REPORT OF TOXIC EFFECTS OF FLUORINE FOLLOWING SHORT-TERM INHALATION

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SUMMARY OF PROJECT

The purpose of this project was to determine the toxic effects from short-term (ranging from 5 to 60 minutes) exposures to fluorine in experimental animals and to determine concentrations which cause irritation in the human subject. Special equipment, including a chamber for exposures, was designed and built to handle fluorine safely. Analytical methods for the determination of the concentration of fluorine in air were developed.

Signs of intoxication from high concentrations of fluorine in air were marked irritation of the mucous membranes of the eyes and respiratory tract, and some irritation to the skin.

The LC50 (concentration calculated to kill 50% of the animals) was determined for 5, 15, 30, or 60 minutes of exposure in rats, mice, guinea pigs, and rabbits. There were no significant differences between the LC50's for the different species of experimental animals. These are tabulated as follows:

LC50 Values For Animals Exposed To Fluorine

Exposure Time (min.)	Rat Concentration mg/cuM	Rat ppm	Mouse Concentration mg/cuM	Mouse ppm	Guinea Pig Concentration mg/cuM	Guinea Pig ppm	Rabbit Concentration mg/cuM	Rabbit ppm
5	1085	700	935	600	-	-	1273	820
15	617	390	545	350	617	390	-	-
30	420	270	350	225	-	-	420	270
60	288	185	234	150	250	160	-	-

Dyspnea, lethargy, red nose, and swollen eyes were observed at concentrations equivalent to 50% of the LC50's. At concentrations which were 25% of the LC50's, there were only mild signs of intoxication, manifested by slight dyspnea and closed eyes. At lower concentrations, there were no gross signs of intoxication. Complete blood counts on these animals did not show significant changes due to fluorine.

Gross pathology following exposures near the LC50's was congestion, hemorrhage, and atelectasis in the lungs and some congestion and/or mottling in the liver. Following sublethal exposures, there was pathology in the lungs, liver, and kidneys. Survivors, sacrificed up to 45 days after such exposures, had congestion in the lungs and occasional congestion in the liver. There was some discoloration of the kidneys in animals sacrificed 7 to 14 days after exposure. When animals were sacrificed six months after exposure, the tissues had returned to normal.

Exposure to concentrations at or below 100 ppm for 5 minutes, 70 ppm for 15 minutes, 55 ppm for 30 minutes, or 45 ppm for 60 minutes caused no apparent effects in the animals.

Exposure of volunteer human subjects caused irritation of the eyes and nose. Irritation of the eyes was the most sensitive index of a subjective effect. Concentrations of 25 ppm were slightly irritating after five minutes of exposure. A concentration of 50 ppm was irritating within one or two minutes and concentrations of 75 to 100 ppm were very irritating within a few seconds.

Rats, mice and rabbits were exposed repeatedly, every 24 hours to one week, to fluorine for five to 60 minutes. Repeated exposures at the same concentration caused no more, and in some cases less, effects than a single exposure to the same concentration. Repeated exposures at low (apparently harmless) concentrations, made the animals less susceptible to the effects from exposure to a higher concentration. Apparently, some tolerance to fluorine was developed in these animals.

Studies on the formation of pulmonary edema indicated that very high concentrations of fluorine (lethal) can cause hemorrhage, but concentrations less than lethal cause edema in the lungs.

Studies of the activity of certain enzymes (succinic dehydrogenase, alkaline phosphatase and glutamic oxalacetic transaminase) in the lung, liver and kidney indicated that there was some increase in activity. In the lung, the activities of succinic dehydrogenase, alkaline phosphatase and glutamic oxalacetic transaminase were increased. In the liver, alkaline phosphatase and glutamic oxalacetic transaminase were increased, but succinic dehydrogenase was unchanged. In the kidney, glutamic oxalacetic transaminase was increased, while succinic dehydrogenase was unchanged. Alkaline phosphatase in the kidney appeared to be unchanged after 15 or 30 minutes of exposure, but appeared to be increased after exposures of 60 minutes.

Studies on the influence of the age of the animal upon the sensitivity to fluorine revealed that neither the very young nor the very old mouse was more susceptible to fluorine than the mature, young adult animal.

Studies on coagulation of blood of rats revealed no influence by the exposures to fluorine used. Apparently insufficient fluoride was absorbed to cause an effect.

Mice were exposed to fluorine in concentrations ranging from those causing no mortality to those causing 100% mortality. They were treated with ascorbic acid either before or after exposure. There was only a very slight indication that ascorbic acid (injected before exposure to fluorine) protected against lethality and pulmonary edema caused by fluorine.

Analyses of blood from dogs and rats were made for blood urea nitrogen, blood glucose, creatinine, uric acid, alkaline phosphatase, total protein and albumin. In rats the blood urea nitrogen and serum alkaline phosphatase levels were increased and there was a slight increase in fasting blood glucose. The creatinine, uric acid, total protein or albumin were not changed significantly. In dogs the blood urea nitrogen appeared to be slightly elevated, but none of the other parameters measured appeared to be changed significantly. It should be emphasized that these dogs were exposed to rather low concentrations of fluorine.

The storage of fluoride in the lungs, liver and kidneys of rats and dogs exposed to fluorine for 5 or 15 minutes was measured to determine the concentration in tissue in relation to concentration in air and to reflect rates of excretion and/or accumulation. At 24 hours after exposure there was poor correlation between the amount of fluoride in rat tissues and the

amount of fluorine in the air of the chamber, but the fluoride in all three tissues was higher than the fluoride in control rats. At 48 or more hours after exposure the fluoride in the lungs was not higher than controls. The fluoride in liver and kidneys was slightly higher than fluoride in control animals as long as one week after exposure. Dogs sacrificed 16 days after a single exposure had no more fluoride in the lungs, liver or kidneys than control dogs. Repeated exposures of dogs and rats for 3 or 4 times (48 hours apart) caused no more fluoride in these tissues than did a single exposure to the same concentration of fluorine. These data indicate that fluoride is found in the lungs, liver and kidneys soon after exposure to fluorine, and that it disappears more rapidly from the lungs than from the liver or kidney. The fluoride does not accumulate in these tissues even after intermittent, repeated exposures.

Urine was collected from rats and dogs, and was analyzed for fluoride. During the first 24 hours after a single exposure there was some correlation between the amount of fluoride excreted in the urine and the amount of fluorine in the air. The rate of excretion of fluoride decreased with time after a single exposure and was nearly normal on about the fourth day. Repeated exposures caused slightly higher rates of excretion than a single exposure. There was, however, no indication of a marked accumulation of fluoride.

Exposures to mixtures of fluorine and hydrogen fluoride were made to determine if additive, less than additive or more than additive

effects occur from the mixture of F_2 and HF. The LC50's in rats, mice and rabbits indicated that the lethal effects were closely related to the total concentration of fluoride. Therefore, the effects were essentially additive. Pathology (gross and microscopic) in the tissues of these animals was very similar to that found in tissues of animals exposed to fluorine or to hydrogen fluoride alone. Tissue concentrations (lung, liver and kidney) were closely related to total fluoride concentration in air, regardless of whether the fluoride was from fluorine or from hydrogen fluoride.

SHORT-TERM LC50s AND ANALYTICAL DEVELOPMENT

I. INTRODUCTION

The use of fluorine as an oxidizer for rocket propellants requires a knowledge of the toxic effects of fluorine following short-term exposures. Since this information had never been obtained, experimental animals were exposed to fluorine for short periods of time (one hour and less) and the lethal and subletal effects determined.

The concentrations in the exposure chamber were extremely important in this study. Since suitable analytical methods were not available, they were developed.

II. MATERIALS AND METHODS

A. Special Handling Equipment

In order to handle fluorine safely in the laboratory, special equipment was purchased from The Matheson Company, Inc. This included a safety enclosure and remote control system for a six-pound gas cylinder, a pressure regulating system (15F-670) with monel adapter (F70M), a hydrogen fluoride trap, a full view rotameter, back pressure indicator, and fluorine resistant metering valves (940F). All connecting tubing was ¼-inch copper with flared fittings. The tube to the backpressure indicator was fluorocarbon (Genetron) tubing. The backpressure indicator, was filled with fluorocarbon oil (Hooker Chemical Corporation, Fluorolube S-30). A nitrogen cylinder was connected to the system through a pressure regulator and needle valves in order to purge the system.

The hydrogen fluoride absorber was a 15-inch section of standard 2-inch steel or monel pipe with caps (¼-inch SAE inlet and outlet), thermometer well, electrical winding (for heating), and asbestos wrapping. The absorber was packed with sodium bifluoride ($NaHF_2$) pellets. Prior to use, it was reactivated by heating to about 300°C while purging with dry nitrogen. The heat treatment drove off HF from the sodium bifluoride to yield a porous, highly absorbent form of sodium fluoride (NaF). Completion of the activation cycle was determined with the aid of blue litmus paper (completed when no more acid formed). The absorber was cooled to room temperature and was then ready for use.

All equipment was assembled, as illustrated in Figure 1, and tested for leaks prior to use.

An additional safety factor was added by using cylinders containing fluorine diluted with nitrogen, instead of essentially 100% fluorine under pressure. These cylinders of specially mixed gas (6 to 9% fluorine in nitrogen) were prepared for our use by Allied Chemical Company, General Chemical Division.

B. Chamber for Exposures

The chamber for animal exposures was designed to meet two special criteria -- 1) materials inert to fluorine and 2) rapid entrance or egress of animals (for accurate short-term exposures) -- in addition to the usual criteria for such a chamber.

The chamber (illustrated in Figure 2) was constructed of stainless steel with neoprene rubber gaskets at movable interfaces. Neoprene rubber reacts only slightly with fluorine at these concentrations.

Rapid entrance and egress of the animals were accomplished by a sliding tray or drawer in the chamber. The tray has solid ends which seal in the open or closed position. With this tray, the animals can be put into the chamber or removed in approximately one second.

Any exposure chamber should have the least possible surface area per unit volume. A sphere is the ideal shape for this, but was not ideal for our working purposes. Therefore, the second-best shape, a cylinder, was selected. A cylinder whose diameter and length (or height) are equal gives the lowest surface-to-volume ratio of this shape. This chamber has a slightly longer length (34 inches) than diameter (24 inches) because of the length necessary for the dog and the

restriction of diameter to go into an existing hood opening. The dimensions, however, are not far from the ideal cylinder.

The volume of the chamber (252 liters) was selected so that the total animal volume in each experiment (with the number to used per group) would not exceed 5% of the chamber volume. The volume ratios of the experimental animals used were as follows:

Species	Individual Body Weight	Chamber Vol. Percent
Dog	11 kg	4.6%
Rabbit	3 kg	1.3%
Guinea Pig	0.5 kg	0.2%
Rat	0.25 kg	0.1%
Mouse	0.03 kg	0.01%

There are eleven ports in the chamber -- one for entry air, one for exit air, one for a thermometer, two for animals (only one used), and six for gas sampling.

The chamber was operated under dynamic conditions. As an added safety factor, a blower was connected to the exit stream to allow operation of the chamber under a slightly negative pressure (0.1 inch of water).

In order to obtain uniform distribution of the fluorine in the air of the chamber, the fluorine and air were first mixed in a mixing chamber before entrance into the exposure chamber. The air was dried by passing over Dierite, to prevent formation of hydrogen fluoride. Instead of single ports for entrance and exit of the air, special copper tubing was constructed with holes sized to allow a nearly uniform volume to flow through each hole. Air entered along the top of the chamber and left along the bottom of the chamber. A mixing fan was built into the rear of the chamber to facilitate uniform distribution.

The contaminated exit air was scrubbed through a 5% solution of potassium hydroxide before being released to the atmosphere (outside the

building).

C. Analysis of Air

There were two general problems of fluorine analyses in these studies. One was to determine the quantity of fluorine in the cylinder, and the other was to determine the concentration of fluorine in the exposure chamber.

1. Fluorine in Cylinder

The concentration of the fluorine in the cylinder was measured by allowing the fluorine-nitrogen mixture to flow through a packed column converter containing a mixture of sodium chloride (large crystal) and sodium fluoride. The chlorine, released by fluorine mole for mole, was measured by a Volhard titration.

The converter was a 24-inch length of black iron pipe with the ends capped. The caps were drilled and tapped for fittings to ¼-inch copper tubing. The mixture of fluorine and nitrogen flowed through the converter into two 500-ml Drechsel bottles connnected in series. The Drechsel bottles contained an alkaline potassium arsenite solution to absorb the chlorine. The Drechsel bottles were connected to a wet test meter for measuring the volume of gas being ananlyzed.

The potassium arsenite solutions from the two Drechsel bottles were combined into a 1000-ml volumetric flask and diluted to volume. A 100-ml aliquot of the diluted solution was withdrawn and nitric acid was added until the solution was acidic. The solution was then titrated with a 0.1 N silver nitrate solution until no further precipitate occurred and the a 10-ml excess silver nitrate was added. Nitrobenzene (10 ml) and ferric ammonium sulfate solution (5 ml) were added to the flask which

was stoppered and shaken. The mixture was then titrated with a standard 0.1 N potassium thiocyanate solution until the supernatant solution showed a faint reddish-brown color.

The amount of fluorine absorbed (in grams) was equal to [(ml of siver nitrate X normality) - (ml of thiocyanate X normality)] X 0.019 X the aliquot factor.

2. Fluorine in Chamber

Several different methods were used for the determination of fluorine in the air of the exposure chamber. It was assumed that some of the fluorine might react to form fluorides, particularly hydrogen fluoride, in the exposure chamber. Therefore, it was desirable to measure both fluorine and fluorides concurrently.

a. Fluorine - Colorimetric

A measured quantity of air containing fluorine was passed through an impinger (fritted glass) containing aqueous potassium iodide solution, (400 mg/l), made alkaline with sodium bicarbonate. The fluorine reacted with the potassium iodide to form potassium fluoride and iodine. The solution was acidified with acetic acid and allowed to stand for 10 minutes. The free iodine in the solution was then measured using a 0.01 or 0.1 N sodium thiosulfate solution and starch indicator. In order to check this method, the fluoride concentration (from potassium fluoride) was also determined by the method described below.

b. Fluoride - Colorimetric

A measured quantity of air from the chamber was passed through

an impinger (fritted glass) containing aqueous sodium hydroxide solution. The total amount of fluoride in the solution was measured by the colorimetric reaction with 4,5-dihydroxy-3-(p-sulfophenylazo)-2,7 naphthalene-disulfonic acid trisodium salt (Eastman Organic Chemicals, No. 7309) following our modifications to the method of E. Bellack and P. J. Schoubee, Anal. Chem., 30, 2032-2034, 1958.

This method briefly is as follows:

Reagent A - Eastman No. 7309 (0.958 gram) was dissolved in distilled water and diluted to 500 ml.

Reagent B - Zirconyl chloride octahydrate (0.133 gram) was dissolved in 25 ml of distilled water. Concentrated hydrochloric acid (350 ml) was added to the zirconium chloride solution, and the mixture was diluted to 500 ml with distilled water.

Equal volumes of reagents A and B were combined to produce a single reagent.

Reference Solution - Ten milliliters of reagent A were added to 100 ml of water. Ten ml of solution containing 7.0 ml of concentrated hydrochloric acid (diluted to 10 ml with distilled water) were added to the diluted reagent A. This solution was used to set the reference (zero) point of the colorimeter.

Standard Curve - The standard curve was prepared with sodium fluoride solutions containing 0.000 to 1.400 mg/l.

A 5-ml water sample (containing standard or unknown fluoride) and 1 ml of mixed reagent (equal volumes of A and B) were mixed well, and the optical absorbance read at 580 microns. If the absorbance reading of the

unknown sample fell beyond the range of the standard containing 1.400 mg/l, the procedure was repeated using a smaller aliquot.

The amount of fluoride in the unknown was calculated as follows:

$$X(mg/l) = \frac{A_o - A_x}{A_o - A_1}$$

A_o is absorbance of 0.00 fluoride standard

A_x is absorbance of unknown

A_1 is absorbance of 1.00 mg/l fluoride standard

c. Fluorine-Gas Liquid Chromatography

1. Preliminary Studies -- Information from other investigators indicated that fluorine could be analyzed by gas-liquid chromatography with a thermal conductivity detector. Therefore, in our first attempt to analyze for fluorine in the air, a thermal conductivity detector was used. Since it was assumed that one of the difficulties of these analyses would be separation of fluorine from air, some preliminary experiments were conducted to test different chromatographic column-packing materials. Although these materials were not the materials of choice for elemental fluorine itself (without being mixed with air), they were tested for separation of fluorine from air. It was thought that a column-packing support of diatomacous earth might eliminate the interference from air. A mixture of approximately 10% fluorine in dry nitrogen was used as the sample gas.

The following procedures describe the various conditions used in the preliminary experiments.

Constant Operating Conditions

Instrument: Micro-Tek GC o 2000-R equipped with a nickel-plumbing system (1) (Figure 3)

Detector: Thermal Conductivity constructed from nickel with Gow Mac filaments

Detector Current: 225 ma.

Detector Temperature: 110°C

Sampling Method: Gas sampling valve with 2 cc sample loop (Figures 4 and 5)

Conditioning Temperature and Flow for Columns: 70°C at 60 cc/min for 12 hours with ends of columns disconnected from detector.

