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THE MAMMALIAN TOXICITY OF FLUOMINE DUST

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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Acute and chronic toxic effects of fluomine on animals were evaluated to establish recommendations for health and safety standards. Fluomine, also known as cobalt-bis(3-fluorosalicylaldehyde)-ethylenediimine, is an alternate oxygen storage source at high altitudes. Fluomine was shown to be an eye and skin irritant and a potent dermal sensitizing agent. Chronic inhalation exposure to 0.5 mg/m^3 produced pulmonary changes in dogs and rats while no significant irritation was noted at an exposure concentration of 0.1 mg/m^3.

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PREFACE

This technical report of the Toxic Hazards Research Unit (THRU) concerns work performed by the Department of Community and Environmental Medicine of the University of California, Irvine on behalf of the United States Air Force under Contract Number F33615-76-C-5005.

The current contract for operation of the Laboratory was initiated in 1975 under Project 6302 "Occupational and Environmental Toxic Hazards in Air Force Operations," Task 01 "Toxicology of Propellants and Materials", Work Unit Number 63020115. K. C. Back, Ph.D., Chief of the Toxicology Branch, was the technical contract monitor for the Aerospace Medical Research Laboratory.

Acknowledgement is made to John Wohlslagel and Gary Fogle for their significant contributions to this research project and for assistance in the preparation of this report.

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GENERAL INTRODUCTION

Over thirty years ago, Calvin and his coworkers, while investigating the properties of metal chelate compounds, characterized the reversible oxygen-absorbing properties of a number of cobalt chelates (Martell and Calvin, 1952) including bis(salicylaldehyde) ethylenediamine cobalt (II) (salcomine) and bis(3-fluorosalicyldehyde) ethylenediimine cobalt (II) fluomine. The structural formula for the oxygen complexed form of fluomine is shown in Figure 1. Their interest in preparing and testing these compounds was to provide models for the naturally occurring oxygen carriers, the hemoglobins and hemocyanins. They found that solid salcomine and fluomine were capable of absorbing and desorbing oxygen for many cycles with little loss of efficiency depending upon temperature and oxygen pressure. Fluomine was superior to salcomine with respect to more rapid absorption of oxygen without a lag phase and the number of regeneration cycles which could be accomplished without severe loss of capacity due to deterioration of the chelate.

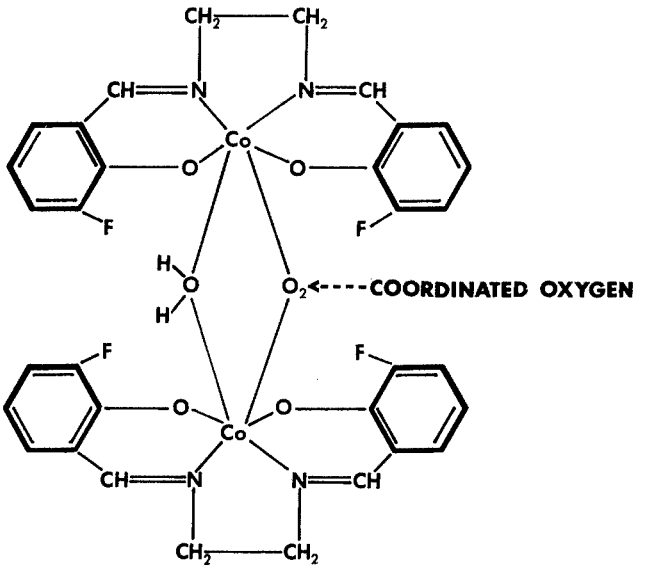

Figure 1. Fluomine: cobalt-bis(3-fluorosalicylaldehyde)-ethylenediimine.

Recent interest in the oxygen absorbing chelates, particularly fluomine, has rested on possible use as regenerable oxygen life-support sources in high-altitude aircraft. Toxicity hazard to crew members or passengers might arise from chemical degradation of the fluomine to form possibly toxic volatile products or from physical degradation of solid

fluomine with formation of smaller particles which might be carried through filters in the air supply system. These smaller particles would also be available for possible dust formation during replacement of cartridges in maintenance of the life-support system. In order to evaluate the possible hazards presented by the use of fluomine, a series of acute and chronic animal toxicity studies were carried out. This report of the results of those tests is divided into two independent sections, one covering acute tests and the other covering chronic exposure.

SECTION I

ACUTE TESTS

INTRODUCTION

A search of the current literature failed to reveal any information concerning the mammalian toxicity of fluomine. However, Coon et al. (1942) reported on the toxicity of salcomine powder inhalation on mice. They report 1 of 6 mice dying following inhalation of 390 mg/m^3 dust for 5.5 hours and 4 of 6 mice dying after 6 hours at 1000 mg/m^3. Exposure to salcomine dust resulted in severe irritation to the tracheo-bronchial system and the lungs. The lungs were hyperemic and contained edema fluid in the peripheral portions of the lobules. Because of the lack of toxicological information on fluomine, a comprehensive series of acute tests was planned. This included acute oral and inhalation toxicity, primary eye and skin irritation, skin and inhalation sensitization.

MATERIALS AND METHODS

Test Substance

The fluomine, as received, was in granular form and had been sieved to a mesh size of 20 to 45 (840 to 350 microns, respectively) for use in oxygen generation systems. Unless otherwise stated in the text, the material was used without alteration.

Animals

The rodents used in acute and chronic experiments were 25 to 30 gram male CF-1 mice, 250 to 400 gram male CFE (Sprague-Dawley derived) rats, and 450 to 600 gram male albino short-hair (Hartley strain) guinea pigs. All of the rodents were obtained from Carworth Farms, Incorporated. New Zealand albino female rabbits purchased from Pel Freeze, Inc., weighing between four and five pounds, were used for the eye and skin irritation studies. Quality control studies on all species during the quarantine period showed the animals to be in good health.

Eye Irritation

The compound was tested for eye irritation using New Zealand albino rabbbits and varying doses of fluomine suspended in normal physiological saline. Twenty-four hours prior to use, the rabbits' eyes were examined for corneal lesions using a 1% solution of fluorescein dye.

