, circulatory distribution, and ultimate fluoride entry into the brain compartment from blood. However, questions persisted not only with respect to how fluoride ions might cross the blood-brain barrier that normally excludes charged molecules, but also why the resultant brain lesions were specifically localized to the basal ganglia. Evidence presented in this Appendix now suggests that fluoride may access the brain directly from the nasal cavity, bypassing both the systemic circulation and the blood-brain barrier. The possibility of a direct nose-to-brain route is predicated on the following observations:

1. Brain-to-plasma fluoride ratios were approximately 20-fold higher after acute inhalation exposure to sulfuryl fluoride than after systemic (*i.e.*, oral, intravenous, or intraperitoneal) exposure to sodium fluoride.
2. Brain lesions induced by sulfuryl fluoride inhalation were confined to the basal ganglia. No such lesions were detected following oral exposure to sodium fluoride.
3. Select proteins (interferon, insulin, oxytocin), aerosol particles (manganese and $PM_{2.5}$), and whole cells (microglia and mesenchymal stem cells) have been shown to enter the brain compartment directly from the nasal cavity, bypassing the systemic circulation.

Sulfuryl fluoride could conceivably enter the brain by any of several non-systemic routes, including intracellular (axonal) and extracellular (paracellular, intercellular, transcellular, perivascular, or perineural) pathways. In addition, a local vascular pathway might also deliver fluoride to the basal ganglia, whereby fluoride first enters the nasal venous plexus, is transferred to the cavernous sinus, and then proceeds to the internal carotid artery and the cerebral arteries. These entry routes are discussed in detail in this Addendum.

Altogether, our analysis indicates that fluoride could be delivered to the brain by an intranasal pathway following inhalation exposure. This mode of action (MOA) does not require distribution via the systemic circulation. As such, there are implications for calculating human equivalent concentrations (HECs) and reference concentrations (RfCs) for sulfuryl fluoride because the underlying assumptions vary significantly from those appropriate to a systemic MOA.

This analysis presents the evidence for direct access of inhaled sulfuryl fluoride to the brain. While sulfuryl fluoride data supporting this route are not yet available, the considerations outlined herein are robust enough to limit our ability to apply a purely systemic MOA for the derivation of regulatory target concentrations.

Analytical Methods, Data Selection, and Analysis

A. Data Sources

Data for all sulfuryl fluoride inhalation studies were derived from registrant submitted studies. Data for oral, intravenous (i.v.), or intraperitoneal (i.p.) administration of sodium fluoride were from published literature. For the comparison of brain tissue-to-plasma (T/P) fluoride ratios, the sulfuryl fluoride inhalation studies analyzed were either acute (2-6 hours) or short-term (11-14 days) in duration. All non-inhalation sodium fluoride studies analyzed were acute (< 11 hours), including gavage (Geeraerts *et al.*, 1986), i.v. (Knaus *et al.*, 1976; Whitford *et al.*, 1979a; Whitford *et al.*, 1979b), and i.p. (Carlson *et al.*, 1960a).

B. Analytical Methods

Sulfuryl fluoride inhalation studies in rat included exposure concentrations of 3, 5, 20, 30, 150 ppm and 300 ppm. In all these studies, tissue fluoride[1] concentration was expressed as the net free fluoride, which was calculated by subtracting the background free fluoride levels measured in control animals. Two methods were employed for free fluoride analysis. One was a direct method using ion selective electrodes (ISE), which was normally used when tissue fluoride concentrations were high. The second was an indirect method that subtracts fluorosulfate from total fluoride (free fluoride + fluorosulfate). This method was used when tissue fluoride concentrations were low. Total fluoride was measured using silver perchloric acid facilitated microdiffusion with ion selective electrode detection (MD/ISE). This indirect method was also occasionally used in cases with high tissue fluoride concentrations. However, tissue free fluoride was not calculable when the measured fluorosulfate concentration was higher than total fluoride, which occurs most often with low-dose (e.g., at 3, 5, 20 or 30 ppm) sulfuryl fluoride exposures where fluoride measurements were close to the limit of quantification. At high doses (150 or 300 ppm), summation of fluorosulfate and free fluoride levels (by ISE) were comparable to the measurement of total fluoride by MD/ISE for both kidney and cerebrum in the rat (Hotchkiss *et al.*, 2011b), indicating the applicability of both methods in tissue free fluoride measurement. For data repeatability across different studies, we also observed more consistent measurements at high doses (> 30 ppm) than at low doses (≤ 30 ppm). To avoid errors introduced by the choice of analytic method, only brain and plasma net free fluoride concentrations from the two higher dose groups (150 and 300 ppm) in rat studies were included in our data analysis.

[1]Unless otherwise specified, fluoride concentration in all inhalation studies refers to net free fluoride concentration.

C. Data Selection and Analysis for Tissue-to-Plasma Fluoride Ratio Calculation

Ideally, T/P ratios should be calculated when fluoride reaches steady state. However, steady state does not exist between plasma and brain following acute inhalation exposure to sulfuryl fluoride (within 6 hours of exposure) or after pulse loading of radiolabeled $Na^{18}F$ through i.v. administration in rats (Whitford *et al.*, 1979b). This is largely due to the rapid elimination of plasma fluoride through bone absorption and urine excretion, both during and after exposure. The only inhalation study that measured plasma fluoride during exposure was an acute 6-hour rabbit study, where fluoride increased with exposure duration and reached peak levels immediately after termination of exposure (Hotchkiss *et al.*, 2011a). To minimize the possibility of misinterpretation due to low plasma fluoride caused by rapid fluoride elimination, we confined our T/P ratio analysis to samples collected during or immediately after the conclusion of the exposure when plasma fluoride concentrations were measured at their highest levels. The selection of peak plasma fluoride has the added value of data reliability due to high fluoride concentrations because of the increase in signal-to-noise ratio.

Comparisons of T/P ratio among different exposure routes were limited to rat studies, as the vast majority of studies were carried out in this species. For the i.v. route of administration, the same criteria of data inclusion were used for T/P ratio determination. However, for the oral route, data points from 1 hour post exposure were used, which was typically when peak plasma concentrations occurred (Whitford *et al.*, 2008). Similarly, for the i.p. route, data points at 80 minutes post exposure were used (Carlson *et al.*, 1960a).

Results

A. Fluoride T/P ratio between inhalation and non-inhalation studies

The following results are categorized by route of experimental exposure and comparative concentrations of fluoride measured in the target tissue. As mentioned above, emphasis was placed on acute studies that included measurements made either during or immediately after exposure, thus enabling the capture of the highest fluoride concentrations in plasma. Because T/P ratios are highly dependent on post-exposure sampling time, a complete time-course of brain and plasma fluoride levels from acute studies was also included to provide a more comprehensive analysis of fluoride pharmacokinetics.

For the inhalation studies conducted in adult rats, T/P ratios ranged between 0.6 and 3.052, with a mean value of 2.02 (Table 1). Most studies reported higher fluoride in brain than plasma (T/P > 1). Such high T/P ratios were in direct contrast to T/P ratios obtained after oral, i.v. or i.p. treatments (Table 1; Figure 1). Similarly, T/P ratio of approximately 2.5 in rat pups after inhalation exposure were 34-fold higher than in pups exposed to fluoride by milk gavage (T/P =

0.074) (Table 1). A low T/P ratio (0.019) was found in rat fetuses exposed to fluoride transplacentally after inhalation exposure of the mothers to 150 ppm sulfuryl fluoride for 6 hours/day, gestation day (GD) 6 to GD20 (Marty *et al.*, 2011).

T/P ratios were typically low (0.02-0.08) after acute oral or i.v. exposure, even when plasma free fluoride levels reached lethal concentrations (>1800 µM) (Whitford *et al.*, 1979a). However, one acute i.v. study in rats reported relatively high T/P ratios (0.087 – 0.3), which accompanied much lower plasma fluoride concentrations (Table 1) (Knaus *et al.*, 1976). If, however, the resulting concentrations of fluoride in plasma from the i.v. administration are limited to the same range as those from inhalation exposure, T/P ratios were also typically low (0.087-0.092) (Knaus et al., 1976). For the i.p. route, the T/P ratio 80 minutes post-exposure was 0.13 (Carlson *et al.*, 1960a). Taken together, these results showed that T/P ratios were ≤ 0.1 for rats exposed to sodium fluoride via oral, i.v., and i.p., or to sulfuryl fluoride via the transplacental route, suggesting limited permeability of fluoride at blood-brain barrier via systemic circulation. All studies using whole-body inhalation to sulfuryl fluoride showed T/P ratios greater than 1 (Table 1).

Two nose-only inhalation studies employed the same single 4-hour exposure to 300 ppm. One study resulted in a T/P ratio of 0.903 (Mendrala *et al.*, 2002), while the other showed a T/P ratio of 2.912 (Hotchkiss *et al.*, 2011c). This difference was mainly due to divergent fluoride concentrations in plasma. The two studies used different analytical methods (direct vs. indirect) and the sample collection method varied (individual vs. pooled), which may have contributed to the differences. A relatively low T/P ratio of 0.6 in the nose-only study was reported after 2 hours of inhalation exposure (Mendrala *et al.*, 2002). On average, there was an approximately 20-fold difference in mean T/P of 2.0 versus 0.1 between inhalation and non-inhalation studies, respectively.

Rats and rabbits exposed by inhalation to 300 ppm sulfuryl fluoride for 6 hours showed a 3-fold difference in plasma fluoride concentration between rats (46.8 ± 6.5 nmol/mL) and rabbits (120 ± 25 nmol/mL) (Rick *et al.*, 2011). However, brain fluoride concentrations were similar (76.2 ± 4.4 nmol/g in rats *vs*. 86.4 ± 2.2 nmol/g in rabbits). A similar magnitude of difference in brain fluoride would be expected if fluoride enters brain via the systemic circulation. This finding lends further support to a non-systemic brain access pathway after acute inhalation exposure to sulfuryl fluoride.