Variable Operating Conditions For Each Experiment

Column Temperature: Ambient to 65°C

Carrier Flow: 25 cc to 150 cc/min

Inlet and Valve Oven Temperatures: Ambient to 75°C

2. Indirect Methods -- Additional experiments were conducted to detect fluorine indirectly by measuring chlorine released from sodium chloride. The chlorine was determined with thermal conductivity and with a thermionic flame detector.

The following operating conditions were established to provide successful separation and detection of fluorine as chlorine within six minutes.

Column: 20' x 1/4" copper, packed with 20% Kel F-10 on Kel F-300 polymer, 45-60 Mesh

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(1) Certain fittings necessary to introduce and direct sample throughout the chromatograph could not be obtained as nickel. Stainless steel or brass fittings were used in such instances. All tubing through which the sample passed was constructed from nickel. The gas sampling valve contained a Hastelloy sampling stem.

Column Temperature: 62°C

Carrier Gas Flow: 60 cc/min helium (40 psi, 6 rotameter setting)

Detector #1: Thermal Conductivity
(a) Temperature, 125°C
(b) Filament current, 300-350 ma.

Detector #2: Thermionic Flame
(a) Temperature: 125°C
(b) Hydrogen Flow: 40 cc/min
(c) Air Flow: 400 cc/min
(d) Background Current: Coil coated with saturated sodium sulfate to give 2 X 10^{-9} amps background with 1.6 amps applied coil current

Fluorine Conversion Column:

(a) 1' x 1/4" nickel, packed with 28-45 mesh sodium chloride
(b) Temperature: 210°C

Inlet Block and Exit Block Temperature: 50°C

Sample Concentration: (a) 10% fluorine in N_2
(b) 1% fluorine in N_2

Recorder: MT-11, 1 mv, 1 sec response, chart speed 1"/min

3. Electron Capture -- A few experiments were conducted using an electron-capture detector. The instrument was the same as described previously and the operating conditions were as follows:

Constant Operating Conditions:

Column: 4' x 1/4" glass, packed with 10% QF-1 on Fluropak
Carrier Gas Flow: 40 cc/min nitrogen
Detector Temperature: 180°C
Detector: Tritiated electron capture
Inlet Temperature: 24°C
Barometric Pressure: 29.98 in.
Sampling Method: Gas-tight syringe

Variable Operating Conditions:

Column Temperature: 24°C to -78°C
Sample Size: 5 to 10 microliters

3. Experimental Animals

The young adult male and female rats (weighing from 150 to 300 grams) used in these studies were Osborne-Mendel strain albino rats raised in our colony from original FDA stock. The mice were young adult Swiss-Webster white, weighing from 20 to 40 grams, raised in our colony. The young adult English-strain guinea pigs weighed from 300 to 500 grams. Rabbits were of a young adult New Zealand strain weighing from 2.0 to 3.5 kg. The dogs were young adult mongrels of mixed breed and weighed from 8.5 to 10.5 kg.

III. RESULTS

A. Fluorine By Colorimetric Method

Since a definite method was not available for the determination of fluorine, the potassium iodide method was developed. Theoretically, the fluorine (F_2) should react with the potassium iodide (KI) to release iodine (I_2) mole for mole in alkaline medium. Upon acidification the iodine could be measured. This was tested and the method proved successful. Theoretically, the KI should not react with hydrogen fluoride (HF). This was tested and checked. The HF did not release I_2 from KI. The pH of the solution was also tested and found to be unchanged, indicating that gaseous HF was not trapped in the KI solution.

The suitability of the KI method for fluorine and the percent recovery were tested over concentrations of fluorine in the air ranging from 5 to 85,500 ppm. Adjustment of the volume of air and/or the concentration of KI solution allowed measurement of fluorine over the entire range. The mean recovery was 99.4% with a standard deviation of 2.4%. (See Table 1).

The air from the chamber was withdrawn with a pump through a short piece of glass tubing connected to the wash bottle by a short piece of rubber tubing. The glass and rubber tubing were passivated with fluorine from the mixing chamber before being used. The same tubing was used for all tests because it was found that "new" tubing apparently reacted with the fluorine causing as much as 40% loss in recovery during the first sampling. The air passed through the fritted glass impinger, the KI solution, and then through a wet test meter (which measured the volume of air).

Although the volume of air sampled depended on the concentration of fluorine, the size of the sample was one to three liters of air in the majority of the samples.

B. Fluorine By Gas Chromatography

1. Preliminary Studies -- The results of the experiments to determine fluorine by gas chromatography using different columns and the thermal conductivity detector were as follows:

a. Experiment No. 1

(1) Column: 20' x 1/4" copper, packed with 33% Kel F-10 oil on 60/80 Chromosorb W. Installed from left inlet to detector.

(2) Sampling: As shown in Figure 3, using sample loop connected to ports shown in Figure 3 (14).

(3) Gas Sampling valve was pulled to out position.

(4) Gas sample bomb was connected to port connection shown on Figure 3 (11) via a 1/8"-diameter copper tube.

(5) A 1/8"-diameter copper tube was connected to (9) (same figure) to direct vent gas eluting from sample loop during purge into fume hood. (Exit end of tube was vented and bubbled into a beaker containing Kel F-10 oil).

(6) Sample bomb was opened slightly to allow about 20 cc/min to purge through sample loop.

(7) After a five-minute purge, sample bomb valve was closed.

(8) After allowing five seconds for sample in loop to reach atmospheric pressure, sample valve stem was pushed "in."

(9) Twenty samples were run in the above manner with no evidence of a fluorine peak as column temperature and flow were adjusted between runs throughout parameters listed above.

b. Experiment No. 2

The possibility of fluorine absorption and retention in the chromatograph was considered. Therefore, an attempt was made as follows to condition the system throughout so that the fluorine would not react:

(1) The carrier gas #1 entrance line was disconnected from port 4 of gas sample valve, and the bomb containing 10% flucrine in air was connected at port 4 of valve.

(2) Sample loop was removed and attached inside valve oven directly across port 2 and port 5 of gas sample valve.

(3) Sample bomb was opened to allow about 20 cc/min of sample to purge directly through valve, loop, column, and detector (filaments off) for two hours.

(4) Sample bomb was disconnected, carrier entrance line again connected to port 4 of sample valve, and bomb again connected to sample "in" port (See Figure 3 (11)).

(5) After flushing carrier through column for 20 minutes, successive injections were made without success.
Note: It was observed during passivation procedure that the bottom six inches of column became extremely hot, and that fluorine was reacting with the Chromosorb W column packing material and that such diatomaceous material would not work.

c. Experiment No. 3

(1) Procedure No. 2 was repeated as described above except a 20' x 1/4" copper column packed with 30% Arochlor 1242 on 60/80 mesh Chromosorb P, acid washed, was used.

(2) No success was obtained on about ten injections.

d. Experiment No. 4

(1) The 1/8" S S line from port 3 of gas sample valve to inlet (9) (Figure 3) was disconnected and a short 1/8"-diameter copper tubing was installed from port 3 of the valve to bottom fitting on valve-oven terminal strip. (See (3)).

(2) A 20' x 1/4" copper column packed with 15% Kel F-10 oil on Fluoropak-80 (a Teflon support) was installed from bottom fitting on valve-oven strip to the detector.

(3) The system was again passivated as in experiment No. 2.

(4) Twenty injections were made with no success.

e. Conclusions with Diatomaceous Earth Columns

From the above work, it became obvious that, due to a reaction in the column, a diatomaceous earth column-packing support would not allow fluorine analysis. Since fluorine is so highly reactive, the use of an inert support, such as a flurocarbon, should be used. A liquid phase, such as Kel F-10, should be the choice. Due to the reactivity of fluorine, there is little possibility that liquid phases other than the Kel-F family will suffice for fluorine analysis.

2. Indirect Method

The early investigations indicated, therefore, that fluorine and air could not be separated on these chromatographic columns. Use of stationary phases or supports capable of effecting such a separation resulted in excessive reaction with fluorine. Consequently, it seemed necessary to attempt the separation either by (1) subambient distillation or (2) converting fluorine to chlorine which could be separated from air by carefully choosing the proper column and determining the optimum operating conditions. The latter choice seemed to offer the most advantages.

The ultimate goal of this investigation was to determine the limit of detectability of fluorine as chlorine using a thermal conductivity detector. However, since a thermionic flame detector, specific for halides, showed promise for this application, such a detector was connected in series with effluent gases from the thermal conductivity detector to check the lower limits of fluorine detection with such a detector.

A one-foot, 1/4-inch diameter nickel column was packed with 28-45 mesh sodium chloride, wrapped with electrical heating tape, and installed between the gas sampling valve and the column inlet system. The sodium

chloride had been melted at 1500°F, cooled, crushed, and meshed to obtain uniform particle size. The column was packed and purged for 24 hours with nitrogen. The column was heated to 325°C during the purging procedure.

The chromatographic column-packing material was prepared using the conventional bowl method (see operating conditions for stationary phase and support). The column was installed in the gas chromatograph and allowed to condition overnight at 70°C with a 60 cc/min helium purge. The exit end of the column was disconnected from the detector throughout the purging step. A 10% mixture of chlorine in the air was connected at the carrier gas entrance port on the chromatograph and allowed to flow at 10 cc/min through the chromatographic column and vented to a fume hood. The sodium chloride column was then installed in the system.

A 10% mixture of fluorine in nitrogen was used for part of this project. The mixture was further diluted to a concentration of 1% fluorine for the remainder of the studies. It was assumed that major quantities of fluorine would be easy to detect and would offer no problems, providing the sample size was limited to avoid damage to filaments by chlorine. It was believed that fluorine concentrations less than 10% could offer problems. Consequently, emphasis was directed toward the successful analysis of lower concentrations. At the 1% concentration level, the

following results were reproducibly obtained. (A chromatogram comparing the peaks from both detectors is shown in Figure 6.

a. Thermal Conductivity Detector

(1) Operating Conditions:

Attenuation: X1 (maximum sensitivity)

Filament Current: 350 ma. (maximum sensitivity)

(2) Observations:

(a) A peak height of 11 centimeters was obtained (25.5 cm represents full scale).

(b) At X1, the attenuation necessary to obtain the Cl_2 peak, the response for nitrogen was such that it interfered with the chlorine peak. The N_2 peak had recovered to within 4.2 cm of the baseline when chlorine began to elute.

(c) Ten injections resulted in complete reproducibility of the peak heights for chlorine.

(d) Evidence indicated that the filament current was being operated too high. The baseline displayed about one division of noise after 24 hours operation at 350 ma. with successive sample injections. It is suggested that about 300 ma. filament current be used for sustained operation with large-sample, frequent injections. At 300 ma., the sensitivity would be diminished somewhat from that shown on the attached chromatogram.

b. Thermionic Flame Detector

(1) Operating Conditions: (See conditions section)

Attenuation: 10^2 Input, 256 Output

(2) Observations:

(a) A peak height of 20.2 centimeters was obtained for chlorine.

(b) The attenuation could be increased by 100 fold with less than two divisions of baseline distortion.

This indicated that fluorine, 100-fold less concentrated, could be detected easily at the same operating parameters using the thermionic flame.

(c) Response for N_2 was so small that no interference on the fluorine peak was obtained. Carrier flow rate and column temperature could be advanced to allow Cl_2 to elute much earlier, thus reducing analysis time and favoring detection of even lower concentrations of fluorine.

Using either detector, the chromatographic system required about three 2-cc injections of chlorine gas to passivate the inlet system and column before successful results were obtained. Time did not allow for a thorough study to determine the frequency of passivation required, but evidence indicated that the system required passivation whenever two or three hours elapsed between sample injections. With a single 2-cc chlorine injection, peaks from sample injections would grow with successive injections and finally stabilize. The number of sample injections before stabilization occurred depended upon the fluorine concentration in the sample. For example, a 10% fluorine mixture stabilized in peak height after four or five samples when preceded by a single 2-cc chlorine injection. Brief investigations indicated that two or three 2-cc chlorine injections were adequate to passivate the system regardless of the fluorine concentration in the sample.

c. Conclusions from Indirect Method

Although time did not permit a complete investigation of all variables involved, results indicated that little difficulty will be encountered analyzing for fluorine in the air, O_2 or N_2 at concentrations

down to the 1% level by gas chromatography, using a thermal conductivity detector. Determination of concentrations down to the 100-ppm level should be obtainable providing sample holdup at reaction sites can be minimized or eliminated with periodic injections of chlorine or fluorine to passivate the system when using a thermionic flame detector.

Cursory studies indicated that the displacement of chlorine by elemental fluorine from a heated sodium chloride column appears quantitative. However, time did not allow for a thorough study of this possible variable. Further work should be done along this line. At any rate, the displacement method appears to be a feasible approach to fluorine analysis.

In order to recommend the choice chromatographic sampling and detection system for fluorine analysis, several things must be considered. These investigators would select the systems based on (1) the detection level of fluorine required, and (2) the impurities contained and whether or not they must be separated and detected. If only the detection and quantitation of fluorine is of interest, the two major considrations would be (1) the sample size to inject (dependent on concentration) and (2) the detector required. If the separation and quantitation of impurities are required, the sample size, the detector, and the column arrangement are significant.

If the separation and quantitation of air components (O_2 and N_2) or other fixed gases such as CO and CO_2 are required, the thermal conductivity detector is required since the thermionic detector is specific only for halogen and would not respond to other components. A column-switching

and plumbing arrangement capable of switching the air peak into a 6' x 1/4" molecular sieve column is also necessary since O_2 and N_2 have identical retention times on the Kel-F Column. Sample sizes would apply as above.

3. Electron Capture

Another approach to the analysis for fluorine in air was made. The sample of dilute mixture (about 1%) of fluorine in air was taken with a gas-tight syringe and injected directly into a chromatographic column (Fluropak with 10% QF-1). Sample sizes were quite small and ranged from 5 to 10 microliters of air. The gases were measured with a tritiated-electron capture detector. When the column was at ambient temperature (24°C), there was no separation of fluorine from air. After cooling the column to -78°C (with dry ice and acetone), there was a separation of fluorine from air. It was concluded, therefore, that with this technique, fluorine and oxygen were resolved at low temperatures provided both were in microgram quantities. It has not been determined if the column can be modified so that fluorine may be measured at lower concentrations in air using the electron-capture detector.

C. Exposures of Animals

1. Preliminary Studies

After the special handling equipment, exposure chamber, and analytical methods were ready, exposure of experimental animals was to be started. The chamber and stainless steel cages to hold the animals were passivated first with the fluorine-nitrogen mixture from the cylinder. Then air was mixed with the fluorine to prepare a concentration of approximately 5000 ppm. This mixture was also passed through the chamber containing the empty

cages. Following this passivation, the air in the chamber was analyzed for fluorine. It was found that the theoretical (nominal) and analyzed concentrations agreed well over a wide range of concentrations of fluorine in air (See Table 1). It was also found that the concentration in the chamber remained constant under the dynamic conditions required for exposure.

The influence of opening and closing the drawer of the chamber on the concentration of fluorine in the chamber was determined. Closing the drawer, after the desired concentration was reached with the drawer in the open (sealed) position, caused only a slight decrease in concentration. Depending on the magnitude of the initial concentration, between 92 to 99% of the total remained after the door was closed. The concentration was then maintained constant by slightly adjusting the fluorine and air flow rates after the animals were introduced.

The animals were removed by quickly reopening the drawer after the desired exposure time had elapsed. The effect of reopening the drawer did necessitate adjusting the concentration for subsequent exposures.

Since the volume of 10 rats and 10 mice was only about 1% of the volume of the chamber, it was assumed that they could all be exposed at the same time. However, during the exposures, the analytical concentrations of fluorine in the chamber were much lower than the theoretical (nominal) concentrations. Although the fluorine was being introduced into the chamber at a rate which should have held the concentration constant, the concentration (as analyzed) decreased rapidly during the first five minutes and continued to decrease at a different rate during the rest of

at a different rate during the rest of the exposure. Obviously this caused considerable consternation.

After again checking all aspects of the analytical methods to ensure their accuracy, it was concluded that the fluorine concentration was actually decreasing during these exposures. A systematic check of the chamber and all components revealed that the theoretical concentration could be maintained very accurately in the empty chamber, as found previously.

The effect of opening and closing the drawer was again checked. Since the concentration decreased when the drawer was opened, this did not create a problem because the animals were being removed from the chamber anyway. Closing the door (by following the techniques specified in the procedure) ca ed a very slight decrease in concentration, as found previously.

Introduction of the empty animal cages, previously passivated, did not change the concentration!

It was then concluded that the animals themselves were causing the decrease in concentration. The next question was whether the fluorine was reacting with the moisture in the expired air, adsorbing or absorbing to the fur and skin, reacting with the moist mucous membranes, or with all of these. An attempt was made to compare the loss due to reaction with the skin and fur to the loss by all possibilities combined. The loss du to skin and fur was determined by exposing nonbreathing (dead) animals. The results, shown in Figure 7, indicated that approximately

50% of the loss under these conditions was due to reaction with the skin and fur. Therefore, the other 50% lost was due to reaction with the expired water vapor and/or to reaction with the mucous membranes of the respiratory tract.

The problem then was to determine the operating conditions for exposing the animals to a constant concentration of fluorine. Since some fluorine was reacting with the animals, it was believed that adjustment of the concentration being introduced into the chamber and/or the number of animals might compensate for the loss. The number of rats was reduced to five (with 10 mice) and fluorine was introduced at a faster rate. With an initial concentration of 400 ppm, the concentration decreased during the first 15 minutes of exposure and then remained constant, as shown in Figure 8. When the initial concentration of fluorine was higher (550 ppm), the decrease in concentration was even more rapid, and it was lower before becoming constant (also shown in Figure 8).

