Incapacitation and Treatment of Rats Exposed to a Lethal Dose of Sulfuryl Fluoride

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Incapacitation and Treatment of Rats Exposed to a Lethal Dose of Sulfuryl Fluoride. NITSCHKE, K. D., ALBEE, R. R., MATTSSON, J. L., AND MILLER, R. R. (1986). Fundam. Appl. Toxicol. 7, 664-670. Rats exposed to 4000 ppm sulfuryl fluoride (VIKANE gas fumigant, SO₂F₂) were incapacitated within 45 min and died within several hours after exposure. Exposure to higher concentrations resulted in a shorter time to incapacitation and death occurred within minutes. Treatment with calcium gluconate before exposure to 4000 ppm SO₂F₂ for 45 min resulted in 80% survival. However, calcium gluconate did not alleviate SO₂F₂-induced convulsions. Administration of phenobarbital before or after exposure to 4000 ppm SO₂F₂ for 45 min effectively reduced the frequency and severity of convulsions and resulted in survival of all animals. Exposure of rats to 10,000 ppm SO₂F₂ for 15 min followed by treatment with phenobarbital reduced the frequency of convulsions and delayed death, but did not prevent death. Diazepam was less effective than phenobarbital while diphenylhydantoin had no beneficial effect and, in fact, made the convulsions more severe and longer in duration. The results of this study indicate that phenobarbital was effective in ameliorating the acute toxic effects of an overexposure to SO₂F₂ in rats.

Sulfuryl fluoride (VIKANE gas fumigant, SO₂F₂) is used for control of drywood termites and other structural pests in southern California and the Gulf Coast area. The material is applied as a gas using a tarpaulin to seal the structure. Because SO₂F₂ is a colorless and odorless gas, chloropicrin (an irritant warning gas) is released in the structure prior to fumigation. During most fumigation procedures, a concentration of 4000 ppm SO₂F₂ is achieved (as per labeling instructions). However, for certain applications, a concentration of 40,000 ppm may be reached. Consequently, inhalation is the most likely potential route of human exposure. The current threshold limit value (TLV) is 5 ppm with a short-term exposure limit (STEL) of 10 ppm (ACGIH, 1985).

Over the years, there have been a few poorly documented cases in which human fatalities were reported to result from overexposure to SO₂F₂ (unpublished observations). Although the information is largely anecdotal, most victims seemed to have intentionally entered sealed buildings during fumigation and were later found dead or dying in or near the building. The “time to incapacitation” or “escape capability” of these victims was not known.

In rodents, the 4 hr LC₅₀ for SO₂F₂ was 991 and 1122 ppm for male and female Fischer 344 rats, respectively (R. R. Miller, personal communication). Convulsions, red staining around the eyes, nares, and mouth, and cyanosis were the most notable observations for animals overexposed to SO₂F₂. His-
topathologic examinations revealed edematous lungs and changes in the liver and kidneys of animals which died after exposure to SO$_2$F$_2$.

The metabolism, disposition, and the relationships, if any, of the metabolites to the mechanism of action of SO$_2$F$_2$ have not as yet been determined in mammals. However, studies by Meikle et al. (1963) indicated that extensive dehalogenation of the $^{35}$S-labeled SO$_2$F$_2$ occurs in termites. Hence, the fluoride ion may play a role in the mechanism of action of SO$_2$F$_2$ in insects. Furthermore, many of the observations in rodents overexposed to SO$_2$F$_2$ seem to be typical of acute fluoride poisoning (Drill, 1954; Goodman et al., 1980; Greenwood, 1940). Fluoride has been reported to decrease serum calcium and magnesium levels and also to decrease serum cholinesterase activity (Kahlson and Urnas, 1935; Drill, 1954).

The first objective of this study was to determine the “time to incapacitation” in rodents. A second objective of the study was to determine if calcium gluconate (CaG) alleviated the effects of exposure to a lethal dose of SO$_2$F$_2$. Calcium gluconate was initially examined since it is the therapeutic treatment for fluoride ion toxicity (Rabinovitch, 1954; Burke et al., 1973). Due to the results observed with calcium gluconate, additional studies focused on the use of one of several anticonvulsants.

**MATERIALS**

SO$_2$F$_2$ (lot WP030680-217) was obtained from the Agricultural Products Department, Dow Chemical U.S.A. Gas chromatographic analysis of the test material revealed the sample to be 99.8% sulfuryl fluoride.