Equal numbers of left and right eyes were tested using the following method. The lower lid was pulled away from the eye and 0.1 ml of the fluomine suspension was instilled into the lower conjunctival sac. The lid was held open for a few seconds then raised to close with the upper lid. The eye was not washed following dosing. The other eye of each tested rabbit was not treated and served as a control.

The eyes were examined at 1, 24, and 72 hours and again at 7 days after application of the fluomine. Staining with fluorescein was done on any of the eyes which showed irritation after 24 and 72 hours and 7 days. Grading of eye irritation was done following the standard method of Draize (1944) which provides a maximum total numerical score derived from the sum of corneal, conjunctival, and iris irritation.

Primary Skin Irritation

A patch-test method was done to measure the degree of primary skin irritation of fluomine on the intact and abraded skin of New Zealand albino rabbits. All rabbits were clipped of hair on the backs and flanks 24 hours prior to exposure. The abrasions were minor incisions through the stratum corneum, but not sufficiently deep to disturb the derma or to produce bleeding. These were made in a "tic, tac, toe" pattern with a syringe needle used to make the incisions.

One-half gram of fluomine was applied to each area of intact skin and abraded skin and covered by a one-inch square of surgical gauze, two layers thick. The gauze patches were held in place with strips of nonirritating elastic tape. The entire area was then covered with a rubber dental dam strip and secured with more elastic tape. The patches remained in place on the rabbits for 24 hours. During that time, the rabbits wore leather restraining collars to prevent disturbance of the patch area, while allowing the rabbits freedom of movement and access to food and water during the test period.

After 24 hours, the wrap and patches were removed and the test areas evaluated for irritation using the Draize table (Table 1) as a reference standard. Readings were also made at 72 hours (48 hours after the first reading). The values for erythema and eschar formation were added to the combined values for edema formation. The mean of these values is the primary irritation score.

TABLE 1. EVALUATION OF SKIN REACTIONS*

	Value
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extended beyond exposed area)	4

*Draize et al. (1944).

Acute Oral Toxicity

Fluomine was administered orally to rodents as a suspension in corn oil. The suspensions were kept in a turbulent state prior to dosing by magnetic stirring. Glass syringes with 18 gauge intubation needles were used to administer the suspensions to the rodents. The animals were fasted for at least 16 hours prior to dosing to allow for more uniform absorption since the amount of food in the stomach varies greatly from animal to animal in the unfasted condition.

Five rats and five mice were dosed at each level, and the LD_{50} with its 95% confidence limits was calculated using the moving average interpolation method of Weil (1952) and/or the probit analysis method of Finney (1971). Deaths which occurred during the 14 days immediately following the administration of the single dose were included in the final mortality tally.

Acute Inhalation Toxicity

Groups of 10 rats and 10 mice were exposed to varying concentrations of airborne fluomine dust particles for 1-hour and 6-hour periods and observed for 14 days postexposure to determine mortality and/or signs of toxic stress. The concentrations were established using a Wright Dust Feeder® and adjusting gear ratios to vary the amount of suspended dust entering the 120 liter rectangular, plexiglas chamber.

A constant flow of 20 liters per minute was maintained through the chamber. Concentrations were measured by gravimetric sampling using a 47 mm membrane filter with a mean pore size of 0.45 μ.

Dust Analysis

Particle size analysis was performed on the dust sampled from the chamber using a plastic overlay on a microphotograph as detailed in the procedure of Vooren and Meyer (1971). The chamber air sample, taken at animal breathing level, was drawn through the filter at 5 liters/minute (370 cm/minute), a rate calculated to be capable of collecting particles with aerodynamic diameters of up to 35 microns.

After sampling, the filter was covered with immersion oil and the slide placed under the microscope. Photographs taken of the filter and of a stage micrometer were enlarged to the same degree. The particles were then measured using a plastic overlay on which concentric circles had been drawn corresponding to particle diameters of 1, 2, 5, 10, and 20 microns. These measurements were calculated directly from the photomicrograph of the stage micrometer. A pinhole was made in the center of the circles of the template, enabling the technician to pierce each particle image as counted.

A total of 455 particles was measured by the above method (Table 2). No attempt was made to classify the particles below one micron in diameter, instead they were combined to form one group. Seventy-one percent of all particles were five microns or less in diameter and, therefore, of respirable size.

TABLE 2. PARTICLE SIZE DISTRIBUTION OF FLUOMINE DUST

Particle Size Range (Microns)	Number of Particles	Percentage of Particles	Cumulative Percentage of Particles
<1	88	19.3	19.3
1-2	94	20.7	40.0
2-5	144	31.7	71.7
5-10	72	15.8	87.5
10-20	37	8.1	95.6
>20	20	4.4	100.0

Skin Sensitization

A modified Landsteiner (1937) technique was used to determine whether fluomine would cause an antigen-antibody reaction in male albino guinea pigs. The experimental group consisted of 18 guinea pigs. Another group of three guinea pigs was used to determine the primary irritation properties of the compound.

The primary irritation test consisted of injecting 0.05 and 0.10 ml quantities of a 0.1% suspension of fluomine in peanut oil into the closely clipped scapular and sacral areas of the three guinea pigs. Similar injections of peanut oil alone were also made. The injection sites were examined at 24 and 48 hours and indicated that the 0.1% suspension was sufficiently irritating to cause inflammatory reactions. A repeat of the test using a 0.01% suspension did not cause irritation, and this concentration was used for the intradermal sensitization regimen.

For the sensitization test, an injection of 0.05 ml of the 0.01% suspension of fluomine was made intradermally into the upper right scapular area of each animal. A similar injection of peanut oil was made concurrently into the upper left scapular area. The injections were examined at 24 and 48 hours and the numerical scores (determined as shown in Table 3) recorded.