The fluorine would have to be introduced very rapidly at first, with a decreasing rate, to compensate for such losses. It is practically impossible to change the concentration in an exposure chamber rapidly enough to compensate for such losses and still keep the concentration constant. With the available equipment, the adjustments could not be made. By adding the fluorine as rapidly as possible and still keeping other desirable criteria, the theoretical concentration was increasing as shown in Figure 9. Under these conditions, the concentration of fluorine became constant for the period of 20 to 60 minutes after introduction of the animals. Such a variance in concentration was unacceptable.

Another problem also arose from the attempt to expose 10 rats and 10 mice at the same time. The fluoride concentration (which did not decrease as rapidly as the fluorine concentration) was very high and essentially the same as the fluorine concentration. (An example is illustrated in Figure 9.) Under these conditions it would be impossible to distinguish effects due to fluorine from those due to fluoride or from those due to the mixture.

Therefore, in order to maintain a constant concentration of fluorine in the chamber, the number of animals was reduced so that no more than two rats (usually only one) were exposed at any one time. An example of the theoretical and analytical concentrations of fluorine in the chamber during the exposure of one rat is illustrated in Figure 10. It should be added that with one rat in the chamber, the fluoride concentration was negligible. The only animals exposed in groups were mice. When 10 mice were exposed at the same time, fluorine concentrations could be maintained.

Although the data from these preliminary tests could not be used for the determination of effects from exposure to constant concentrations of fluorine, they were valuable for establishing criteria for the additional exposures to follow. They also gave considerable insight into the behavior of fluorine in air after contacting organic matter.

After approximately 50 different exposures (utiliziig 220 mice, 200 rats, 3 guinea pigs, and 3 rabbits), operating criteria were established, and the tests to determine effects from fluorine were started.

2. Signs of Intoxication and Dose Response

a. Lethal Concentrations

The concentrations causing fatalities after 5, 15, 30, and 60 minutes of exposure were determined first in rats, using 10 animals per group. From these concentrations, the LC_{50} (Lethal Concentration for 50% of a group of animals) was calculated for each time interval, using the method of Litchfield and Wilcoxon (J. Pharm. Exper. Therap. 96: 99-113, 1949). The LC_{50}'s (shown in Table 2) were: 5 minutes -- 700 ppm, 15 minutes -- 390 ppm, 30 minutes -- 270 ppm, and 60 minutes -- 185 ppm.

The LC_{50}'s for mice, using 10 animals per group at each of the four intervals of exposure time, were then determined and are shown in Table 2. They were: 5 minutes -- 600 ppm, 15 minutes -- 375 ppm, 30 minutes -- 225 ppm, and 60 minutes -- 150 ppm. While the LC_{50}'s for mice were lower than those for rats, the differences were not statistically significant.

The LC_{50}'s for guinea pigs, using five animals per group, were determined at two intervals of exposure time to compare effects between species and to compare the slope of the LC_{50}'s (plotted against time) to the other species. The LC_{50}'s (shown in Table 2) were 15 minutes -- 395 ppm and 60 minutes -- 170 ppm. These LC_{50} values and the slope were essentially the same as the rat.

The LC_{50}'s for rabbits, using five animals per group, were determined at two intervals of exposure time, like the guinea pigs, to compare effects and slope of LC_{50} vs. time to the other species. The LC_{50}'s (shown in Table 2) were: 5 minutes -- 820 ppm and 30 minutes -- 270 ppm.

The LC_{50} following five minutes of exposure was higher (820 ppm) than for the rat (700 ppm). The difference, however, was not significantly different statiscically. The LC_{50} following 30 minutes of exposure was the same as the rat (270 ppm).

When the LC_{50}'s for all four species at the different exposure times were compared, they were essentially the same and not significantly different statistically, with one exception. Following five minutes of exposure, the difference between the mouse (600 ppm) and the rabbit (820 ppm) was significant at 95% confidence limits. While these two values are significantly different "statistically", it is doubtful that the difference is real.

The survivors were observed daily and weighed weekly for 14 days after exposure, and were then sacrificed for pathology.

Signs of intoxication at the lethal concentrations included irritation of the eyes and nose, as shown by conjunctivitis, pawing at the nose, increased secretions, and sneezing. Dyspnea, loss of body weight, general weakness, and death were observed. The loss of weight appeared to be nonspecific, apparently due to anorexia from a "sick" animal. At concentrations in the general range of LC_{40} to LC_{50}, very few signs of intoxication were observed immediately after exposure. Frequently, the animals looked quite well (with some irritation of the eyes and nose) when removed from the chamber. Dyspnea and lethargy were not observed until several hours after exposure.

With the exception of very high concentrations (LC_{90} to LC_{100}), death occurred approximately 12 to 18 hours after exposure. A few deaths were recorded about 24 hours after exposure. In general, if an animal lived for 48 hours, he survived the 14-day observation period.

b. Sublethal Concentrations

After the LC_{50}'s were established in all four species for the different periods of exposure, animals were exposed at lower concentrations to observe effects. The concentration for each period of exposure (5, 15, 30, or 60 minutes) was reduced each time by one-half, with the LC_{50} as the base value. In other words, animals were exposed to 50%, 25%, 12.5%, and 6% of the LC_{50} as shown in Table 3.

At 50% of the LC_{50}'s, there were marked signs of intoxication manifested by irritation to the eyes and respiratory tract. At concentrations which were 25% of the LC_{50}'s, there were only mild signs of intoxication, manifested by slight dyspnea and closed eyes. At lower concentrations, there were no gross signs of intoxication. Although there were no visible signs of intoxication at concentrations equivalent to 12.5% of the LC_{50}'s, animals were exposed to lower concentrations (6% of the LC_{50}'s) to check for microscopic changes in the organs and tissues.

The signs of intoxication occurring in the different species following exposures of different duration are tabulated as follows: 5-minutes exposure, Table 4; 15-minute exposure, Table 5; 30-minute exposure, Table 6; and 60-minute exposure, Table 7.

Complete blood counts, including hemoglobin, hematocrit, erythrocyte count, total leukocyte count, and differential leukocyte count, were made on rats (5 animals per group) and on dogs (2 animals per group). The blood samples were withdrawn from the tail of the rat and from the saphenous vein of the dog. Rats were exposed for 15 minutes at concentrations of

107, 125, 220, and 329 ppm; and for 60 minutes at concentrations of 98, 104, 111, 134, and 182 ppm. Dogs were exposed for 15 minutes at concentrations of 39, 58, and 93 ppm; and for 60 minutes at concentrations of 38, 68, and 109 ppm. The exposures of the rats in particular were rather severe because it was believed that any effect should be elicited and then a dose-response determined. The blood counts were made before exposure and on the second, seventh, fourteenth, and twenty-first days after exposure. The data did not show significant changes in any of these elements of blood, even after quite severe exposure to fluorine.

It is interesting and worthy to note that the clotting time of the blood appeared to be prolonged. This was a general observation and not carefully measured, but certainly warrants further investigation.

3. Gross Pathology

An autopsy was performed on every animal which was exposed. Animals that succumbed were autopsied immediately after being found dead. Survivors of the LC_{50} determinations were sacrificed 14 days after exposure. Animals exposed at lower concentrations were sacrificed serially -- immediately after exposure as well as 1, 2, 4, 7, 14, 21, and 45 days after exposure. The objects of serial sacrifice were to determine how soon after exposure an effect could be observed and how soon after exposure a damaged tissue would revert to normal.

a. Rats

The lungs of the rats showed by far the greatest change of any organ or tissue following exposure to fluorine. The rats showed very slight evidence of damage to the kidneys, which became apparent 14 or more days after exposure. There was some discoloration (mottling)

in the livers of some animals, however the incidence occurred at random and could not be correlated with degree of exposure to fluorine.

The gross pathology in the lungs, graded from 5 (severe) to N (no change), and the ranges of concentrations related to these changes are summarized in Table 8. Grade 5 changes in the lungs occurred at concentrations near the LC_{50}. Lower concentrations at each period of exposure caused less effects in the lungs. There were no gross changes observed in the lungs at concentrations of 100 ppm for 5 min., 70 ppm for 15 min., 55 ppm for 30 min., or 45 ppm for 60 min. (and lower).

It can be seen from Figure 11, which illustrates the data in a graph, that the slopes of the lines between the different grades of lung pathology are slightly different from the slope of the LC_{50}. This indicates possibly that lung damage is more closely related to concentration than to dose (concentration X time) when compared to lethal effects.

b. Mice

From the LC_{50}'s it appeared that the mouse was at least as susceptible to fluorine as any other species tested, if not more susceptible. Therefore, more mice than most of the other species (about as many rats as mice) were exposed at sublethal concentrations to observe effects. Another reason for using mice was that 10 animals could be exposed at one time, assuring uniform exposure to all animals in the group. The gross changes in the lungs and their relation to concentration also are included in Table 8 and Figure 11.

The mice were sacrificed serially to determine effects following sublethal exposures. The most marked changes were found in the lungs.

The data showing the degree of gross pathology in the lungs, as well as differences in effect up to 45 days after exposure, are presented in Table 9. Since 20 animals were exposed at each concentration, two or three animals were sacrificed at each of the eight periods of time after exposure. The grades of damage to the lung varied somewhat due to differences in the reactions of animals with the same exposure.

In general, the effects increased as the concentration increased at each exposure period. There was a dose-effect relationship between damage to the lung and exposure. The slope of the dose response, however, was slightly different from the slope of the LC_{50} values as shown in Figure 11. This indicates that (like the rat) the degree of lung damage apparently is more closely related to concentration than the lethal effects.

At each period of exposure, the concentration was decreased until no gross effects were found in the lungs. The dividing lines between no gross effect levels in the lungs and very slight congestion (Grade 1) were -- 5 minutes, 100 ppm; 15 minutes, 70 ppm; 30 minutes, 55 ppm; and 60 minutes, 45 ppm. At higher concentrations, some damage was observed in the lungs. At lower concentrations, there were no gross effects.

As expected, concentrations near the LC_{50} caused very marked diffuse congestion with ecchymotic or petechial hemorrhages (Grade 5). Lower concentrations cause diffuse congestion in the lungs ranging from severe (Grade 4), to moderate (Grade 3), to mild (Grade 2), to very mild (Grade 1). This grading system was selected for ease of handling the data. (A number was easier to handle than a description.) The demarcation between any two grades of damage is not sharp because of biological variation among the animals. As seen in Table 9, however, the lung damage is quite well correlated with exposure to fluorine.

Serial sacrifice of the animals revealed that the effects were not appreciably worse after exposure. There was some regression of the changes in the lungs which started about 7 days after the exposure. In most cases, the lungs were normal 45 days after exposure if only mild damage was observed soon after exposure. With more severe damage, however, the lungs showed recovery, but still some effects, 45 days after exposure.

In the mice, there was some evidence of gross damage to the liver and kidneys following sublethal exposures. This appeared mainly as a change in coloration of both organs. At lethal concentrations, there was little or no grossly visible damage to the liver or to the kidney. It is probable that if the damage is due to a cumulative effect, the animals exposed to high concentrations did not live long enough to develop the changes. At sublethal concentrations, there was little or no damage to either organ of animals sacrificed up to two days after exposure. By the seventh day after exposure, however, there was some damage in a few animals. The overall incidences of damage to the liver and kidney were 15% and 17%, respectively. On the fourteenth day after exposure, the incidences of damage to the liver and kidney were 30% and 29%, respectively. On the twenty-first day after exposure, the incidences of damage to the liver and kidney were 48% and 59%, respectively. On the forty-fifth day after exposure, the incidences of damage to the liver and kidney were 75% and 85%, respectively.

It should be emphasized that low concentrations, which caused no effect in the lungs, did not cause effects in the liver or kidneys.

c. Guinea Pigs

The only organ or tissue which showed gross pathology in guinea pigs was the lung. The degree of gross change and its relation to concentration of fluorine are summarized in Table 10. Lethal concentrations caused severe congestion and hemorrhages, as observed with other species. As the concentration was decreased, the degree of gross pathology also decreased. The guinea pigs were no more susceptible to fluorine than rats or mice and actually were slightly less susceptible, although not to any significant degree.

d. Rabbits

The only gross changes observed in the organs and tissues of rabbits were in the lungs. All other organs were normal. Following five minutes of exposure, there were no changes at 134 ppm and lower. Mild, diffuse congestion in the lungs (grade 2) was observed at 386 ppm. At higher concentrations (452 ppm and above) there was marked congestion with hemorrhages. One rabbit exposed to 1588 ppm survived for four days. The lungs showed severe congestion with hemorrhages. These data are summarized in Table 11.

Exposures to rather low concentrations were made because of previous reports (in the literature) that rabbits are more susceptible to fluorine than rats or mice. These results do not indicate that the rabbit is more susceptible.

Serial sacrifice of rabbits at 1, 2, 4, 7, 14, and 21 days after exposure showed little or no change in gross pathology during this period, with one exception. At the lower concentrations (about 150 ppm for 5 minutes or 70 ppm for 30 minutes), some mild congestion (grade 1

or 2) was observed in the lungs one or two days after exposure. By the seventh day after exposure, the changes had disappeared and the lungs were normal.

Sacrifice of rabbits 45 days after exposure to higher concentrations revealed that damage to the lung had regressed. For example, exposure of five minutes at 450 to 770 ppm caused severe congestion and hemorrhage (grade 5) in the lungs one day after exposure. At the same concentration, there was only very mild congestion (grade 1) in the lungs 45 days after exposure.

e. Dogs

The only definite gross pathology related to exposure to fluorine was in the lungs. These data are summarized in Table 12. Following a 60-minute exposure to concentrations from 84 to 109 ppm, there was slight congestion in the bronchi and small, round lesions of congestion in the lobes of the lungs. These lesions were 0.5 to 1.5 cm in diameter and bright red in color. At lower concentrations, the lungs were normal.

Following a 15-minute exposure to concentrations of 92 to 100 ppm, there was very slight congestion in one lung, but the other lung appeared normal. Lower concentrations caused no gross damage to the lungs.

If a concentration produced damage to the lungs, it was not worse on the fourteenth day after exposure. On the other hand, it had not regressed, at least very much, by the fourteenth day. Dogs were not sacrificed serially at 21 or 45 days to check for regression.

4. Histology (Micropathology)

The organs and tissues of the animals exposed to fluorine were fixed in 10% buffered formalin, sectioned and stained with hematoxylin and eosin for histological examination.

a. Lethal Concentrations

(1) Lung: Since the most obvious changes found grossly were in the lungs, sections of lungs showing severe, gross changes were examined to determine the nature of this severe effect. These lungs had necrosis and hemorrhages into the alveolar spaces. In addition to determining the nature of this severe damage, lungs from several species were compared. The changes were similar in the different species.

The following descriptions of sections of lungs are presented as examples.

Rat D-109 (701 ppm for 5 min) Severe hemorrhages into alveolar spaces with exudation. Coagulation necrosis of alveoli with peribronchial lymphocytic proliferation.

Rat D-131 (400 ppm for 15 min) Diffuse hemorrhages throughout alveoli. Focal nodular lymphocytic infiltration. Marked vascular engorgement with hemorrhages into alveoli.

Rat D-107 (556 ppm for 5 min) Nodular lymphocytic hyperplasia. Congestion of alveoli with hemorrhage and exudation. Very little inflammatory reaction.

Rat D-143 (200 ppm for 60 min) Coagulation necrosis of vascular walls with resultant hemorrhages into surrounding tissues and alveoli.

Mouse D-61 (150 ppm for 60 min) Coagulation necrosis of pulmonary vascular walls with hemorrhage into alveolar spaces.

Guinea Pig D-188 (440 ppm for 15 min) Generalized exudation into alveolar spaces. Focal areas of necrosis of alveolar septal cells with infiltration by lymphocytes and macrophages.

Rabbit D-208 (770 ppm for 5 min) (4 Slides) Severe coagulation necrosis One focal area of lymphocytic and macrophage infiltration. Massive hemorrhages into alveolar spaces with exudation.

(2) Liver: Following exposures which were lethal or near the lethal concentrations, some gross pathology was seen also in the liver. The following examples describe the type of change seen microscopically. Tissues from the same animals used as examples previously (lung) are presented to allow correlation of microscopic observations of both liver and lung.

Rat D-109 (701 ppm for 5 min) Coagulation necrosis diffusely scattered throughout entire hepatic tissue. Massive periportal hemorrhages and exudation throughout entire hepatic tissue.

Rat D-131 (400 ppm for 15 min) Diffuse cloudy swelling or parenchymatous degeneration of hepatic tissue.

Rat D-143 (200 ppm for 60 min) Acute congestion and hemorrhage into hepatic parenchyma. Coagulation necrosis of vascular walls resulting in generalized hemorrhage.

(3) Kidney: Following exposure to concentrations which were lethal, there was no apparent damage to the kidneys.

b. Sublethal Concentrations

Tissues from animals exposed to sublethal concentrations, with subsequent sacrifice, also were examined microscopically. This was done to determine the organs and/or tissues damaged, the types of

changes, the difference in effects between species, the correlation of response with concentration and time of exposure, the correlation of degree of gross with histological damage, the onset or disappearance of an effect (by serial sacrifice), and the "no effect" levels.

