Department of Pesticide Regulation

Brian R. Leahy
Director

Edmund G. Brown Jr.
Governor

M E M O R A N D U M

TO: Shelley DuTeaux, PhD MPH, Chief
Human Health Assessment Branch

FROM: Qiaoxiang Dong, PhD, Associate Toxicologist *[original signed by Q. Dong]*
Carolyn M. Lewis, MS DABT, Research Scientist III *[original signed by C. Lewis]*
Svetlana Koshlukova, PhD, Senior Toxicologist *[original signed by S. Koshlukova]*
Risk Assessment Section

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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 Protection 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 Protection 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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