Commercially available medical-grade preparations of calcium gluconate (10% w/v) (lot 16765), phenobarbital (65 mg/ml) (lot 053156), diazepam (5 mg/ml) (lot 1411-09193), and diphenylhydantoin (50 mg/ml) (lot 02534) were purchased from Maurry Biological Company, Los Angeles, California; Elkins-Sinn Inc., Cherry Hill, New Jersey; Hoffman-LaRoche Inc., Nutley, New Jersey, and Parke-Davis, Morris Plains, New Jersey, respectively.

**Animals.** Male Fischer 344 (F-344) rats (6–8 weeks of age) were purchased from Charles River Breeding Laboratories, Kingston, New York. Upon arrival at the laboratory, the health status of the rats was determined by a laboratory veterinarian. The rats were acclimated for at least 7 days prior to initial exposure to sulfuryl fluoride. The animals were randomly assigned by weight to an exposure group using a computer-generated program of random numbers. The animals were uniquely identified with metal ear tags.

Animals were fed Certified Purina Chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum except during exposures. When animals were not exposed to sulfuryl fluoride, they were placed in rooms designed to maintain temperature (22°C), relative humidity (50%), and light cycle (12 hr light and 12 hr dark).

**Induced incapacitation.** The exposure chamber consisted of a 14-liter cylindrical Plexiglas chamber containing a motorized, shaft-driven activity wheel (Fig. 1). The activity wheel was 12 cm wide and 32 cm in diameter and was constructed of 1 cm stainless-steel mesh. Concentrations of 4000, 10,000, 20,000, or 40,000 ppm SO$_2$F$_2$ were prepared by diluting pure SO$_2$F$_2$ with a measured volume of room air in Saran bags. The test material was pumped into the exposure chamber under positive pressure at a rate of 9 liters/min. The concentration of SO$_2$F$_2$ pumped into the chamber for the first minute was approximately double the intended target concentration, thus allowing the targeted concentration to be attained within approximately 1 min.

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2 Fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).
A Miran 1A infrared spectrophotometer (Foxboro/Wilks, Norwalk, Conn.) at a wavelength of 11.8 μm was used to measure the concentration of SO₂F₂ in the chambers. For each exposure, a chamber air sample (1 liter) was obtained with a gas-tight syringe and then diluted in a Saran bag with measured volumes of filtered air. The contents of the Saran bag were then pumped into the Miran 1A and the response compared to known standards. All of the exposure analytical concentrations were within 20% of the target concentration and most of the exposure concentrations were within 10%.

Groups of five rats were exposed separately to 4000, 10,000, 20,000, or 40,000 ppm SO₂F₂. Each rat was forced to walk on the rotating activity wheel (4 rpm) for the first 10 min and then alternately rested (2 to 5 min) and walked (1 min). When an animal was no longer capable of walking on the rotating activity wheel, it was considered to be incapacitated, and the exposure was terminated. The time to incapacitation was recorded for each animal. Surviving animals were sacrificed approximately 150 min following exposure to the test material.

Pre- and postexposure treatment with calcium gluconate (CaG). Animals were exposed to various concentrations of SO₂F₂ in 112 liter Rochester-type stainless-steel and glass chambers under dynamic airflow conditions. Sulfuryl fluoride was metered from Saran bags into the main chamber airflow in a similar manner to that described previously. During the exposure period, the airflow through the chamber was maintained at 30 liters/m. At the end of the exposure period, the airflow was increased to 120 liters/m in order to rapidly vent SO₂F₂ from the chamber and thus provide access to the animals as quickly as possible. For each exposure, the concentration of SO₂F₂ present in the chamber was determined with an infrared spectrophotometer as described previously.

Groups of 25 rats were exposed to 0, 4000, or 10,000 ppm SO₂F₂ for 55, 45, or 16 min, respectively. The exposure interval for rats exposed to 4000 or 10,000 ppm was sufficient to cause incapacitation of some animals in the positive control group which was consistent with the results obtained in the induced incapacitation experiments previously described. Each group of 25 rats was subdivided into five subgroups of 5 animals each: subgroup 1 (positive control group) was exposed only to the test atmosphere of SO₂F₂; subgroup 2 received 500 mg/kg CaG ip prior to exposure to SO₂F₂; subgroup 3 received 500 mg/kg CaG ip after exposure to SO₂F₂; subgroup 4 was exposed to the test atmosphere of SO₂F₂ and used only for serum analyses; and subgroup 5 received 500 mg/kg CaG ip prior to exposure to SO₂F₂ and used only for serum analyses. Surviving animals were retained for 3 days after exposure to SO₂F₂.