The following descriptions from microscopic examinations of the lungs were selected to show that the changes in a small section of tissue sometimes were similar following exposure to different concentrations. However, the total area involved (from gross observations) was quite different. All the animals were exposed for 15 minutes to fluorine at different concentrations.

Rat D-53 (350 ppm for 15 min) Sacrificed day 1. Grade 5 gross. Massive hemorrhages throughout lung parenchyma. Infiltration of leukocytes (mainly lymphocytes) superimposed over coagulation necrosis of alveoli.

Rat D-53A (350 ppm for 15 min) Sacrificed day 7. Grade 5 gross. Nodular lymphoid hyperplasia throughout lung parenchyma. Marked proliferation of septal cells, macrophages, and lymphocytes. Vascular congestion with perivascular hemorrhage.

Rat D-53B (350 ppm for 15 min) Sacrificed day 14. Grade 5 gross. Nodular lymphocytic infiltration. Nearly complete atelectasis due to proliferation of septal cells, lymphocytes, and macrophages. Vascular and perivascular congestion and exudation with hemorrhages into tissues.

These 3 preceding tissues show a progressive proliferative inflammation proceeding from hemorrhage to leukocytic response, finally resulting in an abundance of septal cells, macrophages, and lymphocytes with concurrent loss of alveolar spaces.

Rat D-135 (322 ppm for 15 min) Sacrificed day 45. Grade 4 gross. Peribronchial lymphocytic nodular infiltration. Necrosis of vascular walls with hemorrhage and exudation into surrounding tissues. Scattered focal areas of alveolar necrosis with infiltration of alveolar spaces by macrophages, lymphocytes, and septal cells.

Rat D-162 (98 ppm for 15 min) Sacrificed day 45. Grade 1 gross. Peribronchial and perivascular nodular lymphocytic infiltration. Hemorrhage and exudation into alveoli caused by coagulation necrosis of vascular walls. Slight to moderate proliferation of septal cells, macrophages and lymphocytes.

Mouse D-63 (140 ppm for 15 min) Grade 2 gross. Pulmonary vascular congestion with hemorrhage into alveolar spaces.

Mouse D-99 (128 ppm for 15 min) Grade 1 gross. Pulmonary vascular congestion.

Guinea Pig D-182 (232 ppm for 15 min) Grade 2 gross. Partial atelectasis caused by increase of number of septal cells and macrophages. Dilatation of blood vessels with exudation and slight hemorrhage into surrounding tissues.

Dog D-251 (100 ppm for 15 min) Grade 1 gross. Slide 1: Reduction in size and number of alveolar spaces causing partial atelectasis. Proliferative changes with increase in fibrocytes, septal cells, and macrophages. Some edema and hemorrhages into alveolar spaces. Bronchi relatively normal. Slide 2: Same as preceding, but with more pronounced lesions and proliferation of mucous cells lining bronchi with mucous nearly filling the bronchus.

The following descriptions from microscopic examination of the lungs were selected to illustrate any similarities or differences

between the different species tested when exposed to approximately the same concentration (100 ppm) for the same length of time (60 minutes) and sacrificed at the same time following exposure (14 days).

Rat D-157 (98 ppm for 60 min) Hemorrhage into alveolar spaces with exudation. Proliferation of septal cells, macrophages, and lymphocytes. Perivascular lymphocytic infiltration.

Guinea Pig D-176 (100 ppm for 60 min) Nodular lymphoid hyperplasia throughout tissue. Generalized alveolar proliferation of septal cells and macrophages. Dilatation of blood vessels with hemorrhage and exudation into surrounding tissue.

Dog D-252 (100 ppm for 60 min) Slide 1: Reduction in size and number of alveolar spaces due to hyperplasia of alveolar walls. These are composed of septal cells. Congestion of the alveolar walls with hemorrhage and exudation. Slide 2: Same as preceding. Slide 3: Same as preceding, except one area of intense infiltrations of eosinophilic granulocytes and polymorphonuclear granulocytes on right border of lobe.

Other examples of descriptions of findings in sections of lungs are presented below to show that there is a correlation between dose (concentration X time) and response of tissues. While the response is more dependent on concentration than is the lethal response, there is still a correlation between dose and effect.

Rabbit D-220 (134 ppm for 5 min) Slight hemorrhage into alveolar spaces along periphery of lobe. Narrowing of alveolar spaces due to septal cell proliferation. Dilatation and congestion of blood vascular system.

Rabbit D-228 (70 ppm for 30 min) Slight alveolar hemorrhages with some narrowing of alveolar spaces due to proliferation of septal cells and macrophages.

Dog D-253 (92 ppm for 60 min) Congestion of pulmonary vessels. Increase in number of septal cells, with corresponding loss of alveolar space. Alveolar infiltration by lymphocytes and macrophages.

Guinea Pig D-151 (82 ppm for 60 min) Peribronchial alveolar proliferation with some fibrosis. Proliferative inflammatory reaction with loss of alveolar space causing marked atelectasis. Inflammatory cells are septal cells and macrophages.

Mouse D-66 (115 ppm for 30 min) Pulmonary vascular congestion.

One of the important aspects of this study was to determine if an effect of fluorine would be worse, would regress, or remain constant for a time after exposure. Pathology in the lung was of primary importance, but damage to any other organ or tissue also was important. Following sublethal exposures, animals were sacrificed serially immediately (within 5 minutes) after removal from the chamber, one hour after exposure and 1, 2, 7, 14, 28, and 45 days after exposure. The organs which showed gross damage were lung, liver, and kidney. These were sectioned and examined under the microscope.

While the changes were similar in the several species used, the descriptions of tissues from mice are presented below. Since the

mice were exposed in groups of ten mice each, exposure of a certain group was constant. Changes in the lungs, liver, and kidneys were as follows:

Exposure -- 130 ppm for 60 minutes.

Mouse D-61-1 (Day 1 sacrifice) Lung: Coagulation necrosis of pulmonary vascular walls with resulting hemorrhage into alveolar spaces. Kidney: Numerous focal areas of lymphocytic infiltration throughout cortex and medulla. Focal areas of coagulation necrosis in cortex. Liver: Necrosis.

Mouse D-61-7 (Day 7 sacrifice) Lung: Pulmonary vascular congestion and hemorrhage into alveolar spaces. Kidney: Focal areas of lymphocytic infiltration in cortex and medulla. Several areas of necrosis in cortex. Liver: Several areas of necrosis.

Mouse D-61-14 (Day 14 sacrifice) Lung: Vascular congestion and some hemorrhage into alveolar spaces. Kidney: Focal nodular lymphocytic infiltration in the cortex. Coagulation necrosis with eosinophilic cast formation in cortex and medulla. Liver: Slight necrosis and congestion.

Mouse D-61-21 (Day 21 sacrifice) Lung: Vascular hemorrhage with exudation into alveolar spaces. Kidney: Focal lymphocytic infiltration mainly in cortex. Tubular hemorrhages. Liver: Normal

Mouse D-61-45 (Day 45 sacrifice) Lung: Pulmonary vascular congestion and hemorrhage into alveolar spaces. Kidney: Vascular congestion and nodular lymphocytic infiltration mainly in cortex. Liver: Normal.

Exposure -- 82 ppm for 60 minutes.

Mouse D-69-7 (Day 7 sacrifice) Lung: Pulmonary congestion. Kidney: Vascular congestion. Liver: Passive congestion.

Mouse D-69-14 (Day 14 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Some infiltration of lymphocytes in cortex. Liver: Passive congestion.

Mouse D-69-21 (Day 21 sacrifice) Lung: Very slight pulmonary congestion. Kidney: Slight infiltration of lymphocytes in cortex. Some vascular congestion. Liver: Normal.

Mouse D-69-45 (Day 45 sacrifice) Lung: Normal. Kidney: Focal nodular lymphocytic infiltration with vascular congestion and hemorrhages into tubules. Liver: Normal.

Exposure -- 55 ppm for 60 minutes.

Mouse D-215-7 (Day 7 sacrifice) Lung: Slight vascular congestion. Kidney: Cystic degeneration of tubules and lymphocytic infiltration mainly in cortex. Liver: Normal.

Mouse D-215-14 (Day 14 sacrifice) Lung: Normal. Kidney: Two small focal areas of lymphocytic infiltration. Liver: Passive congestion.

Mouse D-215-21 (Day 21 sacrifice) Lung: Normal. Kidney: Several focal areas of lymphocytic infiltration. Liver: Normal.

Mouse D-215-45 (Day 45 sacrifice) Lung: Normal. Kidney: Several focal areas of lymphocytic infiltration. Liver: Normal.

Exposure -- 115 ppm for 30 minutes.

Mouse D-66-7 (Day 7 sacrifice) Lung: Bronchial pneumonia. Kidney: Nodular focal infiltration of lymphocytes. Vascular congestion. Liver: Passive congestion.

Mouse D-66-14 (Day 14 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Focal areas of nodular lymphocytic infiltration in cortex. Liver: Passive congestion.

Mouse D-66-21 (Day 21 sacrifice) Lung: Pulmonary congestion. Kidney: Vascular congestion of cortex with focal nodular lymphocytic infiltration. Liver: Normal.

Mouse D-66-45 (Day 45 sacrifice) Lung: Very slight pulmonary vascular congestion. Kidney: Vascular congestion and focal infiltration of lymphocytes in cortex. Liver: Normal.

Exposure -- 51 ppm for 30 minutes.

Mouse D-225-7 (Day 7 sacrifice) Lung: Normal. Kidney: Normal. Liver: Normal.

Mouse D-225-14 (Day 14 sacrifice) Lung: Normal. Kidney: Normal. Liver: Normal.

Mouse D-225-21 (Day 21 sacrifice) Lung: Normal. Kidney: Normal. Liver: Normal.

Mouse D-225-45 (Day 45 sacrifice) Lung: Normal. Kidney: Normal. Liver: Normal.

Exposure -- 140 ppm for 15 minutes.

Mouse D-63-7 (Day 7 sacrifice) Lung: Pulmonary vascular congestion and hemorrhage into alveolar spaces. Kidney: Vascular congestion and nodular focal lymphocytic infiltration in cortex. Liver: One or two areas of necrosis with neutrophilic infiltration.

Mouse D-63-14 (Day 14 sacrifice) Lung: (3 sections) Slide 1: Congestion and hemorrhage into alveolar spaces. Slide 2: Infiltration of entire lobe with lymphocytes and macrophages. Slide 3: Slight congestion and hemorrhage into alveolar spaces. Kidney: Several focal areas of lymphocytic infiltration in cortex. Liver: Normal.

Mouse D-63-21 (Day 21 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Congestion and focal areas of lymphocytic infiltration. Liver: Normal.

Mouse D-63-45 (Day 45 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Vascular congestion in two areas of focal lymphocytic infiltration. Liver: Normal.

Exposure -- 82 ppm for 15 minutes.

Mouse D-71-7 (Day 7 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Focal lymphocilic infiltration in cortex. Liver: Normal.

Mouse D-71-14 (Day 14 sacrifice) Lung: Some congestion. Kidney: Two small focal areas of lymphocytic infiltration. Liver: Normal.

Mouse D-71-21 (Day 21 sacrifice) Lung: Normal. Kidney: Several small focal areas of lymphocytic infiltration. Liver: Normal.

Exposure -- 174 ppm for 5 minutes.

Mouse D-64-7 (Day 7 sacrifice) Lung: Pulmonary vascuaar congestion and hemorrhage into alveolar spaces. Kidney: A few focal areas of lymphocytic infiltration of cortex. Liver: Normal.

Mouse D-64-14 (Day 14 sacrifice) Lung: Pulmonary vascular congestion with hemorrhages and exudation into alveolar spaces. Kidney: Some focal infiltration of lymphocytes in cortex. Liver: Normal.

Mouse D-64-21 (Day 21 sacrifice) Lung: Slight pulmonary vascular congestion. Kidney: Some focal infiltration of lymphocytes and vascular congestion. Liver: Normal.

Mouse D-64-45 (Day 45 sacrifice) Lung: Pulmonary vascular congestion. Kidney: Focal nodular infiltration of lymphocytes in cortex. Vascular congestion, hemorrhage into tubular spaces. Liver: Normal.

Exposure -- 114 ppm for 5 minutes.

Mouse D-72-1 (Day 1 sacrifice) Lung: Slight congestion. Kidney: Normal. Liver: Normal.

Mouse D-72-7 (Day 7 sacrifice) Lung: Normal. Kidney: Congestion in parenchyma with focal areas of lymphocytic infiltration. Liver: Normal.

Mouse D-72-14 (Day 14 sacrifice) Lung: Normal. Kidney: Numerous focal areas of lymphocytic infiltration throughout parenchyma. Liver: Normal.

Mouse D-72-21 (Day 21 sacrifice) Lung: Normal. Kidney: One small area of focal lymphocytic infiltration. Liver: Normal.

Mouse D-72-45 (Day 45 sacrifice) Lung: Normal. Kidney: Slight infiltration of lymphocytes mainly into cortex. Liver: Normal.

An attempt was made to correlate the degree of damage in the lung, liver, and kidney. It was apparent that the lung showed more gross damage than the other two organs. While the kidney and liver were damaged, the degree was less than in the lung following the same exposure. The damage to kidney and liver became apparent 7 to 14 days after an exposure.

A more definitive comparison of pathology in these three organs could be made from the micropathology. The concentrations which

caused any micropathology in the lung, the liver, or the kidney at each period of exposure are summarized in Table 13. It can be seen from the data in this table that pathology in the lung or kidney occurred at almost the same concentration. The pathology in the liver did not occur until the animal was exposed to concentrations higher than those which produced pathology in the lung or kidney. Pathology in the liver first occurred at about the same concentrations which caused Grade 2 gross changes in the lung.

Tissues from animals exposed to concentrations which caused no gross effects were examined histologically. In general, if there were no gross effects, there was no effect found microscopically. In addition to the mice described above (exposure -- 51 ppm for 30 minutes), a few more examples are described as follows:

Rat D-205 (80 ppm for 5 min) Lung: Essentially normal. Kidney: Essentially normal.

Rat D-206 (48 ppm for 30 min) Lung: Essentially normal. Kidney: Essentially normal.

Rat D-233 (40 ppm for 60 min) Lung: Essentially normal. Kidney: Essentially normal.

c. Summary of Histology

Inhalation of lethal concentrations of fluorine caused massive hemorrhages in the lung tissue. This was brought about by coagulation necrosis of the pulmonary vascular system with extravasation of blood. Degeneration and necrosis of hapatic tissue were also present in some animals. These changes were found in all species tested -- rats, mice, guinea pigs, and rabbits.

Sublethal concentrations of fluorine caused similar gross and microscopic changes, but to a lesser extent. An attempt at body repair was shown by the leukocytic response. Kidney and liver involvement was seen after several days had elapsed following exposure to sublethal concentrations.

IV. SUMMARY

Special equipment, including a chamber for exposures, was designed and built to handle fluorine safely.

Analytical methods for the determination of the concentration of fluorine in air were developed. Colorimetric methods were used for the measurement of the concentrations of fluorine and fluorides in the air of the exposure chamber. Analytical methods for fluorine in air, using gas-liquid chromatography, were pursued. A thermal conductivity detector measured the fluorine, but was not sensitive enough for these purposes. Indirect measurement of fluorine, by conversion to chlorine, with a thermionic flame detector appeared to be satisfactory.

Signs of intoxication from high concentrations of fluorine in air were marked irritation of the mucous membranes of the eyes and respiratory tract. The skin of the animals showed some irritation at the concentrations used.