TABLE 3. GRADING OF SKIN REACTIONS IN THE GUINEA PIG SENSITIZATION TEST

The product of the width and length of the wheal (in mm) is multiplied by the following reaction scores:

0 = needle puncture, no wheal
1 = very faint pink
2 = faint pink
3 = pink
4 = red
5 = bright red
6 = edema, <1 mm in height
7 = edema, >1 mm in height
*8 = necrosis, <1 square mm
*9 = necrosis, >1 square mm

*The product of width and length of the necrotic area multiplied by 8 or 9 is added to the numerical value of any of the foregoing reactions that are present.

Following the initial injection, doses of 0.1 ml of the same dilutions (freshly prepared) were injected into the clipped dorsal lumbo-sacral areas of guinea pigs every other day (every third day over weekends) until a total of seven injections were administered. Care was taken to ensure the repeated doses were not injected into the same site.

The guinea pigs were rested for three weeks (incubation period), weighed, and given a challenge dose of 0.05 ml of the 0.01% suspension into the lower right scapular area. A control injection of peanut oil was

made into the lower left scapular area. The reactions were again read and graded after 24 and 48 hours. Any irritation reactions occurring after the initial injection and any caused by the vehicle were subtracted from the challenge test scores.

Sensitizing response and potential of the test agent may be inferred from the numerical grade and the number of animals affected according to the following categories.

Final Grade	Sensitizing Response	Number Sensitized (N=20)	Sensitizing Potential
0-25	None	1-3	Slight
26-99	Mild	4-10	Moderate
100-200	Moderate	11-20	Severe
>200	Severe		

Inhalation Sensitization

The experimental plan as originally devised intended that a regimen similar to that used in the skin sensitization study be employed so that 20 guinea pigs were to be exposed to fluomine dust every other work day for a total of seven exposures. The concentration, 30 mg/m^3, and exposure time, 2 hours, were chosen after acute exposures of rats and mice indicated that these conditions would be nonlethal. However, the first exposure of guinea pigs under these conditions resulted in the deaths of six animals. These were replaced and the group exposed two days later to a reduced concentration of 12 mg/m^3 for two hours. Following this, eight more of the original group died. The exposure time was then reduced to one hour for the remainder of the exposures without further loss of animals. To determine whether sensitization response could be elicited after only a few exposures, an additional three guinea pigs were added for the last three inhalation exposures.

Three groups of guinea pigs resulted from this series of exposures:

A. Six animals that had been initially exposed to 30 mg/m^3 fluomine for 2 hours, then to 12 mg/m^3 for 2 hours, and following this, five times to 12 mg/m^3 for one hour, following the regular sensitization regimen.

B. Six animals that had been initially exposed to 12 mg/m^3 fluomine dust for 2 hours and then five times to 12 mg/m^3 for one hour.

C. Three animals that were exposed three times to 12 mg/m^3 for one hour.

Table 4 summarizes the exposure regimen used for the above groups.

TABLE 4. INHALATION EXPOSURE REGIMEN FOR GUINEA PIG SENSITIZATION STUDY

Exposure Time, Hrs.	Fluomine Conc., mg/m^3	No. of Guinea Pigs in Group A	B	C
2	30	20		
2	12	14	6	
1	12	6	6	
1	12	6	6	
1	12	6	6	3
1	12	6	6	3
1	12	6	6	3

To the three groups listed in Table 4 were added groups D and E, consisting of 6 guinea pigs each, remaining from the original intradermal sensitization study. These animals did not receive any of the dust inhalation exposures. Two control groups, also consisting of 6 guinea pigs each, were included in the challenge regimen. Three weeks after the last inhalation sensitization exposure and 2 months after intradermal challenge for groups D and E, the following treatments were given:

Groups A, D, and controls: A 1-hour inhalation exposure to 15 mg/m^3 fluomine dust.

Groups B, C, E, and controls: An intradermal injection of 0.05 ml of a 0.01% suspension of fluomine in peanut oil.

The fluomine used for the inhalation sensitization and for the highest concentration acute inhalation exposure was ground in a mortar before loading into the Wright Dust Feeder®. Examination of particle size by the method previously detailed gave the results shown in Table 5, indicating that the number distribution had shifted significantly to lower diameters when compared with the unground dust.

TABLE 5. PARTICLE SIZE DISTRIBUTION OF GROUND FLUOMINE DUST SAMPLED FROM EXPOSURE CHAMBER

Particle Size Range (Microns)	Number of Particles	Percentage of Particles	Cumulative Percentage of Particles
<1	132	34.6	34.6
1-2	93	24.4	59.0
2-5	87	22.8	81.8
5-10	50	13.1	94.9
10-20	15	3.9	98.8
>20	4	1.0	99.8

EXPERIMENTAL RESULTS

Eye Irritation

The first fluomine suspension used for determination of eye irritation had a concentration of 33% (w/w). This proved to be extremely irritating to the conjunctivae, causing marked chemosis and considerable discharge. Due to severe swelling of the conjunctivae and nictitating membranes, examination of corneal and iris tissue was impossible except in one case where definite corneal opacity was noted at 72 hours. Therefore, the experiment was repeated using a 3% (w/w) suspension. A comparison of the results of the two tests is shown in Table 6. As can be seen in the table, the 1-hour and 24-hour responses are comparable for the two mixtures tested. The values after 24 hours, however, indicate the higher concentration resulted in irritation of increased duration and severity. No residual fluomine particles were noted after 24 hours in those eyes treated with the 3% suspension whereas undissolved fluomine was observable in eyes receiving the higher dose up to 7 days after treatment. The difference noted is probably due to the amount of retained particles capable of dissolving in the ocular fluid and serving to provide a constant source of chemical irritation.

TABLE 6. IRRITATION RESPONSE FROM INSTILLATION OF FLUOMINE IN RABBIT EYES

Concentration of Suspension	Draize* Scores			
	1 Hour	24 Hours	72 Hours	7 Days
33%	4.3	12.7	23.3	52.5
3%	1.3	8.7	1.0	0

*Draize et al. (1944).