Blood samples for serum analyses were collected from severed cervical blood vessels from animals in subgroups 4 and 5 within 5 min following termination of exposure. Serum calcium and cholinesterase activity were measured with a CentriflexChem system (Union Carbide Corp., Rye, N.Y.). Serum fluoride was measured with a fluoride specific electrode (Orion Research, Cambridge, Mass.). Serum magnesium was measured by atomic absorption spectrophotometry (Perkin-Elmer Corp., Norwalk, Conn.).

Pre- and postexposure treatment with anticonvulsants. Groups of five rats were individually exposed to 4000 or 10,000 ppm SO₂F₂ under similar conditions described for induced incapacitation. Groups were either pretreated with an ip injection of an anticonvulsant 1 hr prior to exposure to SO₂F₂. Phenobarbital (35 mg/kg), diphenylhydantoin (80 mg/kg), and diazepam (5 mg/kg) were chosen as anticonvulsants due to their recognized efficacy. The dose levels of anticonvulsants were selected based on the literature (Barnes and Eltherington, 1973). Rats receiving phenobarbital following exposure to SO₂F₂ received part of the dose (approx 20 mg/kg) iv via the tail vein to more rapidly increase the blood concentration of phenobarbital. Rats were forced to walk continuously on the activity wheel at a rate of 1 rpm until incapacitated. Exposure of rats pretreated with an anticonvulsant was terminated after 50 min exposure (mean ± 2 standard deviations for time to incapacitation in part one of this study) to 4000 ppm SO₂F₂. Rats were observed for convulsions during the exposure and for 2 hr after the exposure period; the time and duration of each convolution was recorded. The animals were maintained for up to 3 days postexposure.

Statistics. Due to the known effects of fluoride toxicity, a one-sided two-way analysis of variance and Bonferroni-corrected t test, α = 0.05, was used to analyze calcium, magnesium, and fluoride concentrations.

RESULTS

Induced Incapacitation

A dose-related decrease in the time to incapacitation was observed as the concentration of SO₂F₂ increased (Table 1). Rats exposed to 20,000 or 40,000 ppm were unable to walk within 12 min and died within 10 min after terminating exposure. Rats exposed to 10,000 ppm remained functional for a slightly longer period and lived approximately 60 min after the exposure was discontinued. Rats exposed to 4000 ppm were able to walk for approximately 45 min and survived for approximately 2.5 hr after the exposure terminated.

The animals exhibited a marked behavioral change shortly after exposure to SO₂F₂.
The normal walking behavior consisted of walking on the screen floor of the activity wheel with only occasional exploratory behaviors. Three to four minutes after SO₂F₂ was presented, the rats began to cling to the floor or sides and ride the wheel rather than walk. The rats made postural adjustments, while clinging to the screen, to remain in an upright position. This behavior continued until the rats became incapacitated. Rats exposed to 4000 ppm did not exhibit this clinging behavior until after 20 min of exposure.

Rats exposed to the three highest concentrations appeared to be slightly cyanotic shortly after exposure started. The bluish skin discoloration of rats exposed to 10,000 ppm disappeared within 10 min after the chamber was purged with room air. Tonic convulsions were observed in 5 of 20 rats during the exposure to SO₂F₂, whereas the remainder of the animals were incapacitated prior to any convulsive activity (Table 1). Eighteen of twenty animals had tonic convulsions either during or after exposure to SO₂F₂. These convulsions were approximately 10 sec in duration. In many cases, respiration ceased for 15–30 sec after the convulsion. Several rats died immediately after a convulsion.

### Table 1

<table>
<thead>
<tr>
<th>Concentration SO₂F₂ (ppm)</th>
<th>Time to incapacitation (min)³</th>
<th>Survival⁴</th>
<th>Time to death (min)³</th>
<th>Number of rats with tonic convulsions⁸</th>
<th>During</th>
<th>Postexposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,000</td>
<td>41.5 ± 4.3</td>
<td>0/5</td>
<td>124 ± 24</td>
<td>3/5</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>10,000</td>
<td>16.3 ± 1.7</td>
<td>0/5</td>
<td>42 ± 19</td>
<td>1/5</td>
<td>4/5</td>
<td></td>
</tr>
<tr>
<td>20,000</td>
<td>10.3 ± 1.5</td>
<td>0/5</td>
<td>5 ± 5</td>
<td>1/5</td>
<td>3/5</td>
<td></td>
</tr>
<tr>
<td>40,000</td>
<td>6.4 ± 0.2</td>
<td>0/5</td>
<td>4 ± 1</td>
<td>0/5</td>
<td>5/5</td>
<td></td>
</tr>
</tbody>
</table>

³ Mean ± standard deviation.
⁴ Number with effect/number exposed.