The LC_{50} (concentration calculated to kill 50% of the animals) was determined for 5, 15, 30, and 60 minutes of exposure in both rats and mice. The LC_{50} for guinea pigs was determined for 15 and 60 minutes of exposure, while the LC_{50} in rabbits was determined after 5 and 30 minutes of exposure. There were no significant differences between the LC_{50}'s for the different species of experimental animals. These, expressed as mg/cuM and as ppm (by volume), are tabulated as follows:

LC_{50} Values For Animals Exposed To Fluorine

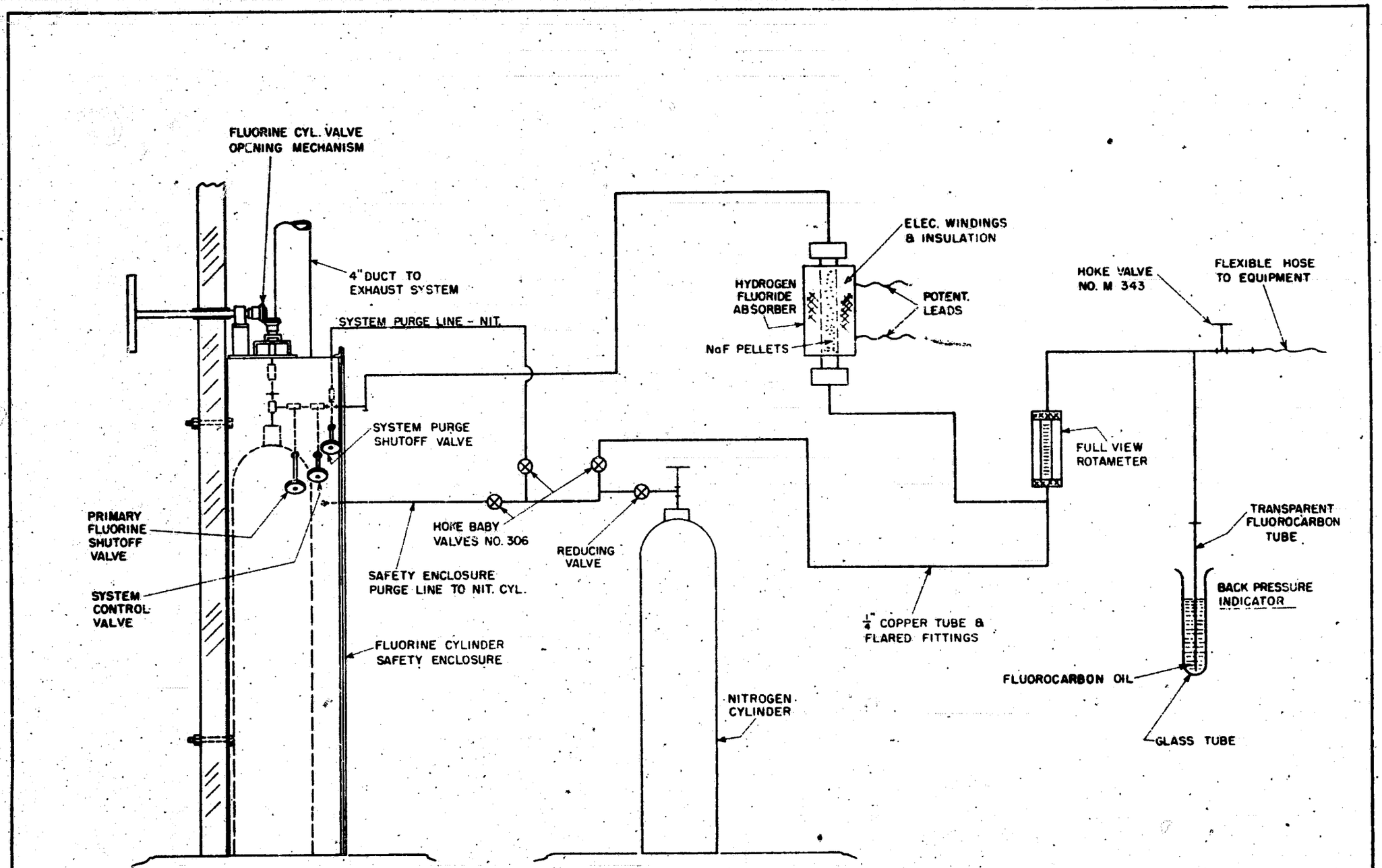
Exposure Time (min)	Rat Concentration mg/cuM	Rat ppm	Mouse Concentration mg/cuM	Mouse ppm	Guinea Pig Concentration mg/cuM	Guinea Pig ppm	Rabbit Concentration mg/cuM	Rabbit ppm
5	1085	700	935	600	----	---	1273	820
15	617	390	545	350	617	390	---	---
30	420	270	350	225	----	---	420	270
60	288	185	234	150	250	160	---	---

At lower concentrations, there were fewer signs of intoxication. Dyspnea, lethargy, red nose, and swollen eyes were observed at concentrations equivalent to 50% of the LC_{50}'s. At concentrations which were 25% of the LC_{50}'s, there were only mild signs of intoxication, manifested by slight dyspnea and closed eyes. At lower concentrations, there were no gross signs of intoxication. Complete blood counts on these animals did show significant changes due to fluorine.

Gross pathology following exposures near the LC_{50}'s was congestion, hemorrhage, and atelectasis in the lungs and some congestion and/or mottling in the liver. Survivors, sacrificed up to 45 days after such exposures, had congestion in the lungs and occasional congestion in the liver. There was some discoloration of the kidneys in animals sacrificed 7 to 14 days after exposure.

Following sublethal exposures, there was pathology in the lungs, liver, and kidneys. Effects in the lung were observed immediately after exposure. Effects in the kidney were observed (and in the liver) first on the seventh to fourteenth day following exposure. Pathology in the lung or kidney occurred from exposure to almost the same concentration. Exposure to higher concentrations was necessary before patholgy was observed in the liver.

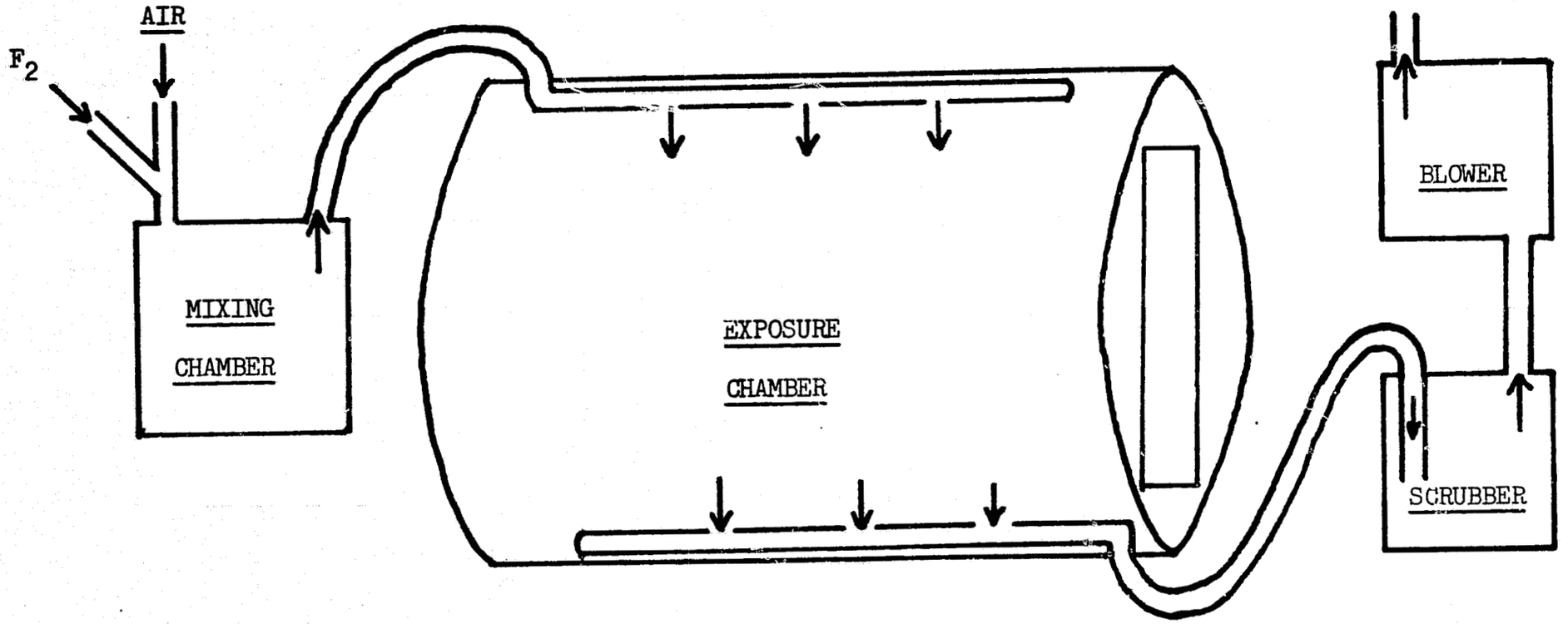
Expesure to concentrations at or below 100 ppm for 5 minutes, 70 ppm for 15 minutes, 55 ppm for 30 minutes, or 45 ppm for 60 minutes caused no apparent effects in the animals.



NOTE -- All valves were Matheson 940 F

FLUORINE CYLINDER LABORATORY SET UP

FIGURE 1



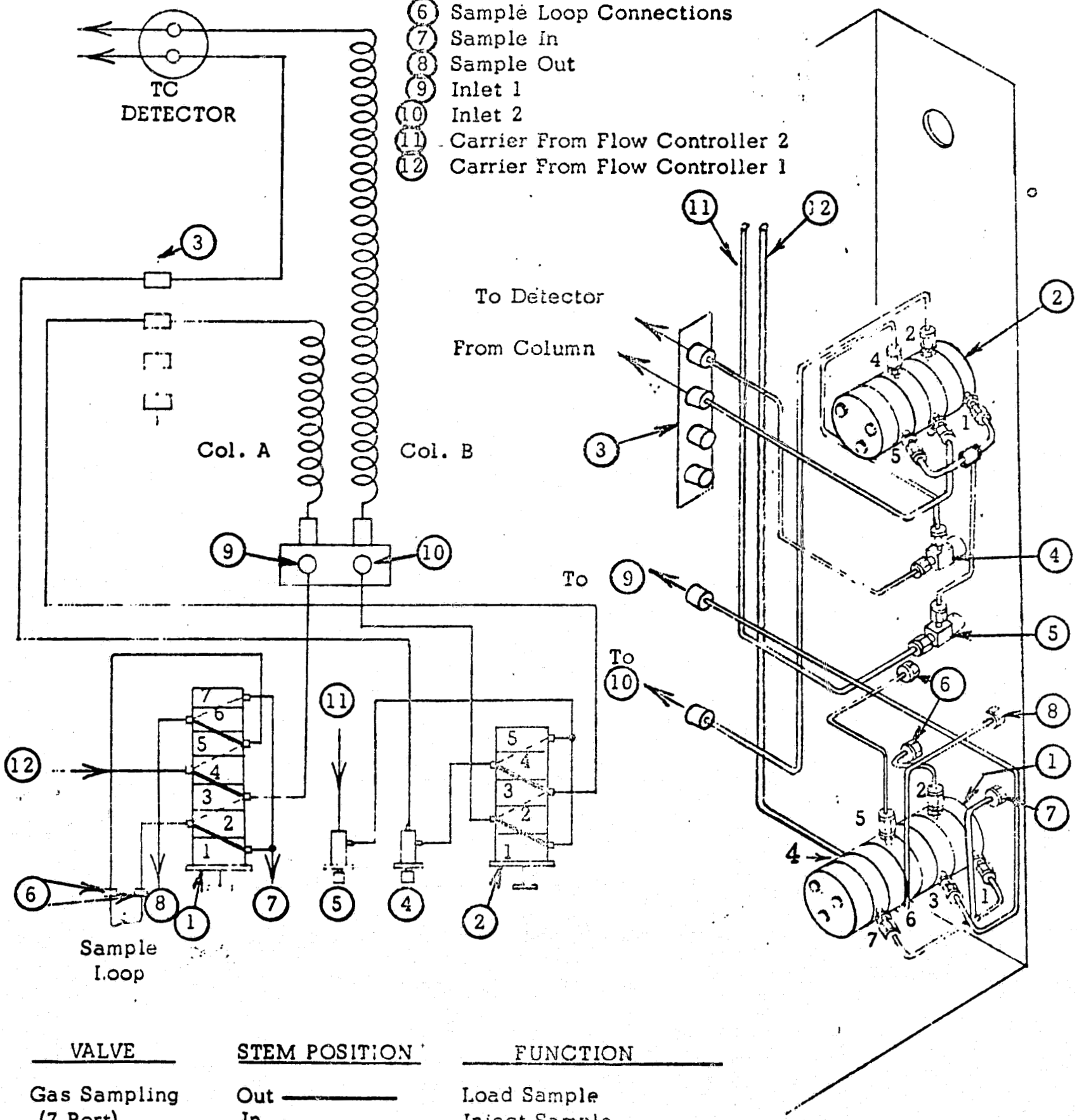
SCHEMATIC OF CHAMBER AND EQUIPMENT

FIGURE 2

INSTALLATION OF GAS SAMPLING AND COLUMN SELECTOR VALVE

For Series or Parallel Operation of Two Columns

1. Gas Sample Valve (7 Port)
2. Column Selector Valve (5 Port)
3. Thru Fitting to Column Oven
4. Variable Restrictor #1
5. Variable Restrictor #2
6. Sample Loop Connections
7. Sample In
8. Sample Out
9. Inlet 1
10. Inlet 2
11. Carrier From Flow Controller 2
12. Carrier From Flow Controller 1



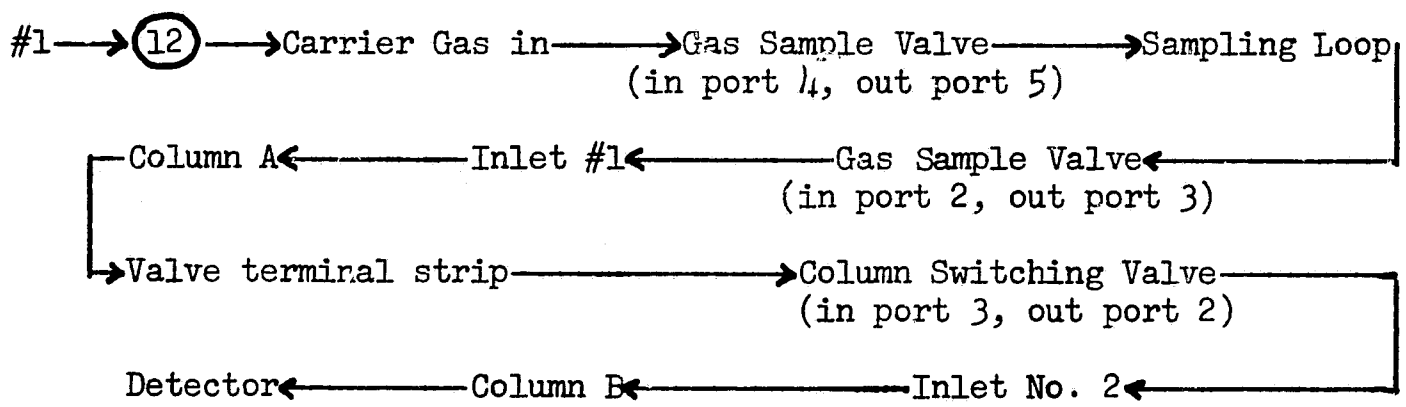
VALVE	STEM POSITION	FUNCTION
Gas Sampling (7 Port)	Out ———	Load Sample
	In - - - - -	Inject Sample
Column Selector (5 Port)	Out ———	Columns in Parallel
	In - - - - -	Columns in Series

FIGURE 3

FLOW PATTERN USING PLUMBING ARRANGEMENT

Condition A.

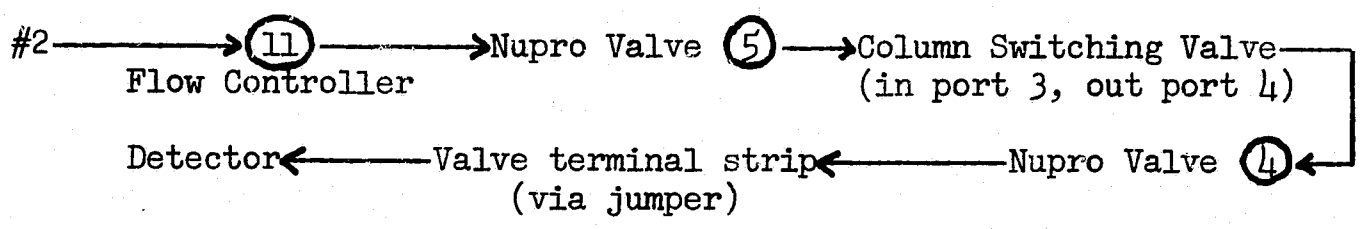
Gas Sampling Valve in, Column Switching Valve in



Result: As sample is picked up by carrier gas in sample loop, sample is directed through both columns; hence, column series.

Condition A^1.

Simultaneously, flow is directed to other side of detector as follows:



Note: Nupro valves are used to compensate for flow (pressure drop) in columns to prevent major base line shifts when switching the column switching valve in or out.

FIGURE 4

FLOW PATTERN USING PLUMBING ARRANGEMENT

Condition B.

Gas Sample Valve in, Column Switching Valve out

#1 → Flow Controller → (12) → Carrier gas in → Gas Sample Valve (in port 4, out port 5) → Sample Loop → Gas Sample Valve (in port 2, out port 3) → Inlet #1

Column A → Valve Terminal Strip → Column Switching Valve (in port 4, out port 4) → Nupro Valve (4) → Valve Terminal Strip → Jumper → Detector

Result: Sample Flow through column A only.