The diffuse characteristic of the irritation, seen also in the trachea of rats exposed by inhalation, indicated that it was chemical in nature rather than mechanical abrasion, which implied that some solubilization of the fluomine was occurring to provide the irritant. In order to test this hypothesis, fluomine was milled with physiological saline for 1.5 hours and filtered to yield a 0.71 g/100 ml solution. This solution was tested for eye irritation potential by an instillation of 0.1 ml into one eye of each of six rabbits. Examinations at 1 hour, 72 hours, and 7 days were negative, indicating that the solution, as tested, was nonirritating to the eyes.

The result cast some uncertainty on the original interpretation of fluomine eye irritancy residing in the soluble portion, but it is possible that the fluomine solution was quickly washed out of the eye while the solids were retained on the conjunctiva providing a continuous source of soluble material. However, mechanical abrasion cannot be ruled out as a contributing factor.

Primary Skin Irritation

Rabbit skin irritation tests (Table 7) show fluomine to be a mild to moderate irritant, more potent in areas of abrasion. This would be consistent with the findings of extreme irritancy to the eye.

TABLE 7. RABBIT PRIMARY SKIN IRRITATION RESULTS

	Reading Time (Hours)	Rabbit Number 1 I	1 A	2 I	2 A	3 I	3 A	4 I	4 A	5 I	5 A	6 I	6 A
Erythema	24	2	2	0	0	0	0	1	1	1	1	1	1
	72	1	1	0	0	0	0	0	1	1	1	1	1
Edema	24	0	3	0	1	0	3	0	3	0	3	0	3
	72	2	2	0	0	0	2	0	2	0	0	0	2

I - Intact Skin
A - Abraded Skin

Acute Oral Toxicity

Mortality data from single dose oral administration of fluomine to mice and rats are given in Tables 8 and 9. Most deaths occurred during the first 24 hours postexposure with the latest deaths occurring at 7 days postexposure.

TABLE 8. ACUTE SINGLE DOSE ORAL TOXICITY OF FLUOMINE FOR MALE MICE

Dose, mg/kg	Mortality Ratio (Number Died/Number Dosed)	Days to Death*
400	5/5	1,1,1,1,4
200	5/5	1,1,1,4,4
100	1/5	6

LD_{50} and 95% C.L. = 123 (93-167) mg/kg

* "1" indicates any death within 24 hours after dosing.

TABLE 9. ACUTE SINGLE DOSE ORAL TOXICITY OF FLUOMINE FOR MALE RATS

Dose, mg/kg	Mortality Ratio (Number Died/Number Dosed	Days to Death*
400	5/5	1,1,3,5,7
200	3/5	2,5,7
100	0/5	-
50	0/5	-

LD_{50} and 95% C.L. = 187 (129-270) mg/kg

* "1" indicates any death within 24 hours after dosing.

Gross examination of mice that died within 24 hours of dosing revealed distended blood-filled stomachs, something not seen in the orally dosed rats. Microscopic examination of both rat and mouse tissue showed areas of necrosis of the lymphocytes within the germinal centers of the spleen. This lesion is not normally found in rodents and is considered to be a result of the chemical insult.

Acute Inhalation Toxicity

The measured inhalation exposure concentrations and resulting mortality are shown in Table 10. The maximum concentration which could be generated, 890 mg/m^3, was not able to produce deaths in mice as a result of a one-hour exposure.

Gross examination of the rats that died following exposure revealed fluomine particles in the trachea and in the lungs. Microscopic examination of the animals revealed that the entire mucosa of the nasal cavity showed erosion and sloughing of the epithelium with a severe, purulent inflammatory response. The trachea also showed an intense inflammation with necrosis of the epithelium which extended downward into the glandular tissue.

The lungs showed signs of perivascular edema, intraalveolar edema, and congestion with diffuse focal areas of lymphoid hyperplasia. Larger air spaces were often filled with a proteinaceous material. The liver showed periacinal congestion with dilation of the sinusoids.

TABLE 10. MORTALITY RESPONSE OF RATS AND MICE TO 1-HOUR AND 6-HOUR INHALATION EXPOSURES OF FLUOMINE DUST

Rats		Mice	
Conc., mg/m³*	Mortality Ratio Number Died/Number Dosed	Conc., mg/m³*	Mortality Ratio Number Died/Number Dosed
	SIX HOURS		
407	10/10	464	6/10
185	10/10	195	1/10
104	3/10	94	1/10
49	0/10		
LC_{50} and 95% (C.L.) = 112 (81-163) mg/m³)		LC_{50} and 95% (C.L.) = 416 (222-780) mg/m³	
	ONE HOUR		
890**	9/10	890**	0/10
750	7/10	750	0/10
507	0/10	507	0/10
LC_{50} and 95% (C.L.) = 712 (639-792) mg/m³			

*Concentration measured gravimetrically.
**This exposure was done with milled fluomine dust.

Sensitization Studies

As detailed in the Materials and Methods section, the guinea pig intradermal sensitization study was done using a 0.01% suspension, one-tenth of the standard concentration, of fluomine in peanut oil. Sensitization responses were obtained in 16 of 18 animals with a mean reaction score of 285, indicating that fluomine is a potent sensitizer for guinea pigs by the intradermal route.

Since the normal route of exposure to this material is expected to be by inhalation, guinea pigs were exposed to a series of inhalation exposures to see if a sensitization response would be elicited. This was performed following the same regimen as the previous sensitization study, with inhalation exposures substituted for the intradermal injections with modifications detailed in the Methods section.

All animals challenged by inhalation of 15 mg/m³ fluomine dust for one hour exhibited eye and nose irritation. However, no group, whether pretreated intradermally or by inhalation, showed any signs of lung hypersensitivity reactions after inhalation challenge. Three of six guinea

pigs in Group B (exposed six times to 12 mg/m^3, initially for two hours and subsequently for one hour each) showed a response to intradermal challenge with a mean reaction score of 72 after 24 hours. At 48 hours, the response had subsided giving a mean reaction score of 41. No response to intradermal challenge was given by the three animals in Group C (exposed three times to 12 mg/m^3 of fluomine dust for one hour) or controls.