### Table 2

<table>
<thead>
<tr>
<th>Concentration SO₂F₂ (ppm)</th>
<th>Length of exposure (min)</th>
<th>Calcium gluconate treatment</th>
<th>Survival⁴</th>
<th>Time to death for nonsurvivors (min)³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55</td>
<td>None</td>
<td>5/5</td>
<td>N.D.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Preexposure</td>
<td>5/5</td>
<td>N.D.</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Postexposure</td>
<td>5/5</td>
<td>N.D.</td>
</tr>
<tr>
<td>4,000</td>
<td>45</td>
<td>None</td>
<td>0/5</td>
<td>126 ± 32</td>
</tr>
<tr>
<td>4,000</td>
<td>45</td>
<td>Preexposure</td>
<td>4/5</td>
<td>90</td>
</tr>
<tr>
<td>4,000</td>
<td>45</td>
<td>Postexposure</td>
<td>0/5</td>
<td>93 ± 11</td>
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<tr>
<td>10,000</td>
<td>16</td>
<td>None</td>
<td>0/5</td>
<td>42 ± 16</td>
</tr>
<tr>
<td>10,000</td>
<td>16</td>
<td>Preexposure</td>
<td>0/5</td>
<td>54 ± 11</td>
</tr>
<tr>
<td>10,000</td>
<td>16</td>
<td>Postexposure</td>
<td>0/5</td>
<td>75 ± 27</td>
</tr>
</tbody>
</table>

³ Mean ± standard deviation.
⁴ Number surviving 3 days after exposure/number exposed.
³ Mean ± standard deviation.
⁴ Number with effect/number exposed.
⁵ N.D. = none died.

Note: N.D. = none died.

TABLE 3
SERUM CHEMISTRY VALUES* OF RATS EXPOSED TO SULFURYL FLUORIDE

<table>
<thead>
<tr>
<th>Concn SO₂F₂ (ppm)</th>
<th>Pretreatment with calcium gluconate (mg/kg)</th>
<th>N</th>
<th>Calcium (mg/dl)</th>
<th>Cholinesterase (U/ml)</th>
<th>Fluoride (ppm)</th>
<th>Magnesium (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>5</td>
<td>10.4 ± 0.6</td>
<td>8.6 ± 1.6</td>
<td>0.30 ± 0.08</td>
<td>23.0 ± 0.6</td>
</tr>
<tr>
<td>0</td>
<td>500</td>
<td>5</td>
<td>11.3 ± 0.5b</td>
<td>8.5 ± 1.7</td>
<td>0.30 ± 0.12</td>
<td>24.9 ± 1.4</td>
</tr>
<tr>
<td>4,000</td>
<td>0</td>
<td>5</td>
<td>10.0 ± 0.9</td>
<td>7.9 ± 0.4</td>
<td>17.3 ± 2.2c</td>
<td>21.2 ± 4.3c</td>
</tr>
<tr>
<td>4,000</td>
<td>500</td>
<td>4</td>
<td>11.0 ± 0.5b</td>
<td>7.4 ± 0.7</td>
<td>19.9 ± 0.9c</td>
<td>21.2 ± 0.9c</td>
</tr>
<tr>
<td>10,000</td>
<td>0</td>
<td>5</td>
<td>10.1 ± 0.4</td>
<td>7.2 ± 0.4c</td>
<td>33.0 ± 2.9c</td>
<td>18.8 ± 1.5c</td>
</tr>
<tr>
<td>10,000</td>
<td>500</td>
<td>5</td>
<td>13.3 ± 1.6b</td>
<td>7.5 ± 0.3c</td>
<td>37.5 ± 2.6c</td>
<td>19.7 ± 3.0c</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.

bSignificantly increased from group exposed to same concentration sulfuryl fluoride but no calcium gluconate, one-tailed test.

cSignificantly different from animals exposed to 0 ppm sulfuryl fluoride.