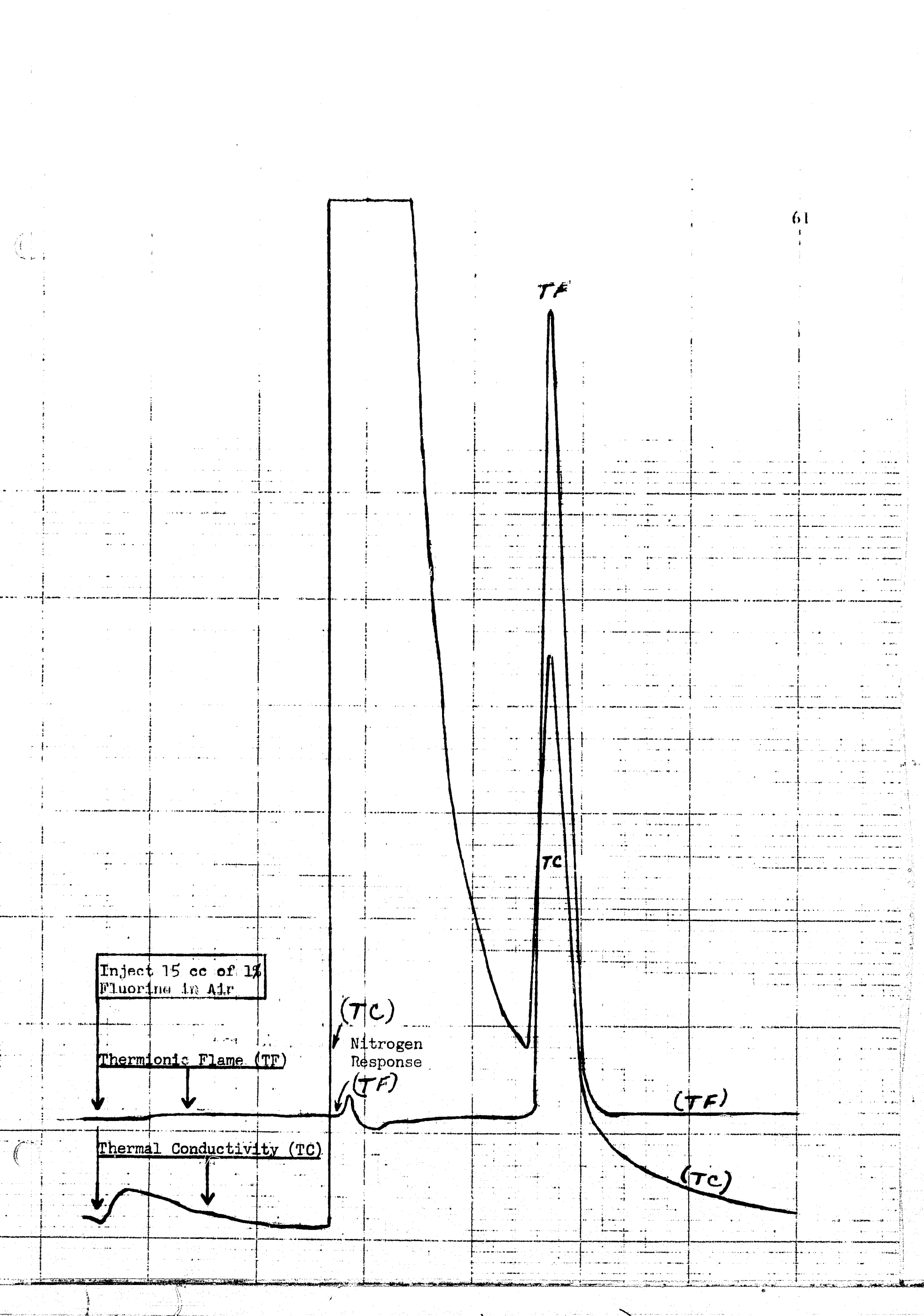
Condition B^1.

Simultaneously

#2 → Flow Controller → (11) → Nupro Valve (5) → Column Switching Valve (in port 1, out port 2) → Inlet No. 2 → Column B → Detector

Note: Nupro valve (5) Compensates for pressure drop in Column A.
Nupro valve (4) Compensates for pressure drop in Column B.
These must be manually adjusted.

FIGURE 5


TF
TC
Inject 15 cc of 1% Fluorine in Air
(TC)
Nitrogen Response
(TF)
Thermionic Flame (TF)
Thermal Conductivity (TC)
(TF)
(TC)

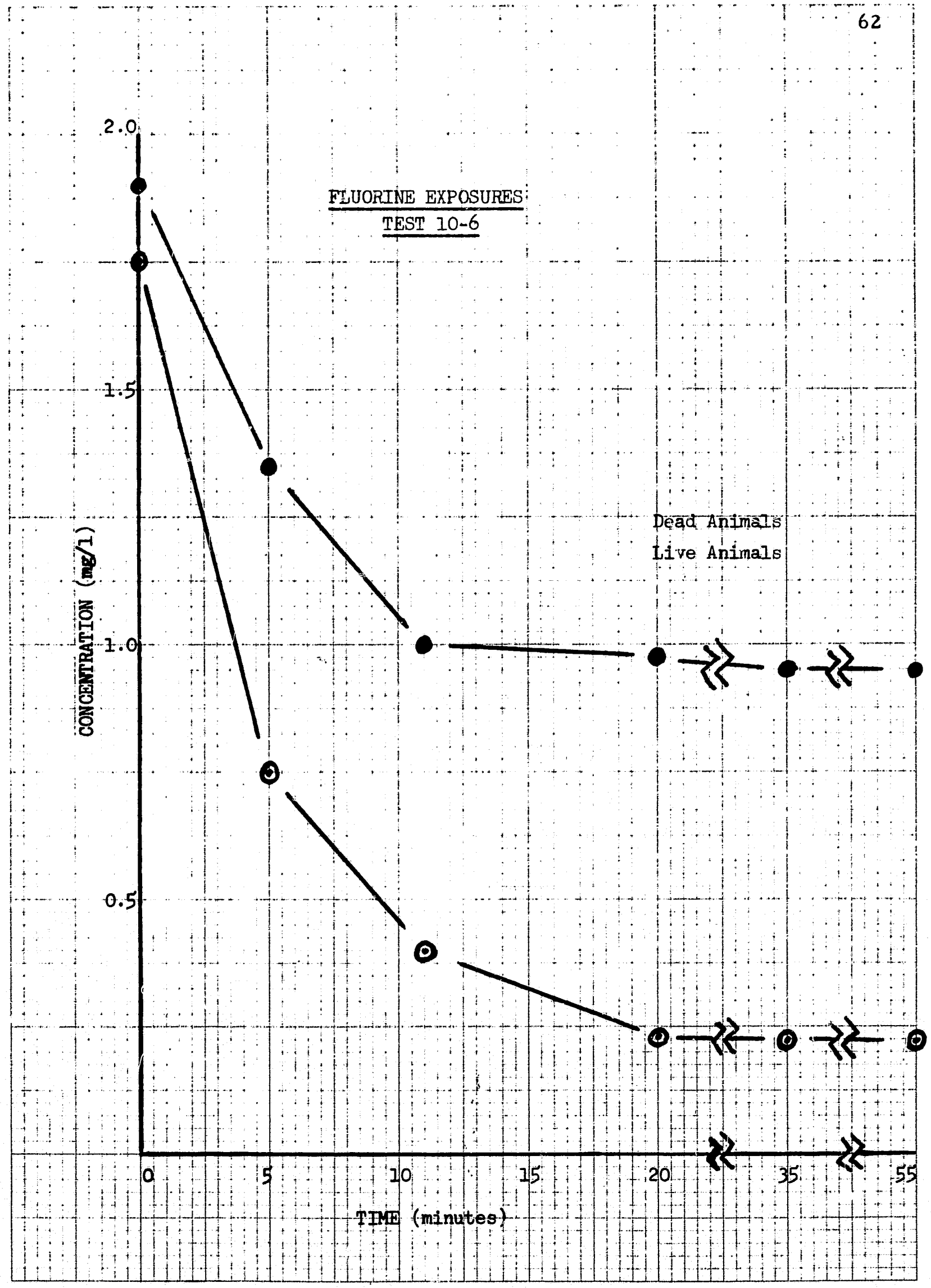

FLUORINE EXPOSURES
TEST 10-6
CONCENTRATION (mg/l)
2.0
1.5
1.0
0.5
0
5
10
15
20
35
55
TIME (minutes)
Dead Animals
Live Animals

FIGURE 7

FLUORINE EXPOSURES

(5 Rats and 10 Mice)

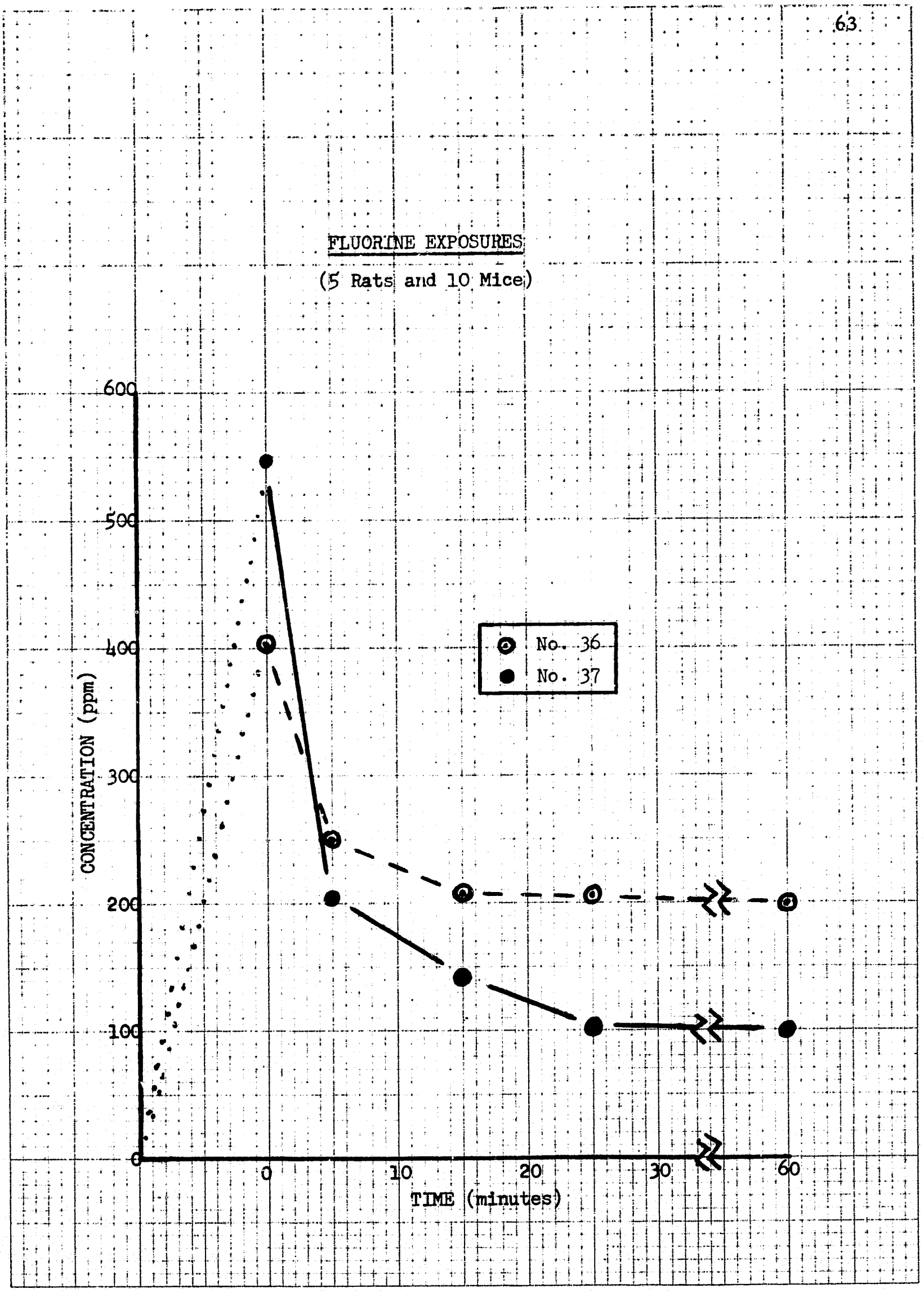


FIGURE 8

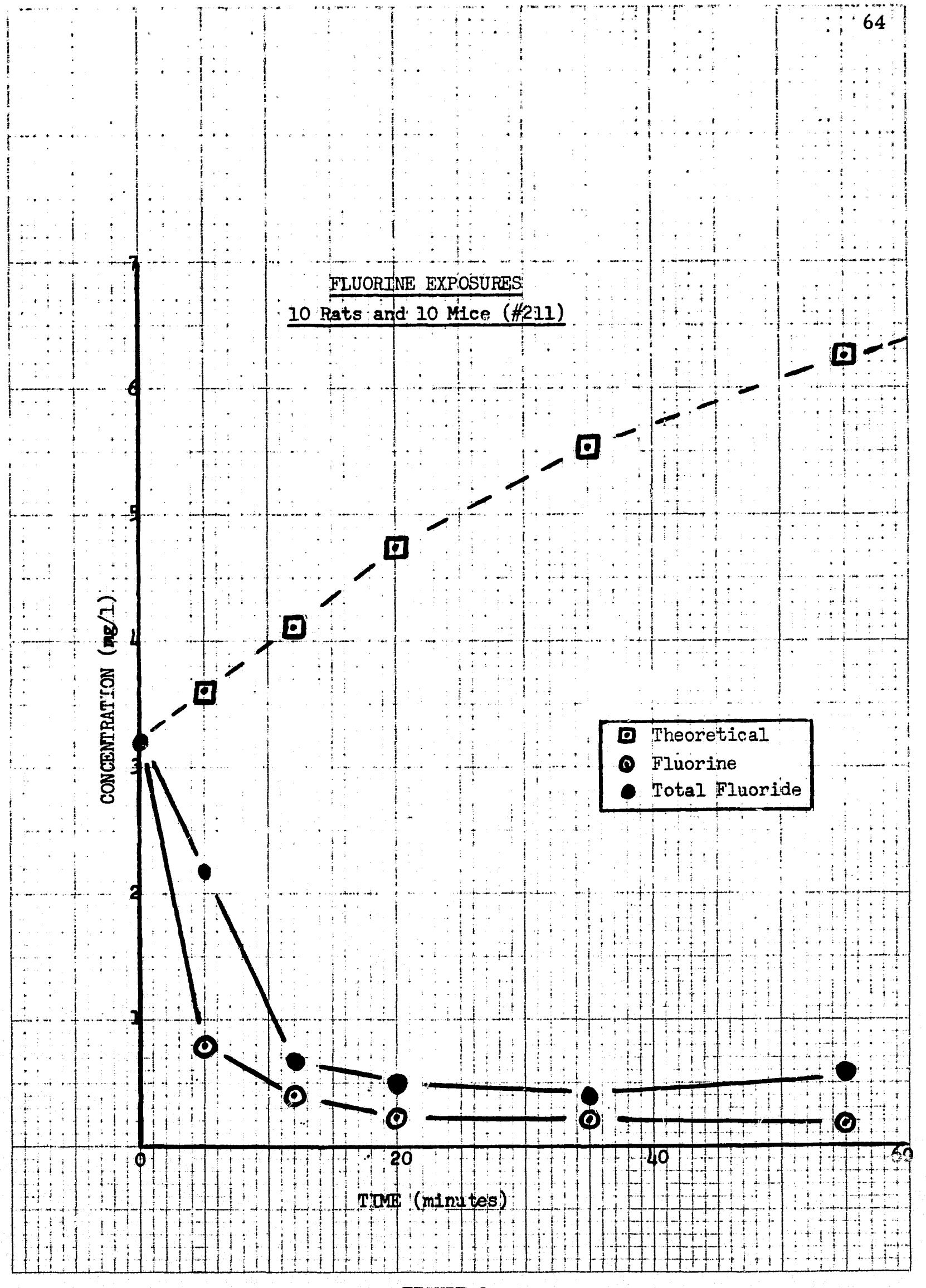

FLUORINE EXPOSURES
10 Rats and 10 Mice (#211)
CONCENTRATION (mg/l)
TIME (minutes)
0
20
40
60
Theoretical
Fluorine
Total Fluoride