Table 1. Fluoride levels in plasma and brain and fluoride brain-to-plasma (T/P) ratio of rats exposed to sulfuryl fluoride (SO_2F_2), fluorosulfate (FSO_3) or sodium fluoride (NaF) by various routes

Strain (sex)	Age group	Route	Dose	Duration	F-plasma (nmol/mL)	F-brain (nmol/g)	T/P	Study
Crl:CD(SD) (M/F)	PND11-21	Inhalation (WB)	150 ppm SO_2F_2	6 hrs/d, PND11-21 (regular diet)	27.7	69.5	**2.5**	Marty *et al.* (2015)
Crl:CD(SD) (M/F)	PND11-21	Inhalation (WB)	150 ppm SO_2F_2	6 hr/d, PND11-21 (low-F diet)	28.8	68.2	**2.4**	Marty *et al.* (2015)
Fischer 344 (M)	Adult	Inhalation (NO)	300 ppm SO_2F_2	Single, 2 hrs	132.3	77.7	**0.6**	Mendrala *et al.* (2002)
Fischer 344 (M)	Adult	Inhalation (NO)	300 ppm SO_2F_2	Single, 4 hrs	131.7	119.7	**0.903**	Mendrala *et al.* (2002)
F344/DuCrl (M)	Adult	Inhalation (NO)	300 ppm SO_2F_2	Single, 4 hrs	49.8	145	**2.912**	Hotchkiss *et al.* (2011c)
F344/DuCrl (M)	Adult	Inhalation (WB)	300 ppm SO_2F_2	Single, 6 hrs	39	117	**3.052**	Hotchkiss *et al.* (2011b)
F344/DuCrl (M)	Adult	Inhalation (WB)	300 ppm SO_2F_2	6 hrs/d, 5 d/wk, 2 wk	46.02	98	**2.14**	Hotchkiss *et al.* (2011b)
F344/DuCrl (M)	Adult	Inhalation (WB)	300 ppm SO_2F_2	Single, 6 hrs	46.8	76.2	**1.653**	Rick *et al.* (2011)
Crl:CD(SD) (M/F)	PND10	Gavage	20 µg FSO_3 + 20 ug F	Single, 60 mpe	8.6	0.6	**0.074**	Marty *et al.* (2011)
Crl:CD(SD) (M/F)	PND10	Gavage	40 µg FSO_3 + 40 ug F	Single, 60 mpe	19.6	1.5	**0.074**	Marty *et al.* (2011)
Crl:CD(SD) (M/F)	GD20 fetus	Transplacental	150 ppm SO_2F_2 (Dams)	6 hrs/d, GD6-20	17.1	0.327	**0.019**	Marty *et al.* (2011)
Wistar (M)	Adult	Gavage	4.5 mg F/kg	Single, 60 mpe	63.2	1.74	**0.028**	Geeraerts *et al.* (1986)
Wistar (M)	Adult	Gavage	9 mg F/kg	Single, 60 mpe	94.7	2.26	**0.024**	Geeraerts *et al.* (1986)
Wistar (M)	Adult	Gavage	13.6 mg F/kg	Single, 60 mpe	115.8	2.68	**0.023**	Geeraerts *et al.* (1986)
Wistar (F)	Adult	i.v.	0.75 mg F/kg + 20 µCi of ^{18}F	Single, 5 mpe	124	**3.97**	0.032[a]	Whitford *et al.* (1979b)
Wistar (F)	Adult	i.v. infusion	8 mg F/kg/hr	6 hrs (death occurred)	1814	**105.2**	0.058[b]	Whitford *et al.* (1979a)
Wistar (F)	Adult	i.v. infusion	8 mg F/kg/hr	7.6 hrs (death occurred)	2202	**136.5**	0.062[b]	Whitford *et al.* (1979a)
Wistar (F)	Adult	i.v. infusion	8 mg F/kg/hr	10.3 hrs (death occurred)	1860	**117.2**	0.063[b]	Whitford *et al.* (1979a)
SD (M)	Adult	i.v. infusion	0.9 mg ^{18}F/kg/hr	3 hrs	57.3[c]	5.3	**0.092**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	1.8 mg ^{18}F/kg/hr	3 hrs	121.7[c]	10.5	**0.087**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	3.6 mg ^{18}F/kg/hr	3 hrs	243.4[c]	31.6	**0.130**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	6.0 mg ^{18}F/kg/hr	3 hrs	436.7[c]	89.5	**0.205**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	6.0 mg ^{18}F/kg/hr	1.6 hrs	501.1[c]	73.7	**0.147**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	6.0 mg ^{18}F/kg/hr	1.9 hrs	343.6[c]	79.0	**0.230**	Knaus *et al.* (1976)
SD (M)	Adult	i.v. infusion	6.0 mg ^{18}F/kg/hr	2.3 hrs	386.6[c]	115.8	**0.300**	Knaus *et al.* (1976)
Unspecified (M)	Adult	i.p. injection	1 or 2 mL of 0.2 ppm ^{18}F	Single, 80 mpe	8902[d]	1199[d]	0.13	Carlson *et al.* (1960a)

Abbreviations: SD, Sprague Dawley; M, male; F, female; PND, postnatal day; GD, gestational day; mpe, minutes post exposure; i.v., intravenous; i.p., intraperitoneal; NO, nose only exposure; T/P, tissue-to-plasma ratio; WB, whole-body exposure. All values measured immediately after completion of exposure unless otherwise noted. Values in bold were calculated using original data. FSO_3 derived from $KFSO_3$ and F or ^{18}F derived from NaF. Fluoride concentrations are mean (N > 1) or individual values (N = 1).
[a]Ratio calculated as (counts/min/g wet weight)/(counts/min/ml plasma).

[b]Original T/P ratio using fluoride concentration in brain expressed as tissue water where authors assumed the total water content for brain is 75% (Whitford *et al.*, 1979a). We converted it to T/P ratio based on fluoride concentration in brain expressed as tissue wet weight by multiplying the original T/P ratio with 0.75.
[c]Original fluoride was measured in whole blood (µg F/g), which was converted to plasma concentration by multiplying a converting factor of 0.75/0.55 = 1.36. The converting factor was based on that 75% fluoride of whole blood is in plasma from a dog study (Carlson *et al.*, 1960b) and rat plasma volume on average is 0.55 of whole blood for rats with a body weight of 300g [Table 1 in (Belcher and Harriss, 1957)]. The unit of µg/g was further converted to nmol/g.
[d]Fluoride concentration was expressed as ^{18}F counts/g tissue wet weight.

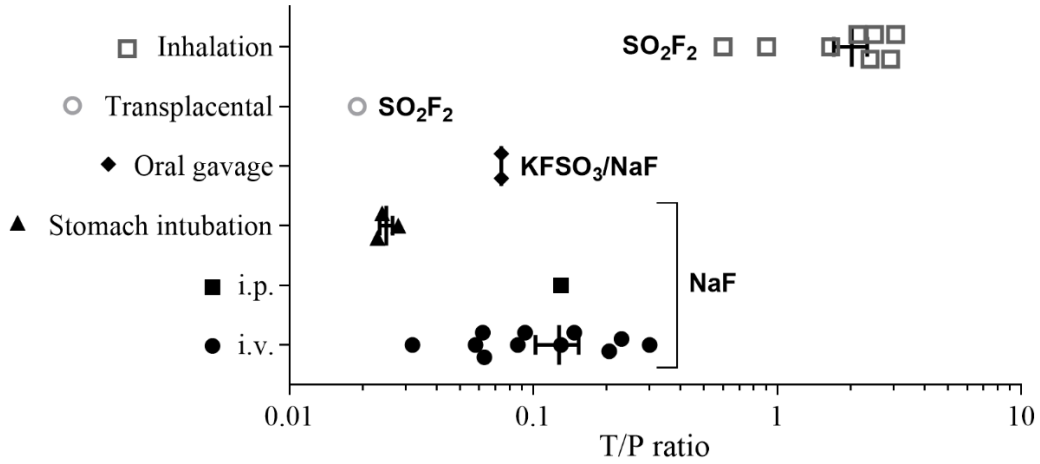


Figure 1. Brain tissue-to-plasma (T/P) ratios of fluoride in adult and young rats exposed by inhalation to sulfuryl fluoride (□), rat fetuses exposed transplacentally to sulfuryl fluoride (SO_2F_2) through dams (○), rat pups exposed orally to fluorosulfate and fluoride (in the form of $KFSO_3$/NaF) through milk gavage (♦), adult rats exposed to sodium fluoride (NaF) via stomach intubation (▲), i.v. (●) or i.p. (■).

Fluoride T/P ratios are highly dependent on post-exposure sampling time, thus a more comprehensive analysis of fluoride pharmacokinetics should use complete time-course graphs of brain and plasma fluoride levels. Because sulfuryl fluoride inhalation studies usually involve continuous exposure of 4-6 hours before the post-exposure sampling, studies employing continuous i.v. infusion over several hours represent more relevant comparison than those employing oral, i.p. or i.v. bolus administration. Therefore, the sodium fluoride continuous i.v. infusion study of Knaus *et al*. (1976) and the sulfuryl fluoride inhalation study of Hotchkiss *et al.* (2011c) were used for comparison.

Figure 2 shows time-course of brain and plasma concentration from adult rats exposed to 6 mg F/kg/hr sodium fluoride via i.v. infusion for 3 hours or to 300 ppm sulfuryl fluoride (SO_2F_2) for 6 hours via inhalation based on data from DeSesso *et al.* (2019) and Hotchkiss *et al.* (2011c). When fluoride concentrations in plasma and brain were plotted over the entire time course of the study, higher fluoride levels were established in plasma than brain in non-inhalation studies (Figure 2A), with the opposite trend occurring in inhalation studies (Figure 2B). Fluoride elimination half-lives between plasma and brain were similar in the inhalation study (2.6 vs. 2.1 hours), but differed in the i.v. infusion study (1.0 vs. 2.1 hours).

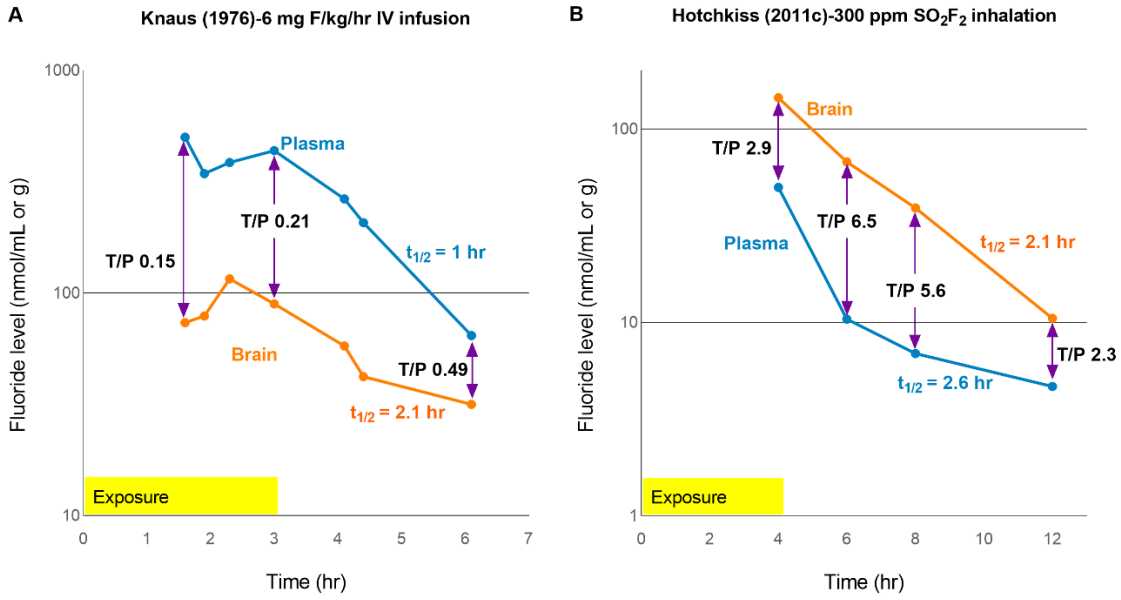


Figure 2. Time-course of brain and plasma concentration from adult rats exposed to 6 mg F/kg/hr sodium fluoride via i.v. infusion for 3 hours (A) or to 300 ppm sulfuryl fluoride (SO_2F_2) for 6 hours via inhalation (B). T/P refers to brain-to-plasma ratio and $t_{1/2}$ refers to half-life. Graph A was modified from Fig. 10 in (DeSesso *et al.*, 2019), and graph B was created by DPR using data from Hotchkiss *et al.* (2011c).