Pre- and Postexposure Treatment with Calcium Gluconate

Rats exposed to 4000 or 10,000 ppm SO₂F₂ alone did not survive (Table 2) and died within the same time period as rats in the previous study (3 and 1 hr, respectively). Four of five rats pretreated with CaG before exposure to 4000 ppm SO₂F₂ survived for at least 3 days. Treatment with CaG after exposure to SO₂F₂ was ineffective; all five animals died within 3 days. While survival was increased in rats receiving CaG before exposure to 4000 ppm SO₂F₂, the surviving animals were extremely debilitated and there was no apparent protection from convulsions. Animals pretreated with CaG did not survive exposure to 10,000 ppm SO₂F₂.

Pretreatment with CaG resulted in a slight increase in serum calcium levels at all exposure levels of SO₂F₂ (Table 3). Serum cholinesterase activity in rats exposed to 10,000 ppm SO₂F₂ was slightly decreased from control values. Serum fluoride levels of rats exposed to SO₂F₂ were significantly increased from control values. Magnesium levels in the serum of rats exposed to 4000 or 10,000 ppm SO₂F₂ were slightly decreased from control values.

Pre- and Postexposure Treatment with Anticonvulsants

Rats pretreated with an anticonvulsant (phenobarbital, diazepam, or diphenylhydantoin) did not have a convulsion during the exposure to SO₂F₂. Following exposure to SO₂F₂, rats pretreated with phenobarbital were alert, active, and without convulsions for the 2-hr postexposure observation period. In fact, all five of the animals pretreated with phenobarbital survived without any evidence of a convulsion during the entire 3-day postexposure period (Table 4).

After the SO₂F₂ exposures, two rats pretreated with diphenylhydantoin had convulsions of longer duration (approx 30 sec) and increased severity in comparison to rats exposed to sulfuryl fluoride alone. Both rats died within 24 hr following exposure to SO₂F₂. Thus additional studies with diphenylhydantoin were not conducted.

After exposure to SO₂F₂, rats pretreated with diazepam required an additional dose of 2.5 mg/kg diazepam to control convulsions. Animals receiving a supplemental dose prior to any convulsions recovered (3/3) and had either very short (approx 5 sec) mild convulsions or no convulsions whatsoever. Only one of two rats given supplemental doses of...
**DISCUSSION**

Survival Indices for Rats Pre- and Post-Treated with Anticonvulsants

At concentrations of 4000–20,000 ppm SO\(_2\)F\(_2\) rats were quickly incapacitated and died shortly after the exposures terminated. Nearly all of these animals had convulsions during or after exposure to SO\(_2\)F\(_2\).

The response observed in rats exposed to SO\(_2\)F\(_2\) was similar to that previously described for fluoride. Moreover, the serum fluoride levels in rats exposed to 4000 or 10,000 ppm SO\(_2\)F\(_2\) were much higher than the 10-ppm serum fluoride levels reported for...
rats acutely poisoned with sodium fluoride (de Lopez et al., 1976). In addition, serum cholinesterase activity and magnesium levels were decreased in rats exposed to a lethal dose of SO$_2$F$_2$, consistent with results that have been previously observed in rats receiving sodium fluoride (Kahlson and Urmas, 1935). Thus, the effects observed in rats overexposed to SO$_2$F$_2$ are probably the result of fluoride toxicity. However, other factors may also be involved since CaG, a known fluoride ion antagonist, was only marginally effective when given prior to exposure to SO$_2$F$_2$ and then the animals were extremely debilitated. These animals had excess calcium available for binding with fluoride since the serum calcium levels were elevated approximately 10% above control values. There was no beneficial effect in animals treated with calcium gluconate following exposures to SO$_2$F$_2$, possibly because the calcium may not have reached the site of action rapidly enough to be beneficial.

The three anticonvulsants used in this study produced remarkable differences. Of the anticonvulsants, phenobarbital was the most effective followed by diazepam, while diphenylhydantoin apparently accentuated the adverse effects of SO$_2$F$_2$. The reason for this is unknown but is most likely due to differences in mechanism of action of the anticonvulsants.

Phenobarbital was so effective in treating rats exposed to 4000 ppm SO$_2$F$_2$ that coadministration of calcium gluconate and phenobarbital would possibly be more beneficial than phenobarbital by itself since these two compounds exert their effects by different mechanisms. In any event, the results of this study indicate that phenobarbital was most effective in ameliorating the acute toxic effects of an overexposure to SO$_2$F$_2$ in rats.

REFERENCES

American Conference of Governmental Industrial Hygienists (ACGIH) (1985). *Threshold Limit Values for Chemical Substances in the Work Environment*. 