In contrast, in the group sensitized intradermally (Group E), skin hypersensitivity responses were found in 4 of 6 guinea pigs at 24 hours and 5 of the 6 at 48 hours. The mean reaction scores at these periods were 600 and 405, respectively. The conclusions of the study may be summarized in the following way:

1. Guinea pigs sensitized by six repeated inhalation exposures to fluomine dust demonstrate a mild to moderate response to an intradermal challenge. However, three inhalation exposures do not elicit this response.

2. Guinea pigs do not show a hypersensitivity or anaphylactic type response when challenged by an inhalation exposure of fluomine dust whether pretreated intradermally or by inhalation.

3. Intradermal challenge presented two months after termination of a fluomine intradermal sensitization study elicits a strong reaction.

DISCUSSION

Single peroral doses of fluomine are toxic to both rats and mice at very low levels. The mouse appears to be more susceptible to the irritating properties of the compound, evidenced by the lower LD_{50} and the incidence of bloated, blood filled stomachs found in the mice but not seen in any of the rats.

Fluomine particles are highly irritating to the eyes of rabbits. The compound causes severe irritation to the conjunctivae resulting in chemosis and marked swelling. If the particles are not removed from the eye, they provide a source of constant irritation to the eye and surrounding membranes. A similar irritation is produced when the compound is in contact with broken or abraded skin. Little or no effect is noted on intact skin.

Single inhalation exposure LC_{50} results indicate that fluomine is highly toxic by this route. Guinea pigs were the most sensitive species to the lethal effects of fluomine with deaths occurring after a single two-hour inhalation exposure to 30 mg/m^3. Rats are considerably less affected than guinea pigs but are more susceptible than mice. Fluomine dust has a highly irritating effect on the entire respiratory systems of all species tested. Also, a systemic action is evidenced by the effect on livers and the necrosis of lymphoid elements within the germinal centers of the spleen.

The dust of this compound is a potent sensitizer of guinea pigs by the intradermal route. Although an anaphylactic-type response was not elicited by the guinea pigs given an inhalation challenge, a positive response after intradermal challenge showed that it is possible for the animals to produce antibodies if exposed to repeated inhalation of low concentrations of fluomine.

SECTION II

CHRONIC TOXICITY OF FLUOMINE DUST

INTRODUCTION

This study was designed to determine the chronic effects of low level, nonirritating concentrations of fluomine particulates to rodents and dogs for use in recommending safe exposure limits. It was designed to examine effects immediately following a six-month industrial-type exposure as well as effects up to one year postexposure. Preliminary two-week intermittent exposures were carried out to determine the optimum concentrations for use in the chronic study. The same strains of test animals were used in these studies as in the acute studies.

METHODS

Generation and Analysis

The fluomine particulates, produced by a Wright Dust Feeder®, were generated into a 200 liter mixing chamber prior to being drawn into the exposure chambers by negative pressure. Regulation of the dust feeder gear ratios and/or the air passing through the mixing chamber controlled the concentration as well as the particle size entering the chambers.

Analysis of fluomine concentration was accomplished by taking hourly filter samples for colorimetric analysis. The fluomine samples were dissolved in 1N HNO_3 and absorbance at 365 nm measured using a GCA McPherson spectrophotometer. Concentration checks were made by counting the particles in the 1.4-3.0 µm range using the Royco® Model 225 particle counter. Since the greater part of the mass of the fluomine was included in this size range, fluctuations in chamber concentration could be easily detected by changes in the channel output representing this range.

Particle size and mass distribution of the chamber aerosol was determined using an Aries® cascade impactor. The impactor was used for this purpose since the Royco® particle counter background noise level was too high at the range of particle diameters below 1.4 µm.

Background contamination did not interfere with the impactor measurements as the stages were washed with 1N nitric acid and the fluomine determined colorimetrically. A summary of the impactor samples taken over the 6-month period is shown below.

Chamber	Parameter	N	Mean (μm)	Range (μm)
A	MMD	20	2.093	1.82-2.39
A	σg	20	1.747	1.64-1.99
B	MMD	16	2.031	1.79-2.25
B	σg	16	1.897	1.59-2.42

Two-Week Exposures

Prior to initiating the long-term study, two-week preliminary exposures were conducted to determine nonirritating concentrations which could be used for the six-month exposure. The animal groups, consisting of 20 male rats and 20 male guinea pigs, were exposed to fluomine particulates, 6 hours/day, 5 days/week, in a 1 m^3 Rochester chamber. Identical numbers of controls were maintained for comparative purposes. At the conclusion of the two-week exposure period, one half of the rats were sacrificed and the lungs precisely excised and weighed after which they were analyzed for fluomine deposition. Statistical comparisons of test and control wet lung weights were made for determination of edematous effects. The remaining rats and one-half of the guinea pigs were sacrificed at the conclusion of the exposure period and given pathological examinations for toxic effects. The remaining guinea pigs (10 test and 10 control) were held for a three-week period after which time they were given an intradermal injection of fluomine to determine their sensitization response.

Chronic Exposures

The animal groups consisted of 100 male rats, 140 female mice, 25 male guinea pigs, and 4 male and 4 female beagle dogs. The test groups were housed in large inhalation chambers (Thomas, 1968) and exposed six hours/day, five days/week. The control group was housed in an animal holding facility within the same building.

Body weights of the rats, guinea pigs, and dogs were measured at biweekly intervals during the exposure period and monthly during the postexposure period. A routine battery of clinical tests was conducted on the dogs on a biweekly basis. This consisted of complete hematology studies, including differentials, and the following clinical chemistry determinations:

Sodium	Albumin
Potassium	Globulin
Calcium	SGPT
Glucose	Alkaline Phosphatase
	Total Protein.

Gross and histopathology examinations were done on all animals that died during the exposure portion of the study or were sacrificed following exposure. Major organs (lung, liver, spleen, kidney, and heart) were examined in all animals as well as a more extensive tissue examination in animals held postexposure.

RESULTS AND DISCUSSION

Two-Week Exposures

The initial concentration examined was 2 mg/m^3 based on the effects of low-level exposure to guinea pigs during the inhalation sensitization studies described earlier. At this concentration, none of the rats died during the exposure nor were any signs of toxic stress noted. All test and control rats gained weight normally during the two-week study.