In addition to the evaluation of T/P ratios, absolute tissue fluoride concentrations in plasma and brain were also evaluated. In samples with similar plasma fluoride levels, brain fluoride levels appeared higher after inhalation than non-inhalation exposure (Figure 3). This finding suggests

the existence of different fluoride accumulation mechanisms between non-inhalation and the inhalation routes.

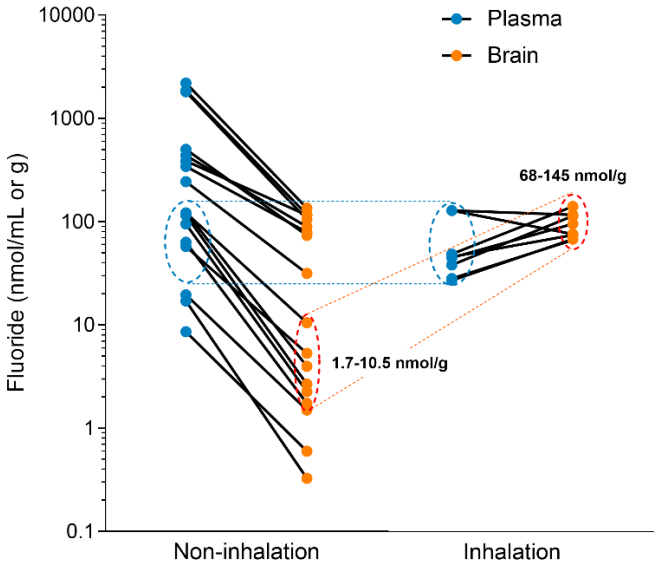


Figure 3. Fluoride concentration in the plasma and brain from studies via non-inhalation or inhalation route of exposure (Data points of plasma and brain fluoride concentrations are from studies listed in Table 1).

B. Brain lesions caused by inhalation vs. non-inhalation studies

Brain lesions characterized by vacuoles, malacia and gliosis resulted from repeated exposure to sulfuryl fluoride in short-term, subchronic and chronic inhalation studies. These lesions were largely confined to the caudate-putamen region of the basal ganglia (Table 2). One exception came from a two-year rat inhalation study where microscopic vacuolation was observed in the cerebral cortex and thalamus/hypothalamus region (Quast *et al.*, 1993b). However, the investigators did not consider those vacuoles to have resulted from sulfuryl fluoride exposure, mainly because they were observed only in females.

Table 2. Brain regional localization of lesions induced by inhaled sulfuryl fluoride

Study type	Exposure	Dose (ppm)	Brain lesion region	Reference
Short-term	CD-1 mouse, 6 hrs/d x 5 d/wk, 9 exposures	0, 30, **100**, **300**	Cerebrum* Medulla	Nitschke and Quast (2002)
	Rabbit, 6 hrs/d x 5 d/wk x 2 wk	0, 100, **300**, **600**	Putamen Internal capsules and external capsules of globus pallidus	Eisenbrandt *et al.* (1985)
Subchronic	F344 rat, 6 hrs/d x 5 d/wk x 13 wks	0, 30, 100, **300**	Caudate putamen	Nitschke *et al.* (1987b)

Table 2. Brain regional localization of lesions induced by inhaled sulfuryl fluoride

Study type	Exposure	Dose (ppm)	Brain lesion region	Reference
	F344 rat, 6 hrs/d x 5 d/wk x 13 wks	0, 30, 100, **300**	Caudate putamen	Mattsson *et al.* (1986)
	CD-1 mouse, 6 hrs/d x 5 d/wk x 13 wks	0, 10, 30, **100**	Caudate putamen External capsule Thalamus/hypothalamus (external capsule)	Nitschke and Quast (1993)
	NZW rabbit, 6 hrs/d x 5 d/wk x 13 wks	0, 30, **100, 600/300**	Putamen Internal and external capsules of globus pallidus (malacia)	Nitschke *et al.* (1987a)
	Beagle dog, 6 hrs/d x 5 d/wk x 13 wks	0, 30, 100, **200**	Putamen (gliosis)	Nitschke and Quast (1992)
Chronic	F344 rat, 6 hrs/d x 5d/wk for 2 years	0, 5, 20, **80**	Cerebral cortex (females only) Thalmus/hypothalamus (females only)	Quast *et al.* (1993b)
	SD rat, 6 hrs/d x 5 d/wk x 10 wks during premating, 7 d/wk during mating, gestation and lactation	0, 5, 20, **150**	Caudate putamen myelinated fiber tracts	Breslin *et al.* (1992)
	CD-1 mouse, 6 hrs/d x 5 d/wk for 18 months	0, 5, 20, **80**	Caudate putamen External capsule	Quast *et al.* (1993c)
	Beagle dog, 6 hrs/d x 5 d/wk x 9 months	0, 20, 80, **200**	Head of caudate nucleus (malacia)	Quast *et al.* (1993a)

*Specific region was not indicated in the study, caudate-putamen region was assumed given that this region was identified in all other species (Quast *et al.*, 1993b); Values in bold are concentrations at which brain lesions occurred.

As noted, preferential accumulation of fluoride in the basal ganglia was not observed after exposure through the systemic circulation. For example, oral administration of sodium fluoride in two chronic studies showed no significant difference in fluoride concentration between cerebellum, medulla oblongata, hypothalamus, striatum, midbrain, hippocampus, and cortex (Mullenix *et al.*, 1995; Whitford *et al.*, 2009). Studies showing fluoride-mediated neurotoxicity after oral exposure (feed or drinking water) did not report brain lesions in the basal ganglia (Table 3). Although most of these studies did not specifically evaluate the basal ganglia, investigators of five studies (Ge *et al.*, 2005; Guan *et al.*, 1986; Jiang *et al.*, 2014b; McPherson *et al.*, 2018; Varner *et al.*, 1998) should have detected lesions in this region based on the brain sections/areas evaluated. Finally, necrosis of the basal ganglia was found in a 9-year old boy who developed choreoathetosis[2] after staying overnight at his sulfuryl fluoride fumigated home (~ 14 hours of acute exposure) (Mulay *et al.*, 2016).

[2] The occurrence of involuntary movements in a combination of chorea (irregular migrating contractions) and athetosis (twisting and writhing).

Table 3. Neuropathological findings induced by fluoride via oral route of exposure (open literature studies)

Species (strain)	Exposure	Route	Neuropathology findings	Brain regions examined	Study
Rat (Wistar)	Neonates/PND28 (NS): 50 ppm F of parental exposure for 62 d prior mating	d.w.	Cerebral cortex: increased neuronal density and %undifferentiated neural progenitor cells; loose cytoplasm; mitochondria & endoplasmic reticulum swelling; dissolved organelles	Mid-sagittal plane (Neonates) *Mid-coronal section (PND28)	Guan *et al.* (1986)
Rat (Long-Evans)	Adults (Male): 2.1 ppm NaF for 52 wk	d.w.	Neocortex/hippocampus: chromatin clumping; enhanced protein staining, pyknosis, vacuolation, and the presence of ghost-like cells; decreased neuronal density Neocortex in right hemisphere: increased immunoreactivity of IgM (indicative of a compromise in BBB) Thalamus: increased immunoreactivity for β-amyloid	*Mid-coronal section (2 mm to 8 mm from bregma)	Varner *et al.* (1998)
Rat (Wistar albino)	PND70 (NS): 30, 100 ppm F (NaF) of parental exposure from last week of pregnancy to the end of lactation, and weanlings exposure for 10 wk	d.w.	All regions examined: decreased in size and number of neurons Cerebellum: decreased Purkinje cells; increased granular cells Motor cortex: chromatolysis and gliosis	Coronal sections of hippocampus, amygdala, motor cortex, and cerebellum (hippocampus has most pronounced changes)	Shivarajashankara *et al.* (2002)
Mouse (Swiss albino)	Adults (Female): 30, 60, 120 ppm F (NaF) for 30 d	d.w.	Hippocampus: decreased in size and number of neurons; cytoplasmic eosinophilia; dark cells; condensed nucleus; cells with low cytoplasma and vacuolization and separation of cells	Hippocampus	Bhatnagar *et al.* (2002)
Rat (albino Wistar)	PND20 (male/female): 45 mg F/L (NaF) of parental exposure for 3 m prior mating to pups at PND20	d.w.	Cortex: neurons shrunken, pyknotic, and darkly stained with small nuclei; decreased cell number; elongated or absent of dendrites; spheroid bodies in neuroplasm	*Right hemispheres of the brains	Ge *et al.* (2005)
Rat (albino Wistar)	F1, F2, F3 PND30: 100, 200 ppm F (NaF) from pregnancy till 1 m old for F1, and continued treatment for F2 and F3	d.w.	Cerebral cortex: necrosis (hyperchromasia and disintegrated cytoplasm); vacuoles; eosinophilia (red neurons) Cerebellum: decreased granular cells; eosinophilic Purkinje cells; gap/space between Purkinje cells and granular layer Hippocampus: degenerating neurons	Cerebral cortex, cerebellum, and hippocampus	Basha *et al.* (2011)
Rat (Wistar)	Adults (Male): 20 ppm NaF for 60 d	Gavage	Cerebellum: dumbbell shaped mitochondrial; crenulated nuclear membrane Neocortex: myelin splitting; vacuolated mitochondria Hippocampus: degenerated cell bodies; granulated	Cerebellum, neocortex, and hippocampus	Reddy *et al.* (2011)

Table 3. Neuropathological findings induced by fluoride via oral route of exposure (open literature studies)