FIGURE 9

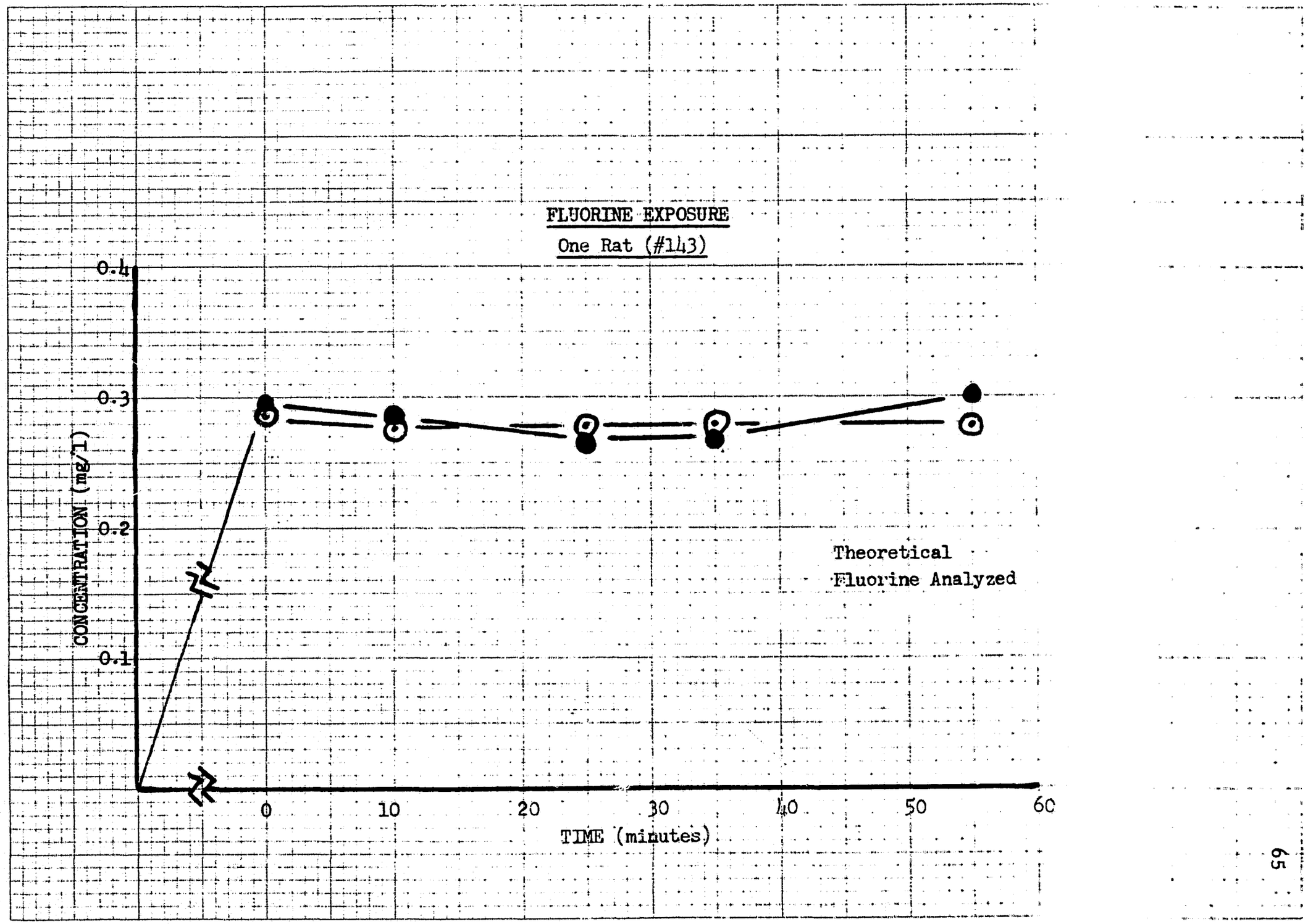


FIGURE 10

SUBLETHAL EFFECTS IN RATS AND MICE EXPOSED TO FLUORINE

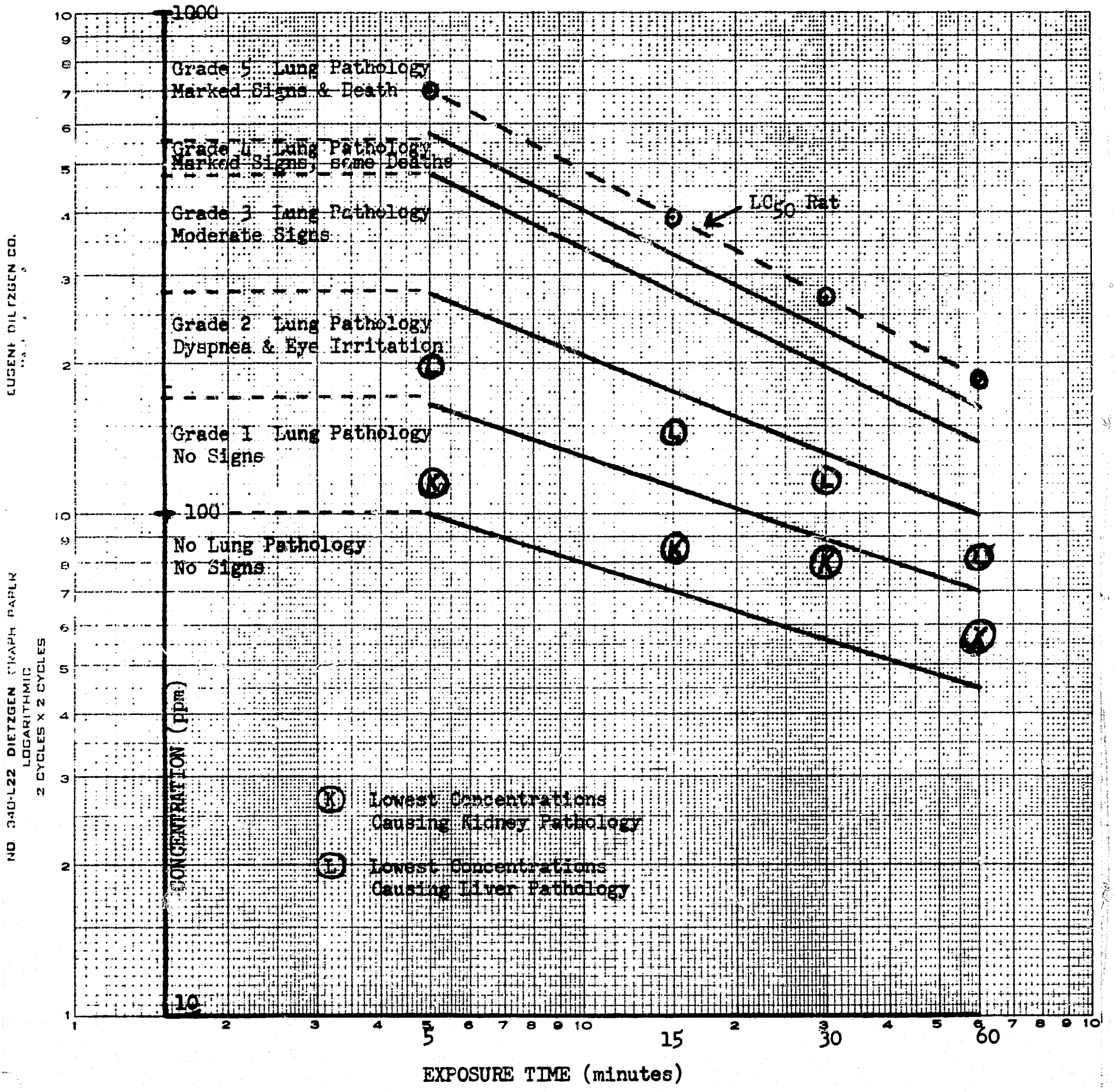


FIGURE 11

TABLE 1

Recovery of Fluorine from Air in Exposure Chamber
Over a Wide Range of Concentrations

Concentration (ppm)		
Theoretical	Analytical	% Recovery
90	90	100
108	108	100
135	128	95
168	171	102
225	225	100
370	370	100
374	386	103
450	430	96
508	515	101
572	555	97
600	597	100
670	685	102
900	890	99
1100	1040	95
1100	1132	103
1350	1320	98
1540	1475	96
1565	1565	100
2020	2000	99
12860*	12720*	99
85500**	84233**	99

Mean ± Standard Deviation 99 ± 2

* From mixing chamber
** F_2 in nitrogen

TABLE 2

LC_{50} Values For Animals Exposed To Fluorine
(10 Rats or mice per group. 5 Rabbits or guinea pigs per group.)

Species	Exposure Time (min)	LC_{50} mg/M³	LC_{50} ppm	19/20 Confidence Limits (ppm)
Rat	5	1,088	700	636-770
Mouse	5	932	600	517-696
Rabbit	5	1,274	820	730-920
Rat	15	606	390	361-422
Mouse	15	583	375	344-410
Guinea Pig	15	614	395	352-443
Rat	30	420	270	232-313
Mouse	30	350	225	199-254
Rabbit	30	420	270	240-310
Rat	60	287	185	142-240
Mouse	60	233	150	139-162
Guinea Pig	60	264	170	152-190

TABLE 3

Exposure Sequences for Various Species to Sublethal Concentrations of Fluorine

Exposure Time (min.)	Species	No. per Group	Concentration of F_2 Approximating % of Rat LC_{50}							
			50%		25%		12.5%		6%	
			(ppm)	(mg/M^3)	(ppm)	(mg/M^3)	(ppm)	(mg/M^3)	(ppm)	(mg/M^3)
5	Rat	10	350	544	175	263	88	132	44	66
	Mouse	10	300	466	174	263	79	119	38	57
	Rabbit	5	410	637	134	201	51	77	26	39
15	Rat	10	195	303	98	147	49	75	25	38
	Mouse	10	188	292	87	131	65	98	32	48
	Guinea Pig	5	198	307	100	150	70	105	*	
	Dog	2	93	144	93	140	39	60	*	
30	Rat	10	140	210	70	105	35	53	18	27
	Mouse	10	113	175	67	101	32	48	16	24
	Rabbit	5	135	210	71	106	32	48	19	29
60	Rat	10	93	144	47	71	28	42	14	21
	Mouse	10	75	117	50	75	30	45	15	23
	Guinea Pig	5	135	232	73	110	*		*	
	Dog	2	93	144	68	104	38	57	15	28

* Not Done

TABLE 4

Sublethal Effects in Animals Exposed to Fluorine for 5 Minutes

Species	Concentration (ppm)	Toxic Signs	Gross Lung Pathology
Rat	500	Marked irritation of eyes and resp. tract, dyspnea	Severe diffuse congestion
	350	Irritation and dyspnea	Moderate diffuse congestion
	175	Eye irritation, sl. dyspnea	Mild diffuse congestion
	88	No effect	No change
	44	No effect	No change
Mouse	467	Marked irritation of eyes and resp. tract, dyspnea	Severe diffuse congestion
	300	Irritation and dyspnea	Moderate diffuse congestion
	174	Sl. dyspnea and irritation	Very mild diffuse congestion
	79	No effect	No change
	38	No effect	No change
Rabbit	410	Irritation and dyspnea	Moderate diffuse congestion
	134	Sl. dyspnea	No change
	51	No effect	No change
	26	No effect	No change

TABLE 5

Sublethal Effects in Animals Exposed to Fluorine for 15 Minutes

Species	Concentration (ppm)	Toxic Signs	Gross Lung Pathology
Rat	195	Irritation and dyspnea	Moderate diffuse congestion
	98	No effect	Very mild diffuse congestion
	49	No effect	No change
	25	No effect	No change
Mouse	188	Irritation and dyspnea	Moderate diffuse congestion
	87	No effect	Very mild diffuse congestion
	65	No effect	No change
Guinea Pig	198	Irritation and dyspnea	Mild diffuse congestion
	100	No effect	Very mild diffuse congestion
	70	No effect	No change
Dog	93	Eye Irritation	Sl. congestion in lungs
	39	No effect	No change

TABLE 6

Sublethal Effects in Animals Exposed to Fluorine for 30 Minutes

Species	Concentration (ppm)	Toxic Signs	Gross Lung Pathology
Rat	140	Irritation of eyes and nose, sl. dyspnea	Moderate diffuse congestion
	70	No effect	Very mild diffuse congestion
	35	No effect	No change
	18	No effect	No change
Mouse	113	Irritation and dyspnea	Mild diffuse congestion
	67	No effect	Very mild diffuse congestion
	32	No effect	No change
	16	No effect	No change
Rabbit	135	Irritation	Mild diffuse congestion
	71	No effect	Very mild diffuse congestion
	32	No effect	No change
	19	No effect	No change

TABLE 7

Sublethal Effects in Animals Exposed to Fluorine for 60 Minutes

Species	Concentration (ppm)	Toxic Signs	Gross Lung Pathology
Rat	93	Irritation and dyspnea	Mild diffuse congestion
	47	No effect	Very mild diffuse congestion
	28	No effect	No change
	14	No effect	No change
Mouse	150	Irritation and dyspnea	Severe diffuse congestion
	75	Dyspnea	Mild diffuse congestion
	50	No effect	Very mild diffuse congestion
	30	No effect	No change
Guinea Pig	135	Irritation and dyspnea	Mild diffuse congestion
	73	No effect	No change
Dog	93	Irritation, cough, sl. dyspnea, vomiting	Small areas of hemorrhage
	68	Eye irritation	No change
	38	No effect	No change
	15	No effect	No change

TABLE 8

Summary of Gross Pathology in the
Lungs of Rats and Mice Exposed to Fluorine

(Concentrations are expressed in ppm)

Degree of Damage	5 (ppm)	15 (ppm)	30 (ppm)	60 (ppm)
5	575+	315+	230+	165+
4	480-575	280-315	200-230	140-165
3	280-480	175-280	130-200	100-140
2	165-280	115-175	90-130	70-100
1	100-165	70-115	55-90	45-70
N	100	70	55	45

Grading System for Gross Pathology in Lungs

Grade	Description
5	Congestion with hemorrhages (petechial or ecchymotic)
4	Severe diffuse congestion
3	Moderate diffuse congestion
2	Mild diffuse congestion (or diffuse congestion without adjective)
1	Very mild diffuse congestion
N	Normal or no change

TABLE 9

Lung Pathology in Mice Following Exposure to Fluorine

Exposure Time	Conc. (ppm)	Days After Exposure							
		Im*	1 hr**	1	2	7	14	21	45
60 min.	30	N	N	N	N	N	N	N	N
	50	1	1	1	1	N	N	N	N
	55	2	1	N	1	2	1	2	1
	80	2	2	2	2	2	2	2	2
	140	4	4	4	4	1	2	2	2
	155	4	4	5	4	2	2	2	2
	200	5	5	5	5	5	3	3	2
30 min.	32	N	N	N	N	N	N	N	N
	51	1	N	N	N	N	N	N	N
	82	1	1	1	1	2	1	1	1
	116	2	2	2	2	1	2	2	2
	181	3	3	3	3	2	2	N	2
	204	5	4	4	4	2	2	2	1
	305	5	5	5	4	4	4	4	3
15 min.	65	N	N	N	N	N	N	N	N
	82	1	1	1	1	2	1	N	N
	108	1	1	1	1	1	2	N	N
	128	1	2	2	1	2	2	1	1
	144	2	2	2	2	2	2	N	2
	208	3	3	3	3	2	2	1	1
	236	3	3	3	3	2	2	1	2
	293	4	4	3	5	2	2	1	1
	310	5	5	5	5	2	N	1	1
	421	5	5	5	5	2	N	1	1
5 min.	38	N	N	N	N	N	N	N	N
	79	N	N	N	N	1	1	N	N
	114	1	1	1	1	1	1	1	N
	129	1	1	1	1	1	N	1	1
	174	1	1	1	1	1	2	2	1
	220	2	2	2	2	2	2	1	N
	467	3	3	3	3	1	2	N	N
	758	5	5	5	5	2	2	2	2
	855	5	5	5	5	5	3	2	1

* Immediately after exposure.
** One hour after exposure.

TABLE 10

Lung Pathology in Guinea Pigs Following Exposure to Fluorine

Exposure Time	Concentration (ppm)	Degree of Gross Lung Pathology
15 min.	70	N
	80	1
	100	1
	195	2
	232	2
	275	3
	367	3
	428	4
	440	5
60 min.	73	1
	82	N
	86	2
	91	2
	100	2
	110	2
	115	2
	200	5
	290	5

TABLE 11

Lung Pathology in Rabbits
Following Exposure to Fluorine

Exposure Time	Concentration (ppm)	Degree of Gross Lung Pathology
5 min.	26	N
	41	N
	51	N
	134	N
	386	2
	452	5
	770	5
	1588	5
30 min.	8	N
	10	N
	19	N
	32	N
	39	N
	71	1
	195	4
	240	5
	325	5
	690	5

TABLE 12

Lung Pathology in Dogs Following Exposure to Fluorine

Exposure Time	Concentration (ppm)	Degree of Gross Lung Pathology
15 min.	39	N
	58	N
	92	1
	93	1
	100	1
60 min.	15	N
	19	N
	38	N
	68	N
	73	1
	84	1
	87	N
	92	N
	97	2
	102	2
	109	2

TABLE 13

Comparison of Pathology in Lung, Liver or Kidney of Mice Exposed to Fluorine

Time of Exposure	Conc. (ppm)	Organ with Pathology		
		Lung	Kidney	Liver
5 min.	38	N	N	N
	79	N	N	N
	114	1	P	N
	129	1	P	N
	174	1	P	N
	195	2	P	P
15 min.	65	N	N	N
	82	1	P	N
	108	1	P	N
	128	1	P	N
	144	2	P	P
30 min.	32	N	N	N
	51	N	N	N
	82	1	P	N
	116	2	P	P
60 min.	30	N	N	N
	50	1	N	N
	55	1	P	N
	80	2	P	P

N = Normal or No Change
P = Some Pathology
1 or 2 = Degree of Lung Pathology

SURVIVAL FOR SIX MONTHS

In the previous studies with fluorine it was found that certain concentrations caused damage to the lungs, liver and kidneys. Although there was a regression of these effects, there were still some changes, particularly in the kidneys, of animals sacrificed 45 days after exposure. The purpose of this experiment was to determine if the damage to the kidneys would regress completely when the animals were allowed to live longer than 45 days after exposure.

Mice and rats were exposed for 5, 15, 30 and 60 minutes to concentrations of approximately 150, 100, 80 and 60 ppm, respectively. Groups of mice and rats were sacrificed serially at 21, 45 and 180 days after exposure. These concentrations caused some inflammation in the lungs and congestion in the kidneys as shown by examination of the tissues at 21 and 45 days after exposure. Six months after the exposure, lung tissue was regenerated as shown by increased septal cells. The kidneys also had returned to essentially normal. The liver did not show any changes caused by fluorine when the animals were examined six months after the exposure.

HUMAN EXPOSURES

After the effects of fluorine in five species of experimental animals were known, the concentrations which would cause certain effects in man could be predicted. Extrapolation of data from experimental animals to predicted effects in man always is difficult. With the irritating effects of fluorine, however, it was rather simple to test for irritation in man.

These tests were designed to test for irritation only. A face mask was connected to the inhalation chamber so that a known concentration of fluorine in air could be pulled through the mask. The concentration of fluorine in the air of the chamber was measured analytically, and the concentration of fluorine in the air leaving the mask also was measured analytically. Both analyses were made to assure that any dilution of fluorine caused by a possible leak in the mask would be measured.

The test was explained carefully to each subject before he volunteered to participate. He was asked to sign an Authorization For Participation and a Consent Form, before he became a test subject. Examples of each form are included at the end of this section.

The volunteer human subject placed his face into the mask which covered the eyes and nose, but not the mouth. The subject could withdraw quickly from the mask and could inhale uncontaminated air through his mouth at any time. This design was utilized to prevent an accidental, harmful exposure.