Two guinea pig deaths occurred, one on the second exposure day, the other at four exposure days. Signs of toxic stress prior to death included labored breathing, diarrhea, and a general unthrifty appearance. The surviving test guinea pigs appeared lethargic and several exhibited intermittent labored breathing during exposure. The guinea pigs appeared normal after conclusion of each day's exposure and removal of fluomine from the chamber atmosphere. The control guinea pigs appeared normal throughout the study.

Gross pathology findings in the guinea pigs that died during exposure included diffuse, mild congestion in all lung lobes and the kidneys. In addition, the animal that died following four exposure days showed dark red consolidation of several lung lobes, with emphysema. No gross findings which could be considered treatment related were found in the remaining guinea pigs. A significant finding in the rats examined grossly at the conclusion of the study was mild congestion and emphysema. The control rats did not show similar signs, and it was therefore considered that the congestion and emphysema were treatment related effects.

A tabular summary of the mean body weights and the mean wet lung weights of the rats is presented in Table 11. The results of guinea pig growth measurements made during exposure to the fluomine aerosol are shown in Table 12.

TABLE 11. EFFECT OF 2-WEEK INHALATION EXPOSURE TO 2 mg/m^3 FLUOMINE AEROSOL ON RAT GROWTH AND LUNG WEIGHT (N = 10)

	Body Weight (grams)		Lung Weight	Lung/Body
	Preexposure	Final	(grams)	Wt. Ratio
Exposed	215	268	1.96*	.0073
Unexposed Controls	211	265	1.35	.0051

*Different from controls at 0.01 level of significance.

TABLE 12. EFFECT OF 2-WEEK INHALATION EXPOSURE TO 2 mg/m^3 FLUOMINE AEROSOL ON GUINEA PIG GROWTH (N = 20)

	Body Weight (grams)	
	Preexposure	Final
Exposed	408	451*
Unexposed Controls	409	482

*Different from controls at the 0.05 level of significance.

Mean body weights of the exposed guinea pigs were adversely affected while mean body weight gains of the male rats were essentially equivalent to controls. In addition, a significant increase was noted in the mean wet lung weights of the exposed rats, relative to controls, which would indicate an edematous effect probably caused by irritation. This, as well as the guinea pig deaths and rat lung pathology, strongly suggest that 2 mg/m^3 is an irritating concentration.

The lungs from six control rats along with lungs from eight exposed rats were wet ashed and analyzed for cobalt by means of atomic absorption spectroscopy. No statistical differences could be found between the mean values for the two groups. Apparently, the deposition of the fluomine dust in the lungs of rats could not be measured by this method.

Guinea pigs given an intradermal injection of 0.05 ml of a 0.1% suspension of fluomine in peanut oil three weeks after the conclusion of exposures failed to show a response that would be indicative of a sensitization reaction.

The 2 mg/m^3 fluomine aerosol concentration was definitely irritating and was ruled out as a possible concentration for the 6-month chronic study. A second 2-week preliminary study was conducted in the same manner using a 0.2 mg/m^3 concentration of fluomine. At the conclusion of the two weeks, the animals were examined as described for the first preliminary study.

No signs of toxic stress occurred in either species, and all animal groups showed normal body weight gains at the end of the two-week exposure period. The mean body weights of the rat groups and the results of the wet lung examination are shown in Table 13. A statistically significant difference was seen in the mean wet lung weights of the test rats when compared to the controls but the biological significance is negated by the fact that the lung/body weight ratios were not different.

TABLE 13. EFFECTS OF 2-WEEK INHALATION EXPOSURE TO 0.2 mg/m^3 FLUOMINE AEROSOL ON RAT GROWTH AND LUNG WEIGHT (N = 10)

	Body Weight (grams)		Lung Weight	Lung/Body
	Preexposure	Final	(grams)	Wt. Ratio
Exposed	195	276	1.53*	.0055
Unexposed Controls	193	265	1.43	.0054

*Different from controls at the 0.05 level of significance.

Chronic Study

Analysis of the results of the two-week exposures indicated that 0.1 mg/m^3 and 0.5 mg/m^3 would be concentrations likely to bracket the pulmonary irritation effect threshold for six-months exposure. During the study, the mean concentration of fluomine aerosol in the nominal 0.5 mg/m^3 exposure study was 0.51 mg/m^3 for the 117 days of exposure with a daily range of 0.37 to 0.79 mg/m^3. In the nominal 0.1 mg/m^3 study, the mean measured concentration of fluomine for the entire study was 0.10 mg/m^3 with daily excursions of 0.06 to 0.17 mg/m^3.

During the six-month inhalation study, none of the animal species showed any outward sign of toxic stress. A significant number of mice died during a twelve-day period in the low level exposure chamber from an infection identified as type G streptococcus. All mice, including controls, were treated for 5 days with tetracycline to prevent an epizootic. This treatment was successful in that deaths were significantly reduced. Deaths in rats and guinea pigs were sporadic and scattered through the three groups. In no case did the gross examinations reveal lesions which could be attributed to exposure.

All dogs and 10% of the rats and mice were sacrificed at the conclusion of the 6-month exposure portion of the study.

The initial protocol for this experiment scheduled that all surviving animals be sacrificed at the end of the 12-month observation period. However, only 16% of the rats and 23% of the mice had died by the 12th month and surviving animals appeared to be in good health. Because holding animals for a longer period might reveal some long-term toxic effects of fluomine aerosol exposure, it was decided to sacrifice 50% of the remaining rats and mice at that time and to hold the remainder for an additional six months. As mortality in the guinea pig group had been much higher, about 70%, all of the guinea pigs were sacrificed. The numbers of animals sacrificed at 12 months postexposure are shown in Table 14.