Species (strain)	Exposure	Route	Neuropathology findings	Brain regions examined	Study
			mitochondria; vacuolation in cytosol; compressed Golgi cisternae; broken myelinated fibers		
Rat (albino)	Adults (NS): 10, 100, 500 ppm F (NaF) for 3 m	d.w.	Cerebrum: decreased neuronal density; neuronal swelling; chromatolysis and vacuolation; pyknotic changes; gliosis	Cerebrum	Hamid *et al.* (2012)
Rat (Sprague -Dawley)	PND60 (Male/Female): 25, 50, 100 mg/L NaF of parental exposure for 10 d prior mating till pups reach 2 m old	d.w.	Ventricles: higher T2 signal in MRI scan Hippocampal CA1 region: acute degeneration in neurons; edema; partial demyelination	*All brain regions (MRI scan; females only) Hippocampal CA1 region (TEM)	Jiang *et al.* (2014a)
Rat (Wistar)	Adults (Male): 2.1, 10 ppm NaF for 30 d	d.w.	Prefrontal cortex: shrinkage of the nuclei; cytoplasmic vacuoles; nuclei fragmentation	Coronal sections at various levels through the prefrontal cortex (Bregma 3.72–2.52 mm)	Akinrinade *et al.* (2015)
Rat (Wistar)	Adults (Male/Female): 60, 120 ppm F (NaF) for 10 wk	d.w.	Cortex/hippocampus: aggregated intranuclear heterochromatin margination; dissolved cellular membrane dissolved; shrinkage of the nuclear and cell volume; dissolved organelle; Increased apoptotic cell, activated microglia and expression of inflammatory factors	Coronal sections of the brain (regions not specified); Hippocampus had higher number of activated microglia than cortex	Yan *et al.* (2016)
Rat (albino Wistar)	Adults (NS): 100, 200, 300 ppm NaF for 40 d	Gavage	Hippocampus: pyknosed nuclei; shrunken and darkly stained nuclei; decreased pyramidal cells; necrosis in neuropil; vacuolation in granular cells; chromatolysis in granule cells and pyramidal neurons	Hippocampus	Shashi and Kumar (2016a)
Rat (albino Wistar)	Adults (NS): 100, 200, 300 ppm NaF for 40 d	Gavage	Cerebrum: chromatolysis; shrunken with vacuolation, eccentric, irregular and spindle shaped nuclei; hyperchromatic and hypertrophic nucleus; aggregated granule cells	Cerebrum	Shashi and Kumar (2016b)
Rat (Long-Evans hooded)	PND70 (Male): 10, 20 ppm F (NaF) from GD6 to PND70	d.w.	Hippocampus: no evidence of neuronal death, glial activation, or astrocyte hypertrophy	*Plane of cut containing the hippocampus (lateral 1.35-1.95 mm)	McPherson *et al.* (2018)

Abbreviations: BBB, blood-brain barrier; d, day; d.w., drinking water; F, fluoride; GD, gestation day; m, month; MRI, magnetic resonance imaging; NS: not specified; PND, postnatal day; TEM, transmission electron microscopy; wk, week. Drinking water contained NaF was available ad libitum 7 d/wk for duration of the exposure period. *Caudate putamen (striatum) of the basal ganglia are part of the sections or brain regions examined by these investigators.

Discussion

Inhalation of sulfuryl fluoride generates neurotoxicity manifested as lesions in the basal ganglia in laboratory animals and in one documented human case. Previous evaluations of sulfuryl fluoride assumed that its neurotoxicity was due to fluoride. The proposed systemic MOA was analogous to that of orally administered fluoride, which is absorbed through the respiratory system into the blood, distributed to target tissues such as brain, teeth and kidney, with resultant toxic effects. However, this systemic MOA does not explain how fluoride ions cross the blood-brain barrier and reach the concentrations observed after the inhalation exposure, nor why the brain lesions resulting from inhalation exposure were specifically localized to the basal ganglia. This suggests that the neuropathological impact of sulfuryl fluoride is inhalation-route specific.

Our analysis revealed marked differences in plasma and brain fluoride concentrations between inhalation and non-inhalation studies. Brain-to-plasma fluoride ratios were approximately 20-fold higher after acute inhalation exposure of rats to sulfuryl fluoride than after systemic (i.e., oral, intravenous, or intraperitoneal) exposure to sodium fluoride. If the systemic route is the preferred pathway, inhalation exposure to sulfuryl fluoride should yield a T/P ratio similar to those achieved following oral, i.v., and i.p. routes of exposure to fluoride or fluoride-containing compounds under similar experimental exposure conditions. However, administration of sodium fluoride by stomach intubation, as well as by i.v. and i.p. administration, all resulted in low T/P ratios (Carlson *et al.*, 1960a; Geeraerts *et al.*, 1986; Knaus *et al.*, 1976; Whitford *et al.*, 1979a; Whitford *et al.*, 1979b). These findings suggest that fluoride is relatively impermeable to the blood-brain barrier via systemic circulation (Whitford, 1996).

Localization of brain lesions in the basal ganglia of several animal species suggests that these structures either serve as a sink or trap for fluoride or are particular vulnerable to fluoride. Whether basal ganglia are equipped with specific properties to trap fluoride is unknown. In comparison to inhalation studies where the basal ganglia is the clear target of sulfuryl fluoride, that is not the case for orally administered fluoride. Rather, fluoride is shown to distribute uniformly among various brain regions when administrated orally albeit at relatively low concentrations of ≤ 20 nmol/g (Mullenix *et al.*, 1995; Whitford *et al.*, 2009). In conclusion, basal ganglia lesions are not associated with fluoride toxicity via systemic circulation.

These findings together point to the existence of alternative, non-systemic pathways that permit direct access of fluoride to the central nervous system (CNS) from the point of contact in the nasal cavity. Several plausible direct nose-to-brain entry pathways are discussed below.

A. Evidence for Intranasal Transport Along the Olfactory or Trigeminal Nerves

The presence of direct nose-to-brain transport of biologically active molecules is well documented in animal models (Crowe *et al.*, 2018; Garcia-Garcia *et al.*, 2005). For example,

intranasally administered insulin reaches the CNS in humans without changing blood glucose concentrations (Pang *et al.*, 2016; Reger and Craft, 2006), suggesting that a direct nasal cavity-to-brain route exists. Similarly, intranasally administered albumin accumulates in the mouse CNS, while little reaches the systemic circulation (Falcone *et al.*, 2014). Moreover, 95% of oxytocin detected in the rat brain after nasal administration was directly transported from the nasal cavity (Tanaka *et al.*, 2018).

The movement of chemicals from the nasal cavity to the brain can occur along the olfactory or trigeminal nerves, both of which innervate the nasal epithelium. The olfactory nerve arises in the olfactory region of the nasal cavity, while the trigeminal nerve innervates the respiratory and olfactory epithelium of the nasal passages.

A recent review supports the possibility of a direct transport by both intracellular and extracellular pathways through olfactory region (Crowe *et al.*, 2018). The intracellular pathway starts with endocytosis by olfactory sensory cells, followed by axonal transport to the olfactory bulb, and subsequent distribution to other brain regions. The extracellular pathway starts with crossing the nasal epithelium through the paracellular space, proceeding through the perineural space, and into the cerebral spinal fluid (CSF) in the subarachnoid space. Supporting data come from studies of inhalation exposure to ultrafine manganese oxide particles in rats with right naris occlusion. These rats showed accumulation of manganese in the left olfactory bulb, confirming a direct translocation route from nasal cavity to CNS along the olfactory nerve (Elder *et al.*, 2006). Likewise, administration of fluorescein isothiocyanate-labelled dextran to rats either through the intranasal or i.v. route showed CSF accumulation occurring only through the intranasal route (Sakane *et al.*, 1995). Similarly, fluoride could enter the brain directly via these intra- or extra-cellular pathways through the olfactory region, leading to high fluoride concentrations in the brain.

The trigeminal nerve, which innervates the nasal cavity, also presents a possible entry path to the brain after intranasal administration. Multiple lines of evidence demonstrate that olfactory nerve pathways are the major anatomical components of intranasal delivery to brain tissue. For example, 1) fluorescent tracers associate predominantly with olfactory nerves as they traverse the cribriform plate[3]; 2) drug concentrations in the olfactory bulbs, which are innervated by the olfactory nerve, are generally higher than in other brain regions following intranasal administration; and 3) a strong positive correlation exists between drug concentrations in the olfactory epithelium and in the olfactory bulbs (Dhuria *et al.*, 2010). Further evidence for a predominantly olfactory nerve route comes from the observation that fluoride was detected in brain tissue within 2 hours of exposure to sulfuryl fluoride. It has been shown that less time is required for substances to reach CNS by way of the olfactory nerve (1-2 hours) than by way of

[3] The cribriform plate is a sieve-like structure between the anterior cranial fossa and the nasal cavity. It is part of ethmoid bone and supports the olfactory bulb.

the trigeminal nerve (>17 hours), presumably due to the longer trigeminal traverse (Lochhead and Thorne, 2012). Nevertheless, the trigeminal nerves cannot be excluded as a minor pathway for brain vacuolation noted in the medulla (lower half of the brain stem) in one mouse study with short-term exposure (Table 2).

In humans, there is both epidemiologic and pathologic evidence that the olfactory region is a major target for many air pollutants (Ajmani *et al.*, 2016). In addition, a cross-sectional study of 123 structural fumigation workers in Florida showed that workers with high sulfuryl fluoride exposure had reduced performance on a pattern memory test and reduced olfactory function (Calvert *et al.*, 1998), suggesting olfactory epithelium could be a major target for sulfuryl fluoride after chronic exposure.

Fluoride entry into the brain via the olfactory/trigeminal nerves may also explain why the basal ganglia are a major target for sulfuryl fluoride. Scranton *et al.* (2011) showed that substances reaching the mouse olfactory bulb can be distributed to the subventricular zone by the rostral migratory stream, a migratory channel for neuroprogenitor cells. The same study reported > 80% reduction in intranasally delivered erythropoietin and calcitonin to the CNS when the rostral migratory stream was surgically resected. Fluoride in the olfactory bulb could be transported to the subventricular zone via the rostral migratory stream. However, this route may not apply to humans, which lack an anatomically similar rostral migratory stream (Maresh *et al.*, 2008).

Additionally, fluoride might reach the lateral ventricles, and thus the basal ganglia, through the CSF. Anatomically, the basal ganglia, in particular the caudate nucleus and putamen, abut the subventricular zone and lateral ventricular system. Accumulation of fluoride in the lateral ventricles through CSF may result in histopathologic damage and vacuole formation in the basal ganglia leading to formation of vacuoles. Alternatively, fluoride may reach the basal ganglia through perivascular convection. A recent study with fluorescent dyes showed directional convection of blood flow within the perivascular space driven by cerebral arterial pulsations from both olfactory and trigeminal nerve-associated brain entry sites (Lochhead *et al.*, 2015).

As noted earlier, necrosis of basal ganglia was found in a 9-year old boy who was exposed to sulfuryl fluoride following structural fumigation of his home (Mulay *et al.*, 2016). That the basal ganglia are anatomical targets for inhaled sulfuryl fluoride is supported by the studies in laboratory animals described in this assessment. Furthermore, work with other compounds suggests a particular susceptibility in this region following intranasal exposure. For example, individuals with occupational exposure to manganese are known to accumulate relatively higher manganese concentrations in this region than in other brain regions (Dobson *et al.*, 2004). Similarly, rats exposed to whole-body inhalation to welding fumes developed astrogliosis in the striatum and globus pallidus (both part of the basal ganglia), where proinflammatory mediators are also localized (Antonini *et al.*, 2009). Intranasal administration of interferon-β1b to

cynomolgus monkeys resulted in greater accumulation in the basal ganglia than in other CNS regions except for the olfactory bulb (Thorne *et al.*, 2008). The striatum is also the main target site for intranasally administered microglia and mesenchymal stem cells (Danielyan *et al.*, 2014). Finally, basal ganglia lesions were reported in human case studies with another fumigant, methyl bromide (Ichikawa *et al.*, 2001).

B. Intranasal Transport to the Brain via the Local Vascular Pathway

The current registrant of Vikane® has suggested an alternative direct fluoride access the brain occurring through a local vascular pathway (DeSesso *et al.*, 2019). In this conception, fluoride from inhaled sulfuryl fluoride is first absorbed into the large venous plexus within the mucosa of the respiratory portion of the nasal passages. From there it is transferred in the venous blood to the cavernous sinus, where it diffuses into the internal carotid artery through a countercurrent exchange. DeSesso and colleagues postulated that fluoride transfer terminates in the basal ganglia because blood from the internal carotid artery flows mainly into the middle and anterior cerebral arteries, both of which branch to supply the basal ganglia. We note that this model did not clarify how fluoride in the internal carotid artery crosses the blood-brain barrier, nor did it estimate the volume of fluoride-rich venous blood draining into the cavernous sinus, or the rate of counter-current exchange at the cavernous sinus-internal carotid complex. Finally, the applicability of this pathway to humans is presently unclear.

Alternatively, it is possible that all of the pathways described above – olfactory/trigeminal, local vascular, and the systemic circulatory – comprise brain entry routes and thus collectively contribute to the lesions associated with sulfuryl fluoride exposure.

Data Gaps

The preceding discussion suggests alternative pathways for delivery of fluoride from the nasal cavity to brain, although confirmative data for any particular pathway are currently lacking. Direct intranasal access could be verified with a study in which the test animals are exposed by blocking one naris, similar to the experiments done with manganese oxide. Further, studies could characterize the disposition of fluoride in various locations – including the nasal cavity, CSF, basal ganglia and other brain regions – during and after inhalation exposure. Homogenous fluoride distribution in all brain regions would suggest that access is not what determines the unique sensitivity of the basal ganglia. Finally, neurobehavioral tests should be performed at peak brain fluoride concentrations to ensure capture of maximal neurological impacts.

There are additional uncertainties and questions, as follows.

1) Do the high fluoride levels in the brain result from entry of fluoride or sulfuryl fluoride gas or sulfonated adducts?

2) Could the high T/P ratio observed after sulfuryl fluoride inhalation be an artifact of different plasma fluoride elimination rates by routes?

3) The chronic oral sodium fluoride studies did not produce lesions in the basal ganglia. Was this due to a true absence of site specificity following oral exposure or were lesions missed upon pathological examination?

4) Was the blood-brain barrier compromised with repeated exposure to sulfuryl fluoride in short-term, subchronic, or chronic settings?

Conclusions

This analysis investigates the likely means by which sulfuryl fluoride exerts its toxicological action in the mammalian central nervous system. A comparative analysis of fluoride disposition following exposure to inhaled sulfuryl fluoride versus oral or i.v./i.p. injected sodium fluoride showed markedly divergent brain-to-plasma concentration ratios by the inhalation and non-inhalation routes. Preferential accumulation of fluoride in the brain along with lesions confined to the basal ganglia suggests direct nose-to-brain access by one or more plausible pathways. A systematic literature review revealed that certain heavy metals, aerosolized particles, some pharmaceuticals, and even stem cells directly access the brain from the nasal cavity. Although there are data gaps preventing a complete mechanistic understanding of sulfuryl fluoride neurotoxicity, additional data may allow for the development of a refined dosimetric model for sulfuryl fluoride that could be used in future risk assessments and in the establishment of regulatory air concentration limits.

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APPENDIX F.

UNCERTAINTIES RELATED TO THE PBPK MODEL

APPENDIX F.

Uncertainties Related to the PBPK Model

The bulk of the studies on sulfuryl fluoride submitted to DPR since publication of its 2006 RCD are attempts to establish the pharmacokinetic parameters necessary for PBPK model construction. The sulfuryl fluoride physiologically-based pharmacokinetic (PBPK) model is used to resolve the interspecies pharmacokinetic difference between animals and humans by calculating human equivalent concentrations based on internal target tissue concentration of the putative toxic species (fluoride). Every PBPK model has strengths and limitations reflecting the current knowledge on metabolic processes, physiologic parameters, and mode of action. Specific uncertainties associated with the sulfuryl fluoride PBPK model include assumptions on the toxic species and mode of action, as well as calibration and validation.

This Appendix builds on explanation of uncertainties related to the model explained in Section V.C. in the main Addendum.

Model assumptions

The sulfuryl fluoride PBPK model was constructed based on many assumptions. Uncertainties related to some of the major assumptions are summarized below.

- The model assumed a systemic mode of action (MOA) regardless of the route of exposure (e.g., oral or inhalation). Specifically for inhalation exposure, the model is analogous to that of orally administered fluoride, which is absorbed through the respiratory system into the blood and distributed to target tissues such as brain, teeth, and kidney, with resulting toxic effects. In addition, the model assumes that sulfuryl fluoride is rapidly hydrolyzed to form fluorosulfate and fluoride at the portal of entry in the respiratory system with the hydrolysis products distributed to all other tissues including brain via systemic circulation. However, the available database suggests that after inhalation exposure, sulfuryl fluoride-induced neurotoxicity may not be mediated through either a classical systemic or a portal of entry mode of action. Rather, the data suggest potential alternative direct entry pathways through the nasal cavity (see Appendix E).

- The model assumed that fluoride is the toxic species that enters CNS and exerts neurotoxicity. The current database does not rule out the possibility that toxic effects may result from other metabolites, such as fluorosulfate or sulfonated adducts (see Appendix G). Sulfuryl fluoride reacts with endogenous nucleophiles such as ammonium and amino moieties on proteins to form sulfonated adducts. However, sulfonated adduct formation in brain tissues was not measured either in young or adult animals. Both fluorosulfate and sulfonated adducts may function as carbonic anhydrase inhibitors, which can affect

neuronal functions through their influence on pH regulation in the brain (Ruusuvuori and Kaila, 2014; Sapirstein *et al.*, 1984). Thus, fluorosulfate and sulfonated adducts may also contribute to the neurotoxicity observed with sulfuryl fluoride exposure.

- The model assumed that the fractional absorption for rabbits to be 45%, i.e., 3x that in rats, in order to accommodate the empirically established differences in plasma fluoride levels. The rationale for this assumption was based on differences in airway geometry and airflow between rats and rabbits (Corley *et al.*, 2009; Minard *et al.*, 2006). However, earlier studies using oral administration of sodium fluoride between rats and rabbits also showed much higher plasma fluoride level in rabbits than rats (Monsour *et al.*, 1985), suggesting that urinary clearance rather than absorption is the key factor. Plasma fluoride clearance is comprised of renal and extrarenal clearance. An early study showed that renal clearance rate in rats (3.61 mL/min/kg) was approximately 3-fold that in rabbits (1.14 mL/min/kg), while there was no significant difference in extrarenal clearance (Whitford *et al.*, 1991). In addition, in the same study that showed plasma fluoride concentration in rabbits to be 3-fold greater than that in rats, there were no differences in plasma fluorosulfate and brain fluoride concentrations (Rick *et al.*, 2011). A similar magnitude of difference in plasma fluorosulfate would be expected if higher absorption occurred in rabbits. The similar level of brain fluoride between these two species despite 3x difference in plasma fluoride either indicates that fluoride clearance in rat brain is slower than in plasma or brain fluoride was not derived from the systemic circulation. Fluoride handling in rat plasma and brain was not significantly different, exhibiting roughly the same half-lives in both tissues (Hotchkiss *et al.*, 2011a). Thus similar brain fluoride concentrations in rats and rabbits further support direct intranasal access to brain. Together, these data indicate that high plasma fluoride in rabbits is mainly due to slower elimination, not higher absorption.

- The model assumed that the fractional absorption for humans to be 15%. The measured fractional inhalation absorption in rats was 12.5-14% (Mendrala *et al.*, 2002). Due to a 3-fold difference in plasma fluoride level detected between rats and rabbits for the same exposure (Rick *et al.*, 2011), a fractional inhalation absorption of 45% was assumed for the rabbit. The investigators attributed this to differences in airway geometry (surface area) and airflow (minute ventilation) between rats and rabbits (Corley et al., 2009; Minard et al., 2006), and use the same logic to assume a conservative estimation of 15% for humans (Poet and Hinderliter, 2011). As stated above, the difference in plasma fluoride between rats and rabbits was mainly due to slower elimination, not higher absorption in rabbits. Thus, use this invalid logic to estimate fractional absorption in humans could be misleading.

Model calibration

The PBPK model was primarily based on pharmacokinetic studies in rats. However, these studies were inconsistent in exposure method (nose-only vs. whole-body) and fluoride measurement technique (direct vs. indirect methods). This resulted in data inconsistencies among studies using similar exposure routes and durations (see Tables 7-8 in Section II.E.5 in the main Addendum). Other issues related to model calibration are summarized below.

- The partition coefficient (PC) describes the relative solubility of a chemical in different tissues. It is based on the plasma concentration in the blood immediately leaving the tissue. This value is a critical parameter in PBPK model calibration. The PC for fluoride was obtained in one of three ways: from the literature, calculated or optimized. All PC values for sulfuryl fluoride and fluorosulfate were calculated using QSAR. There are inherent uncertainties associated with the QSAR prediction method. By the QSAR approach, the average ratio of predicted-to-experimental tissue:plasma PCs was 1.26, with 85% of the 269 predicted values within a factor of three of the corresponding literature values obtained under experimental conditions (Poulin and Theil, 2000). This uncertainty was not addressed in the model, as no sensitivity analysis was done for these PC values.

- The partition coefficient for fluorosulfate in brain:blood (0.35) may be inappropriately high since all studies showed non-detectable or low levels of fluorosulfate in the brain. This was evidenced by the overprediction of fluorosulfate in the brain by the model.

- The partition coefficient for fluoride in the brain:blood (0.71) appears to be low since brain-to-plasma ratios were generally larger than 1 in most samples (see Table 1 in Appendix E).

- Many parameters in the model were optimized to fit the experimental data. These included bone kinetic and metabolic rate constants for sulfuryl fluoride and fluorosulfate, urinary elimination rates for fluorosulfate, and fetal transfer rates for fluorosulfate. No reasoning was provided with respect to how realistic these optimized values are.

- A universal ventilation allometric scaling method was used in cases where species-specific ventilation equations were available. Species-specific equations are likely to provide a better fit than general equations applied across species (U.S. EPA, 1988).

- It is unclear how urinary elimination values were derived based on the study by Whitford (1996). Consequently, it is difficult to assess how well these clearance rates represent the various species regarding renal clearance of fluoride.

Model validation

The rat PBPK model was validated with pharmacokinetic datasets from neonatal (Marty *et al.*, 2011a), weanling (Marty *et al.*, 2011b), and repeated dosing studies (Hotchkiss *et al.*, 2011b). The rabbit PBPK model was validated with the parallel pharmacokinetic comparison study between rats and rabbits (Rick *et al.*, 2011). Although an oral route was built into the model based on a previous published oral fluoride PBPK model (Rao *et al.*, 1995), only inhalation data were used to validate the rat model. Oral rat data were not used for validation. As such, pharmacokinetic comparisons between oral and inhalation routes could not be made in the rat model.

The human model was the same as the rat model, but used human-specific parameters. Because pharmacokinetic data on human exposure to sulfuryl fluoride were not available, the model validation was against data from oral exposure of humans to fluoride, e.g., plasma fluoride data from human oral sodium fluoride studies (Buzalaf *et al.*, 2008; Maguire *et al.*, 2005). In so doing, the model becomes analogous to that of orally administered fluoride, as mentioned in Model Assumptions above. We hold that the best practice is to validate the inhalation model with inhalation exposure data. Substantial uncertainty would be introduced by using the invalidated inhalation route for predicting outcomes from inhalation exposures in humans.