TABLE 14. 12-MONTH POSTEXPOSURE SACRIFICE OF FLUOMINE AEROSOL EXPOSED ANIMALS

Fluomine Conc., mg/m^3	Animal Species & Number Sacrificed	Age at Sacrifice (Weeks)	Number Retained
0.5	9 Guinea Pigs	93	0
	37 Rats	91	37
	50 Mice	91	52
0.1	5 Guinea Pigs	93	0
	35 Rats	91	34
	45 Mice	91	44
0.0	8 Guinea Pigs	93	0
	34 Rats	91	34
	46 Mice	91	46

The numbers of rats and mice alive for sacrifice at 18 months postexposure are shown in Table 15.

TABLE 15. 18-MONTH POSTEXPOSURE SACRIFICE OF FLUOMINE AEROSOL EXPOSED ANIMALS

Fluomine Conc., mg/m^3	Male Rats	Female Mice
0.5	15	41
0.1	11	35
0.0	13	29

Gross examination of the rats, mice and dogs sacrificed immediately postexposure failed to reveal any exposure-related lesions. The only significant changes in the dog organs examined by light microscopy were a higher incidence of diffuse, mild pulmonary congestion with intraalveolar edema and greater severity of hydropic degeneration in the livers of the 0.5 mg/m^3 group. The significant findings are shown in Table 16.

TABLE 16. SIGNIFICANT HISTOPATHOLOGY FINDINGS IN DOGS IMMEDIATELY AFTER 6-MONTH EXPOSURE TO FLUOMINE AEROSOL (N=8)

Organ	Concentration, mg/m^3 0.5	0.1	0.0
Lung:			
Congestion, diffuse, mild	4	0	0
Intraalveolar edema	4	0	0
Liver:			
Vacuolation, hydropic, minimal	0	0	7
Vacuolation, hydropic, mild	2	4	0
Vacuolation, hydropic, moderate	3	0	0
Vacuolation, hydropic, severe	3	1	0

To ascertain whether intermittent exposure to low levels of fluomine is capable of sensitizing guinea pigs, ten animals exposed to 0.5 mg/m^3 were given intradermal injections of fluomine one-month postexposure. These failed to elicit skin reactions which would be indicative of hypersensitization.

No unusual gross lesions were detected in any of the rodent groups either in individuals that died or those sacrificed at any period. Except for tumors which will be dealt with later in this report, no significant micropathological lesions were found in the mice from any sacrifice group. Incidental lesions were randomly distributed with no dose related responses noted.

Chronic inflammation of the trachea was seen in 70% of both groups of exposed rats on histopathologic examination immediately following exposure. No control rats showed this lesion of the trachea at this examination period. Rats that died or were sacrificed during the remainder of the experimental holding period did not evidence any higher incidence of tracheitis than that shown by control animals (Table 17).

TABLE 17. CHRONIC INFLAMMATION OF TRACHEA IN RATS IMMEDIATELY FOLLOWING EXPOSURE TO FLUOMINE DUST

	Incidence		
Sample Period	0.5 mg/m^3	0.1 mg/m^3	Control
Conclusion of Exposure	7/10*	8/12*(1)	0/10
0-12 Months Postexposure	25/53	22/54	23/56
12-18 Months Postexposure	17/37	13/34	16/34
TOTALS	49/100	43/100	39/100

*Different from controls at the 0.01 level of significance [Fisher Exact Test (Bliss, 1967)].
(1) Includes two rats that died during exposure.

The lungs of all rats sacrificed following exposure were carefully excised and weighed to give the values shown in Table 18. As can be seen, the mean wet lung weights of the exposed animals did not differ significantly from that of the control rats.

TABLE 18. EFFECT OF 6-MONTH INHALATION EXPOSURE TO FLUOMINE AEROSOL ON RAT LUNG WEIGHT

Fluomine Conc., mg/m^3	Body Weight (grams)	Lung Weight (grams)	Lung/Body Wt. Ratio
0.5	472.3	2.67	0.565
0.1	497.1	2.47	0.497
0.0	522.9	2.56	0.495

In addition to the chronic inflammation of the trachea seen in the rats examined immediately following exposure, bronchopneumonia was found in 17% of the rats from the 0.5 mg/m^3 group that either died during the 12-month postexposure period or were sacrificed at 12 months postexposure (Table 19). Bronchopneumonia was not seen in any of the control rats examined during this time nor was it noted in any of the rats at the other examination periods.

TABLE 19. BRONCHOPNEUMONIA IN RATS EXPOSED TO FLUOMINE DUST

Sample Period	Incidence		
	0.5 mg/m^3	0.1 mg/m^3	Control
Conclusion of Exposures	0/10	0/12(1)	0/10
0-12 Months Postexposure	9/53	1/54	0/56
12-18 Months Postexposure	0/37	0/34	0/34
TOTALS	9/100	1/100	0/100

(1) Includes 2 rats that died during exposure.

Histopathologic examination of the tissues removed from the guinea pigs at the 12-month postexposure sacrifice period showed no exposure related lesions. No tumors were found nor were any nonneoplastic lesions which could be considered treatment related found in any of the guinea pigs examined.

There were no significant differences in the numbers or types of tumors seen in the exposed rats when compared to the control group at any of the examination periods.

As shown in Table 20, there was no increase in alveolar/bronchiolar carcinomas in exposed versus control mice at any sampling period. There appears to have been a slightly higher incidence of alveolar/bronchiolar adenomas in the high level (0.5 mg/m^3) mice that died between the twelfth and eighteenth month or were sacrificed at 18 months postexposure. No differences can be found for this particular lesion at any of the other sampling periods (Table 21).

TABLE 20. ALVEOLAR/BRONCHIOLAR CARCINOMAS IN MICE EXPOSED TO FLUOMINE DUST

Sample Period	Incidence		
	0.5 mg/m^3	0.1 mg/m^3	Control
During Exposure*	0/22	0/38	0/23
0-12 Months Postexposure	4/66	0/58	3/71
12-18 Months Postexposure	2/52	7/44	6/46
TOTALS	6/140	7/140	9/140

*Includes 14 animals sacrificed at termination of exposure period.