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APPENDIX G.

UNCERTAINTIES ASSOCIATED IN THE TOXIC METABOLITES OF SULFURYL FLUORIDE

APPENDIX G.

Uncertainties Associated in the Toxic Metabolites of Sulfuryl Fluoride

Sulfuryl fluoride exerts its insecticidal action by disrupting glycolysis and the citric acid cycle, thus depriving the organism of the energy necessary for survival (Meikle *et al.*, 1963). However, the modes of action that underlie sulfuryl fluoride's manifold toxic effects remain unclear. The available kinetic data indicate that inhaled sulfuryl fluoride is rapidly hydrolyzed at the site of absorption producing fluorosulfate, fluoride, and sulfate in circulating blood. Because sulfuryl fluoride induced neurotoxicity, nephrotoxicity, and dental fluorosis conform to fluoride-induced toxicities, fluoride was assumed to be the putative toxic species. Examination of potential toxic effect of the other major hydrolysis product fluorosulfate or parent compound sulfuryl fluoride is lacking. In this appendix, DPR evaluates the potential toxic effects of fluoride, fluorosulfate, and sulfuryl fluoride, and proposes possible pathways for sulfuryl fluoride degradation *in vivo*.

Toxic Effects of Fluoride

All registrant-submitted studies attribute the toxicities observed in sulfuryl fluoride exposed laboratory animals to fluoride. The evidence used to support a fluoride-based mode of action is discussed below.

- **Absence of sulfuryl fluoride in the tissues:** In inhalation studies, sulfuryl fluoride was not detected in rat or rabbit blood, nor in rat nasal lavage fluid or bronchoalveolar lavage fluid (Hotchkiss *et al.*, 2008; Hotchkiss *et al.*, 2011a; Marty *et al.*, 2011a). Although sulfuryl fluoride was not analyzed in any other tissues, it was presumed to be absent due to its absence from blood. Thus, the investigators did not consider parental sulfuryl fluoride to be the ultimate toxic species for the observed toxic effects.

- **Absence of fluorosulfate in the brain:** Although the brain is the main target for sulfuryl fluoride, fluorosulfate has rarely been detected in brain tissue. Thus like sulfuryl fluoride, the investigators did not consider fluorosulfate to be the likely toxic species, particularly with respect to neurotoxicity (Hotchkiss *et al.*, 2011b). DPR's analysis of registrant-submitted studies confirmed the general absence of fluorosulfate in brain tissue from adult rats (Table 1), rat fetuses and pups (Table 2), and adult rabbits (Table 3). In the few brain tissue samples where fluorosulfate was detected, the brain-to-plasma ratio (T/P) was low (< 0.06), suggesting that either fluorosulfate did not readily penetrate blood-brain barrier or that it was rapidly cleared from brain via efflux transporters. The impermeability of brain to fluorosulfate has also been shown in mice following tail-vein injections of radiolabeled ^{18}F-fluorosulfate (Khoshnevisan *et al.*, 2017).

- **Neurotoxicity:** The investigators claimed that neurotoxic responses in rats exposed to sulfuryl fluoride (Nitschke *et al.*, 1986) were similar to those previously described for oral exposure to fluoride. However, this is an acute lethal study involving high doses of sulfuryl fluoride (4000-40,000 ppm). Besides death, convulsion was the only observed toxic effect, which was considered as similar to acute fluoride poisoning. The predominant neurotoxic response in animals exposed to sulfuryl fluoride was brain vacuolation. These brain lesions were confined to the basal ganglia region and found in all tested species (rat, rabbit, mouse, dog) as well as in humans (see Appendix E for details). DPR is not aware of any oral acute or chronic studies with fluoride in the literature reporting similar brain lesions at the basal ganglia region.

- **Clastogenicity:** Sulfuryl fluoride affected chromosomes (forming small colonies) in a mouse lymphoma forward mutation assay (Gollapudi *et al.*, 2002) and was associated with chromosomal aberrations *in vitro* (Gollapudi *et al.*, 2005). The investigators considered these findings similar to fluoride's clastogenicity reported in the literature (NRC, 2006). DPR cautions the use of *in vitro* findings to deduce the *in vivo* mechanisms, as sulfuryl fluoride may behave differently between *in vitro* and *in vivo* settings.

- **Nephrotoxicity:** Sulfuryl fluoride inhalation exposure caused various kidney effects, including hyperplasia of the collecting ducts, basophilic epithelial cells in the proximal tubules, increased relative kidney weights, renal papillary necrosis, and degeneration / regeneration of collecting ducts and proximal tubules (Eisenbrandt *et al.*, 1985). The investigators considered these findings similar to fluoride nephrotoxicity reported in oral dosing studies (NRC, 2006). DPR agrees that fluoride could be the underlying cause for sulfuryl fluoride induced nephrotoxicity.

- **Dental fluorosis:** In sulfuryl fluoride inhalation studies, dental fluorosis was reported in two 13-week rat studies (Mattsson *et al.*, 1986; Nitschke *et al.*, 1987), a 1-year dog study (Quast *et al.*, 1993c), and a 2-year rat study (Quast *et al.*, 1993a). The investigators considered dental fluorosis to be a typical effect associated with chronic fluoride exposure (NRC, 2006). DPR agrees that dental fluorosis resulting from sulfuryl fluoride inhalation exposure is most likely mediated via fluoride.

Based on the above analysis, DPR concludes that sulfuryl fluoride-induced nephrotoxicity and dental fluorosis could be mediated via fluoride, but whether fluoride is also responsible for sulfuryl fluoride-induced neurotoxicity is unknown. In 2006, DPR's analysis revealed no correlation between serum fluoride and brain vacuole in rats and rabbits (DPR, 2006). Although this could be confounded by variations in fluoride intake from drinking water and feed or from individual variation in response, it could also imply that fluoride was not the toxic metabolite.

Data on brain fluoride levels in affected regions maybe a better indicator for brain vacuolation. However, the available studies only measured fluoride in the total brain not in region-specific samples.

The toxicology of fluoride has been reviewed extensively by various organizations such as the World Health Organization (WHO, 2006), the National Research Council (NRC, 2006), the European Commission (SCHER, 2011), and the National Toxicology Program (NTP, 2016). Fluoride has diverse actions on a variety of cellular and physiological functions that mainly result from its inhibitory effects on a number of enzymes. For example, it inhibits enzymes critical to glycolysis and oxidative phosphorylation, processes responsible for ATP synthesis (ATSDR, 2003). It also stimulates osteoblast proliferation through inhibition of phosphotyrosyl protein phosphatases (Lau and Baylink, 1998; Thomas *et al.*, 1996). Fluoride may exert its enzymatic inhibition by forming metal fluoride-phosphate complexes that interfere with the activity of enzymes requiring a metal ion cofactor. Fluoride may also cause hypocalcemia and hypomagnesaemia leading to tetany and disturbances of cardiac rhythm by binding irreversibly to calcium and magnesium resulting in precipitation (Birkner *et al.*, 2006; McIvor, 1990; Spittle, 1994). Fluoride intoxication can also lead to hyperkalemia due to the marked potassium efflux from intact cells (McIvor *et al.*, 1985; McIvor and Cummings, 1987).

Table 1. Fluorosulfate levels in plasma and brain of adult rats following inhalation exposure to sulfuryl fluoride – acute and short-term exposures

Strain	Age (wk)	Route	Exposure	Dose (ppm)	Plasma (nmol/mL)	Brain (nmol/g)	Study
Fischer 344	NA (Dam)	NO	Single, 4 hrs	0	--	--	Mendrala *et al.* (2002)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	0	< LLQ = 0.496	< LLQ = 4.83	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	6 hrs/d, 5 d/wk, 2 wks	0	< LLQ = 0.487	< LLQ = 4.84	Hotchkiss *et al.* (2011d)
F344/DuCrl	8	NO	Single, 4 hrs	3	2.87	< LLQ = 3.73	Hotchkiss *et al.* (2011b)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	3	1.25	< LLQ = 4.83	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	6 hrs/d, 5 d/wk, 2 wks	3	3.04	< LLQ = 4.84	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	3	0.928	< LLQ = 4.94	Rick *et al.* (2011)
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20	5	2.85	--	Marty *et al.* (2011a)
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20, LD5-10	5	2.28	--	Marty *et al.* (2011a)
Fischer 344	NA	NO	Single, 4 hrs	30	34.4*	--	Mendrala *et al.* (2002)
F344/DuCrl	12	NO	Single, 2-4 hrs	30	12.1	< LLQ = 5.045	Hotchkiss *et al.* (2011c)
F344/DuCrl	8	NO	Single, 4 hrs	30	39.1	< LLQ = 3.73	Hotchkiss *et al.* (2011b)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	30	14.1	< LLQ = 4.83	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	6 hrs/d, 5 d/wk, 2 wks	30	13.4	< LLQ = 4.84	Hotchkiss *et al.* (2011d)
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20	30	14.4	--	Marty *et al.* (2011a)
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20, LD5-10	30	17.9	--	Marty *et al.* (2011a)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	30	7.85	< LLQ = 4.94	Rick *et al.* (2011)

Table 1. Fluorosulfate levels in plasma and brain of adult rats following inhalation exposure to sulfuryl fluoride – acute and short-term exposures

Strain	Age (wk)	Route	Exposure	Dose (ppm)	Plasma (nmol/mL)	Brain (nmol/g)	Study
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20	150	63.6	--	Marty *et al.* (2011a)
Crl:CD(SD)	NA (Dam)	WB	6 hrs/d, GD6-20, LD5-10	150	38.4	--	Marty *et al.* (2011a)
Fischer 344	NA	NO	Single, 4 hrs	300	134.5[a]	--	Mendrala *et al.* (2002)
F344/DuCrl	12	NO	Single, 2-4 hrs	300	193.7	6.4[b]	Hotchkiss *et al.* (2011c)
F344/DuCrl	8 to 12	NO	Single, 4 hrs	300	169.33	< LLQ = 5.05	Hotchkiss *et al.* (2008)
F344/DuCrl	8	NO	Single, 4 hrs	300	129	< LLQ = 3.73	Hotchkiss *et al.* (2011b)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	300	143	< LLQ = 4.83	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	6 hrs/d, 5 d/wk, 2 wks	300	110	< LLQ = 4.84	Hotchkiss *et al.* (2011d)
F344/DuCrl	9 to 10	WB	Single, 6 hrs	300	103	< LLQ = 4.94	Rick *et al.* (2011)

All values were measured immediately after the completion of exposure unless specified otherwise; GD, gestation day; hpe, hours post exposure; LD, lactation day; LLQ, lower limit of quantitation; NA, not available; NO, nose only; PND, postnatal day; WB, whole body.
[a]Whole blood; [b]Brain tissue-to-plasma (T/P) ratio = 0.033.

Table 2. Fluorosulfate levels in plasma and brain of fetal and newborn rats following inhalation exposure to sulfuryl fluoride – acute and short-term exposures

Strain	Age	Route	Exposure	Dose (ppm)	Plasma (nmol/mL)	Brain (nmol/g)	Study
Crl:CD(SD)	PND22	WB	Single, 4 hrs	3	1.28	< LLQ = 2.52	Marty *et al.* (2011b)
Crl:CD(SD)	Fetus	WB	Dam, 6 hrs/d, GD6-20	5	< LLQ = 0.506	< LLQ = 1.06	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	WB	Dam, 6 hrs/d, GD6-20, LD5-10	5	< LLQ = 0.505	--	Marty *et al.* (2011a)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	5	1.47	< LLQ = 0.454	Marty *et al.* (2015)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	5	1.71	< LLQ = 0.426	Marty *et al.* (2015)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	20	4.75	< LLQ = 0.455	Marty *et al.* (2015)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	20	5.73	< LLQ = 0.437	Marty *et al.* (2015)
Crl:CD(SD)	Fetus	WB	Dam, 6 hrs/d, GD6-20	30	1.77	< LLQ = 1.06	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	WB	Dam, 6 hrs/d, GD6-20, LD5-10 (2 hpe)	30	0.694	--	Marty *et al.* (2011a)
Crl:CD(SD)	PND22	WB	Single, 4 hrs	30	9.28	< LLQ = 2.52	Marty *et al.* (2011b)
Crl:CD(SD)	Fetus	WB	Dam, 6 hrs/d, GD6-20	150	7.87	2.74[a]	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	WB	Dam, 6 hrs/d, GD6-20, LD5-10 (2 hpe)	150	2.73	--	Marty *et al.* (2011a)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	150	75.15	1.88[b]	Marty *et al.* (2015)
Crl:CD(SD)	PND11	WB	6 hrs/d, PND11-21	150	97.47	2.39[c]	Marty *et al.* (2015)
Crl:CD(SD)	PND22	WB	Single, 4 hrs	300	104	3.6[d]	Marty *et al.* (2011b)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (1 hpe)	4 ug F/4 ug FSO_3	1.191	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (3 hpe)	4 ug F/4 ug FSO_3	< LLQ = 1.01	< LLQ = 0.505	Marty *et al.* (2011a)

Table 2. Fluorosulfate levels in plasma and brain of fetal and newborn rats following inhalation exposure to sulfuryl fluoride – acute and short-term exposures

Strain	Age	Route	Exposure	Dose (ppm)	Plasma (nmol/mL)	Brain (nmol/g)	Study
Crl:CD(SD)	PND10	Oral	Single, milk gavage (6 hpe)	4 ug F/4 ug FSO_3	< LLQ = 1.01	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (1 hpe)	20 ug F/20 ug FSO_3	2.59	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (3 hpe)	20 ug F/20 ug FSO_3	3.3	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (6 hpe)	20 ug F/20 ug FSO_3	1.31	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (1 hpe)	40 ug F/40 ug FSO_3	9.61	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (3 hpe)	40 ug F/40 ug FSO_3	6.7	< LLQ = 0.505	Marty *et al.* (2011a)
Crl:CD(SD)	PND10	Oral	Single, milk gavage (6 hpe)	40 ug F/40 ug FSO_3	4.79	< LLQ = 0.505	Marty *et al.* (2011a)

All values were measured immediately after the completion of exposure unless specified otherwise; GD, gestation day; hpe, hours post exposure; LD, lactation day; LLQ, lower limit of quantitation; PND, postnatal day; WB, whole body.
Brain tissue-to-plasma (T/P) ratio: [a] 0.348; [b] 0.025; [c] 0.025; [d] 0.035.

Table 3. Fluorosulfate levels in plasma and brain of New Zealand White rabbits following inhalation exposure to sulfuryl fluoride – acute exposures

Age (mon)	Route	Exposure	Dose (ppm)	Plasma (nmol/mL)	Brain (nmol/g)	Study
20	WB	Single, 6 hrs	3	1.09	< LLQ = 5.02	Rick *et al.* (2011)
20	WB	Single, 6 hrs	30	10.1	< LLQ = 5.02	Rick *et al.* (2011)
20	WB	Single, 6 hrs	300	112	5.93[a]	Rick *et al.* (2011)
NA	NO	Single, 6 hrs	600	392	23[b]	Hotchkiss *et al.* (2011a)
NA	NO	Single, 6 hrs, 18 hpe	600	< LLQ = 5.01	< LLQ = 4.92	Hotchkiss *et al.* (2011a)

All values were measured immediately after the completion of exposure unless specified otherwise; hpe, hours post exposure; LLQ, lower limit of quantitation; NA, not available; NO, nose only; WB, whole body.
Brain tissue-to-plasma (T/P) ratio: [a] 0.053; [b] 0.059.

Toxic Effects of Fluorosulfate

The absence of fluorosulfate in the brain argues that fluorosulfate is not the toxic species in that organ. However, fluorosulfate has been shown recently to be a competitive inhibitor for the sodium-iodide symporter (NIS) in the thyroid, stomach, and salivary gland (Khoshnevisan *et al.*, 2017), while fluoride is not (Waltz *et al.*, 2010). Thyroid hypertrophy was reported in both the subchronic and chronic mouse inhalation studies (Nitschke and Quast, 1993; Quast *et al.*, 1993b). Although fluoride is known to affect thyroid function by disrupting hormonal action in both animals and humans (NRC, 2006), fluorosulfate may also contribute via NIS inhibition to the thyroid effects seen in mice exposed to sulfuryl fluoride.

One unexpected observation from the pharmacokinetic studies was the relatively high brain-to-plasma ratio for fluorosulfate in the fetuses (T/P = 0.348; Table 2) after in utero exposure to sulfuryl fluoride via inhalation-exposed dams. NIS is present in the placenta (Darrouzet *et al.*, 2014; Mitchell *et al.*, 2001), and thus fluorosulfate could be transported from dam plasma to the fetal plasma via placenta NIS, subsequently entering the fetal brain from the fetal circulation at a point when the fetal blood-brain barrier is still not fully developed. Carbonic anhydrases occur widely in the brain and can affect neuronal functions via influence on pH shifts (Ruusuvuori and Kaila, 2014; Sapirstein *et al.*, 1984). The fact that fluorosulfate, a carbonic anhydrase inhibitor (Innocenti *et al.*, 2009), is found in fetal brain raises the specter that it may be associated with neurological effects in fetuses.

Toxic Effects of Sulfuryl Fluoride

Sulfuryl fluoride reacts with endogenous nucleophiles such as ammonium and amino moieties on

proteins (Cady and Misra, 1974; Kigawa *et al.*, 2011; Meikle, 1964). *In vitro* studies indicate that sulfuryl fluoride is capable of forming tyrosine adducts with albumin (Wang *et al.*, 2007). Analysis of FSO_2-albumin adducts in rat bronchoalveolar lavage fluid and plasma showed that these adducts represented a minor degradation pathway of sulfuryl fluoride *in vivo* when compared to hydrolysis to fluorosulfate (Hotchkiss *et al.*, 2008). Still, this study did show the presence of FSO_2-albumin adducts in lung and plasma. However, analysis of these adducts was not attempted in any other tissues, nor was there any attempt to analyze other protein adducts formed by sulfuryl fluoride.

The parent compound sulfuryl fluoride was not detected in nasal lavage fluid, bronchoalveolar lavage fluid, or milk and blood samples collected immediately after termination of exposures, thus systemic distribution of sulfuryl fluoride to other tissues and organs was extremely unlikely. However, it remains possible that the lack of detection was due to methodologic flaws rather than true absence. For example, sulfuryl fluoride's high vapor pressure (15.2 atm at 20 °C; Appendix A) gives it a strong tendency to dissipate into air during sample collection or preparation. Methodologic details concerning how to prevent dissipation were not available[1]. Thus, it is uncertain whether the lack of sulfuryl fluoride was a true representation of its absence in these samples.

An earlier rat inhalation study using ^{35}S-labeled sulfuryl fluoride showed radiolabel in red blood cells, lungs, nasal turbinates, spleen, kidneys, brain, skin, carcass, liver, and fat at 7 days postexposure (Mendrala *et al.*, 2002). The investigators explained this as a result of ^{35}S entering the "sulfur pool" after sulfuryl fluoride degradation, yet no supportive evidence was provided. An oral gavage study in rats using the structurally similar chemical ^{35}S-methylsulfonylmethane showed no radiolabel in blood, liver, kidneys, spleen, brain, and skin at 5 days postexposure (Magnuson *et al.*, 2007), suggesting the "sulfur pool" theory could be invalid. Thus, ^{35}S radioactivity retained in these tissues could represent the parent compound sulfuryl fluoride, the hydrolysis products (fluorosulfate and sulfate), or the FSO_2-adducts. However, sulfuryl fluoride is too reactive *in vivo*, while both fluorosulfate and sulfate are charged at physiological pH. By virtue of their charge, the latter two compounds are hydrophilic, promoting their rapid urinary excretion. In particular, ^{35}S in the brain certainly cannot be attributed to fluorosulfate or sulfate because brain is impermeable to both compounds (Hosoya *et al.*, 2000; Khoshnevisan *et al.*, 2017; Lee *et al.*, 2005; Ohtsuki *et al.*, 2002). This was supported by the pharmacokinetic studies reviewed for this document, which also showed the impermeability of brain to fluorosulfate (Tables 1-3). Therefore, it is likely that ^{35}S detected in these tissues 7 days after sulfuryl fluoride exposure represents FSO_2-adducts. If so, these adducts, similar to other sulfonamide structures,

[1]There was one exception: a brief statement from one study indicating that samples were directly transferred to 20-ml headspace vials (uncapped) containing 5 ml of hexane, followed by immediate capping. In contrast, fortified controls were prepared differently, where matrix standard (whole blood) plus 5 ml of hexane were sealed in a 20-ml headspace vial and sulfuryl fluoride was fortified through the septum directly into the liquid layer via a gas-tight syringe (Hotchkiss *et al*., 2008).

may act as carbonic anhydrase inhibitors (Innocenti *et al.*, 2009). In addition, FSO_2-adducts may retain enough reactivity to undergo another reaction with a nucleophile; e.g., they could bind to amino groups in proteins, acting as crosslinking agents (Wang *et al.*, 2018). It is thus possible that sulfuryl fluoride exerts toxic effects through FSO_2-adduct formation or crosslinking.