TABLE 21. ALVEOLAR/BRONCHIOLAR ADENOMAS IN MICE EXPOSED TO FLUOMINE DUST

Sample Period	Incidence		
	0.5 mg/m^3	0.1 mg/m^3	Control
During Exposure*	0/22	0/38	0/23
0-12 Months Postexposure	15/66	11/58	17/71
12-18 Months Postexposure	21/52	13/44	13/46
TOTALS	36/140	24/140	30/140

*Includes 14 animals sacrificed at termination of exposure period.

Fluomine exposed rats showed a statistically significant depression in mean body weight gain (Figure 2) throughout the study. Although the test rat groups differed from the control groups, they rarely differed from each other, and a dose-response was not established. Following transfer to laminar flow animal rooms, the rat groups showed a slight increase in mean body weight gain. Although all groups showed this increase, the test groups remained below the control group.

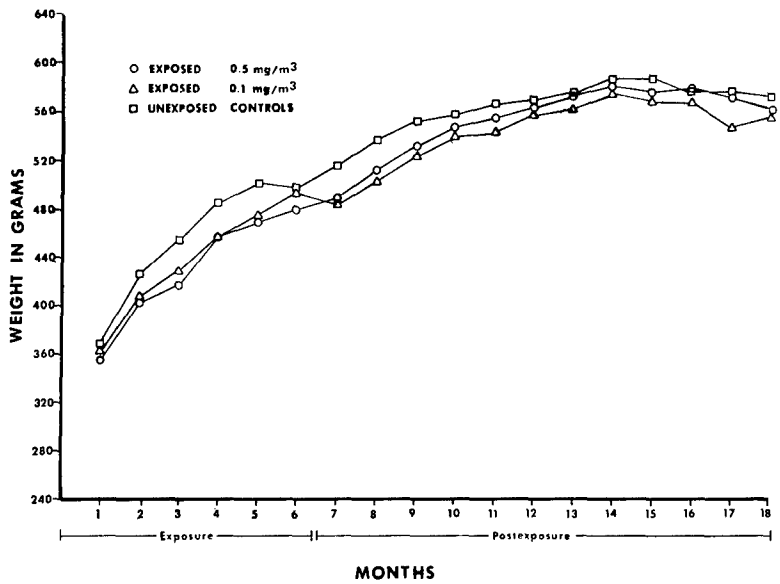

Figure 2. The effect of fluomine aerosol inhalation on growth of male rats.

The guinea pig mean body weights did not differ significantly from the control group during the exposure phase except for short periods of successive weighings as shown in Figure 3. The control group showed a drop in mean weights for a period of four months but then recovered to previous levels. The mean body weight differences noted in the guinea pig groups were transient in nature and not consistent throughout the study. A dose-response relationship was not seen.

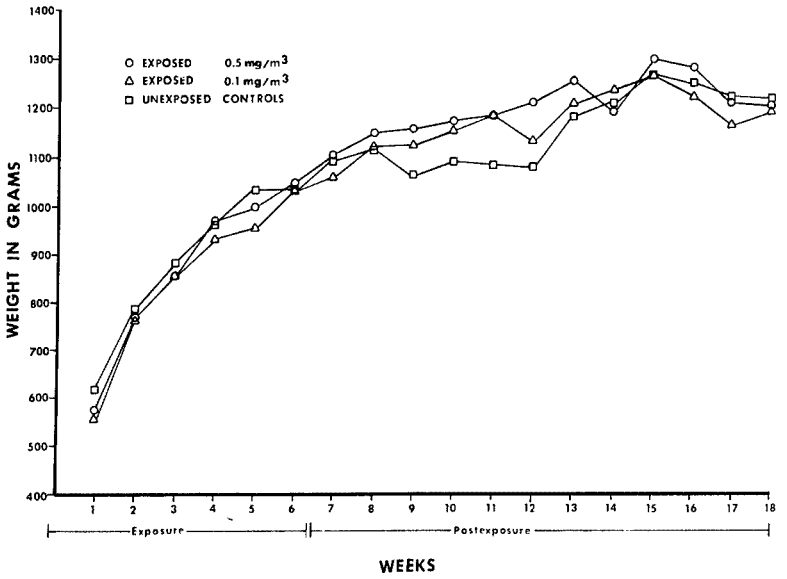

Figure 3. The effect of fluomine aerosol inhalation on growth of guinea pigs.

DISCUSSION

The objective of bracketing the effect threshold of fluomine for a six-month intermittent exposure was attained with the concentrations chosen. Certainly, the increased lung weights in rats exposed to 2 mg/m^3 fluomine for two weeks along with the mortality in guinea pigs demonstrated that this concentration would not be suitable for a six-month exposure. Analysis of all of the data obtained from exposed and control animals showed that exposure to 0.1 or 0.5 mg/m^3 fluomine intermittently for six months had little or no effect on mortality or gross pathology findings. The body weights of rats and guinea pigs were somewhat lower in exposed groups, but there was no correlation with dose.

Histopathology examinations of animals sacrificed immediately postexposure showed that exposure to the higher dose had significant deleterious effects on the lungs and livers of dogs, while effects of the 0.1 mg/m^3 exposure in this species were absent or minimal. Rats in both

the 0.1 mg/m^3 and the 0.5 mg/m^3 exposed groups showed about the same incidence of chronic inflammation of the trachea. Although there was no dose dependency of tracheitis incidence, bronchopneumonia during a 12-month postexposure period developed only in animals exposed to the higher level indicating that this condition is a delayed effect of exposure to fluomine. No other significant nononcogenic lesions were found in any species.

The only tumor which showed any increase in exposed animals was alveolar/bronchiolar adenoma. There was an increased incidence of borderline significance in animals exposed at the higher level in the last six months of postexposure holding.

Challenging guinea pigs with intradermal injections of fluomine suspensions after 2 weeks exposure to 2 mg/m^3 fluomine or after six months exposure to 0.5 mg/m^3 demonstrated that no hypersensitization had occurred in the animals as a result of these exposures.

The concentration of 0.1 mg/m^3 is close to the effect threshold of fluomine at or below which no deleterious sequelae may be expected. Care must be taken in extrapolating these results to man because of the highly irritating nature of the compound to eyes and lungs and because of its potent sensitizing properties.

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