Possible Pathways for Sulfuryl Fluoride Degradation *In Vivo*

Sulfuryl fluoride fate *in vivo* could involve multiple pathways (Figure 1). In the blood, sulfuryl fluoride either hydrolyzes to fluorosulfate (which is further degraded to sulfate) or it attaches as a fluorosulfuryl group ($-SO_2F$) to the amino group ($-NH_2$) in proteins (e.g., N-terminal amino acids) and free amino acids as FSO_2-adducts (FSO_2-A). Concomitantly, these reactions result in release of fluoride. The fluorosulfate pathway dominates the reaction, while adduct formation represents a minor pathway. Although FSO_2-albumin formation was confirmed *in vivo*, adduct formation to other proteins or amino acids were not investigated. In the brain, fluorosulfate was either absent or present at very low levels, while fluoride was present at high levels (relative to plasma fluoride level) following sulfuryl fluoride inhalation exposure. Given the possibility of direct intranasal route of absorption (Appendix E), we propose three possible pathways for brain access of sulfuryl fluoride or its hydrolytic products. In the first pathway, sulfuryl fluoride rapidly hydrolyzes to fluorosulfate and fluoride in the nasal cavity, after which fluoride enters the brain either through olfactory region (intra or extracellular pathways) or through circulating blood via the blood-brain barrier, which may be compromised after repeated exposures in a short-term, subchronic or chronic settings. In the second pathway, FSO_2-adducts formed in the nasal cavity or blood enter brain via specific transporters on the olfactory epithelium or blood-brain barrier. In the third pathway, parental sulfuryl fluoride enters the brain directly through the olfactory nerves, reacting primarily with free amino groups of amino acids and proteins, and nucleophilic sites in the R-groups for tyrosine, serine, and lysine (Wang *et al.*, 2018). Fluoride is released as the byproduct of these reactions and becomes the surrogate for detection as only fluorosulfate and fluoride were measured in the brain. Given the brain tissue is not as watery as the blood compartment, sulfuryl fluoride hydrolysis in the brain could represent a minor pathway where fluorosulfate is either excreted directly or is further hydrolyzed to sulfate and is excreted by efflux transporters, making it nondetectable in the brain. Its role as a carbonic anhydrase inhibitor and crosslinking agent could underlie some of the observed neurological effects.

Overall, DPR's analysis reveal that besides fluoride, other metabolites such as fluorosulfate and FSO_2-A may also contribute to the cassette of toxicities observed with sulfuryl fluoride exposure.

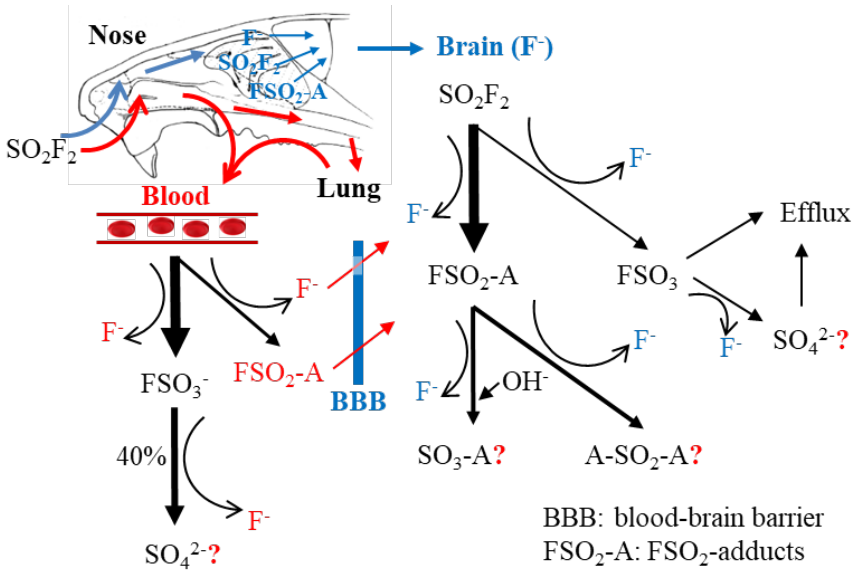

Figure 1. Possible pathways for sulfuryl fluoride degradation in vivo. In blood, sulfuryl fluoride (SO_2F_2) either hydrolyzes to fluorosulfate (FSO_3^-) and sulfate (SO_4^{2-}) (main pathway) or in a minor pathway forming FSO_2-albumin or other adducts (FSO_2-A). To access brain, either sulfuryl fluoride first rapidly hydrolyzes to fluorosulfate and fluoride (F^-) or forms FSO_2-A at the portal of entry and then fluoride/ FSO_2-A enters the brain directly via olfactory nerves or through the systemic circulation via specific transporters on the blood-brain barrier, or sulfuryl fluoride enters brain directly via olfactory nerves and forms FSO_2-A (main pathway) or in a minor pathway forming fluorosulfate. In brain, FSO_2-A may lose its remaining fluoro-group and form the corresponding sulfonate, SO_3-A, or it may react with another amino acid resulting in crosslinking, A-SO_2-A. Fluorosulfate will either be excreted directly or further hydrolyzes to sulfate and then excreted by efflux transporters.

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APPENDIX H.

POINTS OF DEPARTURE,

UNCERTAINTY FACTORS AND REFERENCE CONCENTRATIONS

ESTABLISHED BY US EPA, PMRA, AND EFSA

Appendix H.

Points of Departure, Uncertainty Factors, and Reference Concentrations Established by US EPA, PMRA, and EFSA

Exposure Duration	NOAEL and Endpoint	RfC		Reference
		Occupational	Residential/ Bystander	
Acute				
US EPA (1 day)	No toxicity endpoint for single exposure	--	--	U.S. EPA (2004)
PMRA	**2-Day Inhalation-Rat** (Albee *et al.*, 1993) NOAEL = 291 ppm (291 mg/kg bw) based on no evidence of adverse effects, including sensory evoked potentials which are sensitive indicators of neurotoxicity for sulfuryl fluoride	2.91 mg/kg/day $UF_A = 10$ $UF_H = 10$	2.91 mg/kg/day $UF_A = 10$ $UF_H = 10$	HC PMRA (2006); HC PMRA (2016)
EFSA	**2-Day Inhalation-Rat** (Albee *et al.*, 1993) NOAEL = 300 ppm based on no effect in FOB and electrophysiological tests	AOEC = 3 ppm $UF_A = 10$ $UF_H = 10$	AOEC = 3 ppm $UF_A = 10$ $UF_H = 10$	EFSA (2010)
Short-term				
US EPA (1-30 days)	**2-Week Inhalation-Rabbit** (Eisenbrandt *et al.*, 1985) NOAEL = 100 ppm (30 mg/kg/day) LOAEL = 300 ppm (90 mg/kg/day) based on malacia (necrosis) and vacuolation in brain, inflammation of nasal tissues and trachea	0.3 mg/kg/day $UF_A = 10$ $UF_H = 10$	0.03 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 10$	U.S. EPA (2004)
PMRA	**2-Week Inhalation-Rabbit** (Eisenbrandt *et al.*, 1985) NOAEL = 100 ppm (59 mg/kg/day) LOAEL = 300 ppm (178 mg/kg/day) based on elevated white blood cell counts, decreased liver weights (males only), cerebral vacuolation and malacia (necrosis), altered hepatocellular cytoplasmic homogeneity, inflammation of the nasal mucosa, and hyperplasia of the spleen	0.2 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 3$	0.2 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 3$	HC PMRA (2016)
Subchronic				
US EPA (1-6 months)	**90-Day Inhalation-Rabbit** (Nitschke *et al.*, 1987) NOAEL = 30 ppm (8.5 mg/kg/day) LOAEL = 100 ppm (28 mg/kg/day) based on vacuolation of white matter in the brain of females	0.085 mg/kg/day $UF_A = 10$ $UF_H = 10$	0.0085 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 10$	U.S. EPA (2004)

Exposure Duration	NOAEL and Endpoint	RfC		Reference
		Occupational	Residential/ Bystander	
PMRA (Intermediate -term)	**90-Day Inhalation-Rabbit** (Nitschke *et al.*, 1987) NOAEL = 30 ppm (18 mg/kg/day) LOAEL = 100 ppm (59 mg/kg/day) based on decreased body weight gain, elevated serum fluoride levels, decreased liver weight, and cerebral vacuolation	0.06 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 3$	NA (residential bystander exposure was considered as acute only in ProFume exposure scenario)	HC PMRA (2006); HC PMRA (2016)
EFSA	**90-Day Inhalation-Mouse** (Nitschke and Quast, 1993) NOAEL = 30 ppm LOAEL = 100 ppm	AOEC = 1 ppm $UF_A = 10$ $UF_H = 10$	--	EFSA (2010)
Chronic				
US EPA (6 months-lifetime)	**90-Day Inhalation-Rabbit** (Nitschke *et al.*, 1987) NOAEL = 30 ppm (8.5 mg/kg/day) LOAEL = 100 ppm (28 mg/kg/day) based on vacuolation of white matter in the brain of females	0.028 mg/kg/day $UF_A = 10$ $UF_H = 10$ UF = 3 (duration)	0.0028 mg/kg/day $UF_A = 10$ $UF_H = 10$ $UF_{DB} = 10$ UF = 3 (duration)	U.S. EPA (2004)
EFSA	**18-Month Inhalation-Mouse** (Quast *et al.*, 1993) NOAEL = 20 ppm LOAEL = 80 ppm	AOEC = 0.4 ppm $UF_A = 10$ $UF_H = 10$	--	EU (2009)

AOEC, acceptable operator exposure concentration; EFSA, European Food Safety Authority; FQPA SF, Special FQPA safety factor; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; PMRA, Health Canada's Pesticide Management Regulatory Agency; RfC, reference concentration; UF_A, interspecies uncertainty factor; UF_H, intraspecies uncertainty factor; UF_{DB}, uncertainty factor for incomplete database; US EPA, US Environmental Protection Agency.

Conversion of ppm to amortized daily dose (mg/kg/day)

US EPA: $ppm \times 4.17\, mg/m^3 \times respiration\, rate \times \frac{hours\ exposed}{24\ hours} \times \frac{days\ exposed}{7\ days}$

The default inhalation rates for rats and rabbits are 0.96 m^3/kg/day and 0.38 m^3/kg/day, respectively. The default rabbit respiration rate was based on an allometric equation of inhalation rate in m^3/day = 0.46 body weight $^{0.8307}$ and a body weight of 3 kg from a 1988 US EPA Document (U.S. EPA, 1988). This allometric equation had an error in the coefficient, a correction factor of 1.44 to convert V_E (L/min) to daily inhalation (m^3/day) was missing, the equation with correct coefficient should be m^3/day = <u>0.66</u> body weight $^{0.8307}$, which should result an inhalation rate of 0.55 m^3/kg/day given a default body weight of 3 kg for rabbit. US EPA currently uses mean inhalation rates of 0.55 m^3/kg bw/day and 0.52 m^3/kg bw/day, respectively, for male and female rabbits (U.S. EPA, 1994).

PMRA: $ppm \times 4.17\, mg/m^3 \times respiration\, rate \times \frac{hours\ exposed}{24\ hours}$

The default inhalation rates for rats and rabbits are 0.96 m^3/kg/day and <u>0.57</u> m^3/kg/day,

respectively. The inhaled dose calculation did not adjust for the 5 days/week factor for the 13-week rabbit study. No difference in the total UF for workers and bystanders were mentioned. The additional 3x UF_{DB} for lack of a developmental neurotoxicity was not applied to acute exposures, but applied to both workers and bystanders for both short-term and intermediate-term exposures.

EFSA: Instead of RfC, Acceptable Operator Exposure Concentration (AOEC) was provided in the peer reviewed EFSA document on sulfuryl fluoride. No details on AOEC calculation was provided.

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