At the higher concentrations, the subject was instructed not to inhale through his nose.

The results from single exposures are summarized as follows: (The odor of fluorine was very prominent at all concentrations used.)

Concentration (ppm)	Time (min.)	Effects
10	3	No irritation of eyes and nose.
10	5	No irritation of eyes and nose. Not uncomfortable.
10	15	No irritation of eyes and nose. Inhaled without irritation of the respiratory tract.
23	5	Slight irritation to eyes. Could inhale without respiratory difficulty. (Inhaled intermittently over the five-minute period).
50	3	Irritating to the eyes. Slightly irritating to the nose.
67	1	Irritating to the eyes and nose. Although quite irritant, concentration not unbearable.
78	1	Irritating to the eyes and nose. (Less irritant than cigarette smoke in the eye.) Face slightly irritated following the exposure. Caused coughing when inhaled.
100	1	Very irritating to eyes and nose. Eyes burned after exposure. Felt like "film" over the eyes after exposure. Skin felt irritated after exposure. Subjects did not inhale.
100	0.5	Very irritating to eyes and nose. No "after effects".

The effects of repeated, intermittent exposures are summarized as follows:

Concentration (ppm)	Time (min.)	Repeated	Effects
10	5	Every 15 minutes for 2 hours	Eyes slightly irritated.
10	3	Every 15 minutes for 3 hours	Eyes slightly irritated. Skin slightly irritated. Felt like "film" over one eye reported by some subjects.

These data indicate that irritation to the eye was the most sensitive index of a subjective effect. A concentration of 10 ppm was not particularly irritating for as long as 15 minutes. Concentrations in the order of 25 ppm were slightly irritating to the eyes after a few (5) minutes of exposure. Concentrations as high as 25 ppm could be inhaled without respiratory discomfort. A concentration of 50 ppm was irritating. Concentrations of 67 to 100 ppm were very irritating and became uncomfortable after a few seconds.

It was the opinion of the subjects that 100 ppm was extremely uncomfortable and that they would evacuate an area immediately if such concentrations were present. After exposure to 100 ppm the subjects were asked the questions, "In an emergency do you feel that you would be capable of self-rescue? In an emergency, do you think that you could walk into a room containing 100 ppm fluorine, without a respiratory protective device, and rescue an injured person?". All replies were affirmative.

These data, based primarily on irritation to the eyes, therefore indicate that the human probably should not be exposed above the following:

5 minutes	60 ppm
15 minutes	40 ppm
30 minutes	30 ppm
60 minutes	25 ppm

UNIVERSITY OF MIAMI

AUTHORIZATION FOR PARTICIPATION IN AN INVESTIGATIVE STUDY

Date: September 15, 1966

IDENTIFICATION OF RESEARCH PROJECT: Grant No. NGR 10-007-012

Principal Investigator M. L. Keplinger Granting Agency: NASA

* * * * * * * * * * *

PARTICIPATION CONSENT:

Place of signing: Toxicology Laboratory, South Campus
(Name of hospital, laboratory, etc.)

I hereby consent to the participation of M. L. Keplinger
(Name of Individual)
in an investigational study requiring the following: Exposure of eyes and nose to dilute concentrations fluorine
(Name of procedure or drug to be administered)

It has been explained to me that the study is being conducted for investigational purposes. The explanation of the study on the reverse side hereof has been read by me and also the reason for the study, the risks involved, and the care that will be exercised to avoid complications have been explained to me. I acknowledge that no guarantee or assurance as to the therapeutic value of these studies can be made.

Participating individual _____

* * * * * * * * * * *

WHEN PATIENT IS UNABLE TO AFFIX SIGNATURE:

Person authorized to consent for patient: _____
(Signature)

Authority to consent: _____
(i.e., Father, Mother, Wife, Husband)

* * * * * * * * * * *

WITNESS:

_____ _____
(Signature) (Address)

ATTEST:

_____)
(Signature of Principal Investigator)

PATIENT'S IDENTIFICATION: (Give: Name - last, first, middle; date; and hospital medical facility or institution)
(Use other side)

Consent Form for Investigational Procedure

Willard Machle, M. D., Supervising Physician

NAME:_____AGE:_____M:_____F:_____

"The purpose of this experiment is to determine concentrations of fluorine in air which will cause irritation to the eyes and/or nose. You will place your face in a mask which covers your eyes and nose. Your mouth will be uncovered at all times so that you can inhale through your mouth. The mask will be held snugly in place with your hands. If the concentration becomes irritating, you can quickly remove the mask. In carrying out the test you will not know if you are getting fluorine or air, acting as a control. No inconvenience or hazard from the fluorine is expected and there will be no effects upon health. Please record your consent to take part in this experiment by signing below."

Signature _____

Repeated Intermittent Exposures to Fluorine

Introduction

It was felt that the effects from a second, third and fourth exposure to fluorine should be known. Of particular interest was whether subsequent exposures might cause worse effects than a single exposure. Therefore, experimental animals were exposed for several times to the same concentration of fluorine. It was also felt that in an operational situation men could be exposed inadvertently to low, apparently harmless, concentrations. In such cases we should know if these men would be more susceptible to another exposure at a relatively high level. Therefore, experimental animals were exposed several times to fluorine at different concentrations.

Damage in the lungs from exposures to high concentrations of fluorine has been shown. In order to gain more information regarding the type of change in the lung, experiments were conducted to measure the formation of pulmonary edema and to distinguish edema from hemorrhage. Hemorrhage, or actual bleeding in the lung, of course, indicates more severe effects than edema fluid which escapes through the cell walls.

Results

Same Concentration

Mice, rats and rabbits were exposed to fluorine four times at weekly intervals. Two general levels of exposure were used, one which caused slight effects and one which caused marked effects following a single exposure.

Mice were exposed for five minutes to 103, 109, 116 and 158 ppm, levels which caused slight damage to the lungs from a single exposure. Additional groups of mice were exposed for five minutes to concentrations of 270, 304, 309 and 321 ppm, levels which caused Grade 3 gross damage in the lungs and gross damage to the liver and kidneys. Examples of the changes in the tissues are summarized in Table I. (For comparison, the changes following a single exposure to the same concentration also are presented in Table I.)

At the lower concentrations the gross pathology in the lungs was Grade 1 after a single exposure. Four exposures, one week apart, caused no more damage than a single exposure. The liver and kidneys were essentially normal.

At the higher concentrations, Grade 3 gross damage in the lungs and gross pathology in both the livers and kidneys could have been caused by a single exposure to these concentrations. Instead, the lungs were normal or had only Grade 1 changes. The livers were normal. The kidneys had slight changes in color at 7 and 14 days after the last exposure, and were normal when examined at 21 and 45 days after the last exposure.

The concentrations for the thirty-minute exposures were 64, 49, 44 and 59 ppm. Gross lung, liver or kidney damage was not apparent seven days after the last exposure. At 14, 21 and 45 days after the last exposure some of the animals showed slight (Grade 1) damage in the lungs.

The concentrations for sixty-minute exposures were 43, 50, 56 and 65 ppm. The lungs had Grade 1 changes, but the livers and kidneys were normal. Again the changes in the lung could have been caused by a single exposure. There was no indication of a cumulative effect.

Rats were exposed for five minutes to concentrations which ranged from 85 to 150 ppm. Some of these effects also are included in Table I. The livers and kidneys were normal. The lungs of some of the rats were normal while some of them showed slight damage. The higher concentrations for five minutes of exposure ranged from 256 to 450 ppm. The livers and kidneys were normal. The lungs had slight damage (Grade 1 or 2). A single exposure at a concentration in this range caused Grade 3 lung changes and damage to both the liver and the kidneys.

The lower concentrations for sixty minutes of exposure ranged from 45 to 75 ppm. The livers and kidneys were normal. Gross lung pathology was Grade 1 in almost all animals.

The higher concentrations for sixty minutes of exposure ranged from 88 to 170 ppm. The livers and kidneys were normal. The lungs

had changes of Grades 1, 2 and 3. It should be emphasized, that a single exposure to these concentrations caused very marked changes in the lungs (Grades 4 and 5).

Rats also were exposed for 30 minutes to 46, 59, 64, and 68 ppm, concentrations which should have caused slight damage to the lungs. The rats were sacrificed 14 days after the last exposure. The livers and kidneys were grossly normal. The lungs showed slight gross pathology (Grade 1).

Rabbits were exposed to essentially the same concentrations and for the same intervals of time as the rats. Results were essentially the same, i.e., effects from four exposures, one week apart, were no worse or not as marked as effects from a single exposure to the same concentration.

Rabbits also were exposed 4 times at weekly intervals to concentrations which caused no effects in the lungs following a single exposure. They were exposed for 30 minutes to concentrations of 49 and 55 ppm or for 15 minutes to concentrations of 56 to 73 ppm. There were no apparent effects in the lungs or in any other organ or tissue when examined either grossly or microscopically. This indicated that at least four weekly exposures to fluorine at maximum "no effect" levels following a single exposure caused no additive or cumulative effects in the animals.

Microscopic examination of these tissues revealed that four repeated exposures to fluorine (one week apart) cause no more damage than

a single exposure. In fact the changes in the tissues, particularly the lungs, were slightly less from repeated exposures than from a single exposure to the same concentration.

Different Concentrations

These experiments were conducted to determine if previous exposures to low concentrations of fluorine would change the susceptibility of the animal to subsequent exposures.

Mice were exposed to fluorine at low concentrations (such as 30 ppm for 60 minutes). After a few hours to several days, they were exposed again to fluorine. The pathology in the lungs, which is indicative of changes caused by fluorine, is shown in Table II. Animals exposed at the same time were separated into two groups, one which had been exposed to the low concentration of fluorine and one which had no previous exposure. A number of different concentrations were used, as shown in the first column. The second exposure was 4, 24 or 96 hours after the first exposure, as shown in the second column. It can be seen that the "treated" or previously exposed mice showed less changes than animals which had not been exposed before.

Rabbits were exposed at weekly intervals for four times (50 ppm for 30 minutes). Forty-eight hours after the last exposure, the rabbits were exposed to 400 ppm for 30 minutes. This concentration is considerably greater than the LC_{50}. Therefore, a single exposure should kill the rabbits. Control rabbits exposed to the same concentration died.

Survival of the controls ranged from a few minutes to 18 hours. The pre-exposed rabbits died but the survival time was longer (48 hours) than any of the controls.

A second group of rabbits was exposed to 300 ppm, 48 hours after the fourth exposure to 50 ppm for 30 minutes. All survived for 72 hours even though the concentration was greater than the LC_{50}. On the third day after this exposure (which should have been lethal to 65% of the animals within 24 hours), the same rabbits were exposed again for 30 minutes to a concentration of 327 ppm. All of the rabbits then died within 24 hours after the last exposure.

These data indicate that exposures to sublethal concentrations of fluorine make the animal less susceptible to a lethal concentration. The animals certainly are no more susceptible.

Possible Tolerance

Some of the data, therefore, indicated that effects from exposures to fluorine repeated at weekly intervals (or less) caused no cumulative effects. In fact, it appeared that the effects of these exposures might be less than the effects from a single exposure. A decreased effect indicated some type of tolerance. Mice were exposed to 30 ppm fluorine for 60 minutes (like those described previously). At 4, 24 and 96 hours after the exposure, the LC_{50} of these mice following an exposure of 15 minutes was determined. With each group of "treated" or "pre-exposed" mice, a control or non-exposed group also were exposed.

The $\underline{LC_{50}}$'s of the mice (shown in Table III) determined at 4 and 24 hours following the first exposure were 310 and 350 ppm respectively. These were higher than the LC_{50}'s of mice which had not been exposed previously (260 and 315 ppm). The LC_{50} of the mice determined at 96 hours after the previous exposure (295 ppm) was slightly lower than the LC_{50} of control mice exposed at the same time (315 ppm).

It might be added that microscopic examination of the organs and tissues of these mice also revealed less damage to the lungs of animals which had been subjected to the low concentration of fluorine before being exposed to a concentration which caused damage.

Lower concentrations were then used for repeated exposures. Some mice were exposed to 45 ppm for 30 minutes, while others were pre-treated by exposures to 25 ppm for 15 minutes. (These exposures cause no apparent effects in the animals.) The exposures were repeated every third day for a total of four exposures. At 1, 3 and 7 days following the last exposure different groups were exposed to concentrations ranging from 100 to 500 ppm. At the same time an equal number of untreated (normal or control) mice were exposed to detect any difference caused by pre-treatment.

The LC_{50}'s of the pre-treated mice were greater than the LC_{50}'s of untreated mice exposed at the same time. The pre-treated mice did not show the marked signs of toxicity as the untreated, normal mice. This indicated that the pre-treated mice were less susceptible to the

lethal effects of high concentrations of fluorine and that the apparent tolerance lasted for at least 7 days.

Organ weight - The weights of the lungs of mice which succumbed to fluorine (autopsied immediately) were heavier than normal, indicating the possibility of the formation of edema. At both four and twenty-four hours after the pre-exposure, the lungs of untreated mice were heavier than the lungs of the treated mice. Examples are presented in Table IV where mice were exposed to 30 ppm for 60 minutes, and then four, 24 or 96 hours later were exposed again to fluorine. The difference in lung weights between treated and untreated mice was not apparent at the 96-hour interval. The weights of the lungs of all mice were normal when sacrificed 14 days after these exposures to fluorine.

The weights of the kidneys were increased in animals which succumbed to fluorine (and were autopsied immediately) as shown in Table V. They were normal, however, from animals sacrificed 14 days after exposure. The weights of the kidneys of untreated mice sacrificed two hours after exposure, were slightly heavier than the kidneys of treated or pre-exposed mice when the time between exposures was four or 24 hours. The differences, however, were not very great. With 96 hours between exposures the weights from treated and untreated mice were essentially the same. When sacrificed 14 days after the last exposure there was essentially no difference between the weights of kidneys of treated and untreated animals.

The weights of the livers were not changed by any of these exposures to fluorine; and, therefore, they are not tabulated.

Discussion

These exposures were short-term (up to 60 minutes) and were intermittent or interrupted to simulate potential exposure conditions in launch operations. The operating condition where fluorine is handled must be such that these types of exposures would not occur on a daily basis.

These results indicate that in at least three species of experimental animals the effects from intermittent exposures were no worse than a single exposure to the same concentration.

In this study the effects on the lungs, liver and kidneys were emphasized. It might appear that effects in other organs and tissues were not investigated; however, previous studies showed that these three organs were the target organs for damage from inhalation of fluorine. Other organs and tissues were studies, but no effects were observed.

Some of these results indicated that there may be some type of tolerance or protection developed by exposures to low concentrations of fluorine. The evidence for tolerance was not very remarkable, but provided enough evidence to merit further investigation of this phenomenon.

Summary

Rats, mice and rabbits were exposed to fluorine for five to 60 minutes. The exposures were repeated intermittently at intervals ranging from 24 hours to one week. Repeated exposures at the same concentration caused no more effects than a single exposure to the same concentration. In fact, some of the effects from repeated exposures were less than those from a single exposure. Repeated exposures at low (apparently harmless) concentrations, made the animals less susceptible to the effects from exposure to a higher concentration. These effects were measured by the LC_{50}, onset of death, pathology, and weight of the lungs. Apparently, some tolerance to fluorine was developed in these animals.

Table I

Pathology in Mice and Rats

After Repeated Exposures to Fluorine

Species	Exposures Time (min.)	Exposures Conc. (ppm)	Repeated Exposure Lung (grade)	Repeated Exposure Kidney (grade)	Repeated Exposure Liver (grade)	Single Exposure Lung (grade)	Single Exposure Kidney (grade)	Single Exposure Liver (grade)
Mouse	5	130	1	N	N	1	P	N
	5	321	1	P	N	3	P	P
	30	64	1	N	N	1	P	N
	60	55	1	N	N	1	P	N
Rats	5	150	1	N	N	1	P	N
	5	325	1-2	N	N	3	P	P
	30	68	1	N	N	1	P	N
	60	75	1-2	N	N	2	P	N
	60	140	2-3	N	N	4	P	P

N = Normal or No Change

P = Some Pathology

1, 2, 3 = Degree of Gross Lung Pathology

Table II

Gross Pathology in Lungs of Mice

Following One or Two Exposures to Fluorine for Fifteen Minutes

("Treated" mice were exposed to 30 ppm fluorine for 60 minutes before the second exposure. "Untreated" were exposed at the same time during the second exposure)

Conc. (ppm)	Time of Second Exposure (hrs.)	Grade of Lung Pathology	
		Treated	Untreated
81	96	1	1
118	4	N	1
179	24	N	2-3
183	96	1	2-3
265	24	N	3
285	4	N	3
350	96	N-1	4
359	24	N-1	5
410	4	N-1	5

N = Normal or No change

1 = Very slight congestion

5 = Marked damage with hemorrhages

Table III

LC_{50}'s of Mice Pre-Exposed to Fluorine

Pre-Exposures				Time* of LC_{50}	LC_{50}	
Time (Min.)	Conc. (ppm)	No. of Times	Time Interval		Pre-Exposed (ppm)	Control (ppm)
30	45	1	---	48 hours	270	268
60	30	1	---	4 hours	310	260
60	30	1	---	24 hours	350	315
60	30	1	---	96 hours	295	315
15	25	4	48 hrs.	1 day	290	220
15	25	4	48 hrs.	3 days	310	275
15	25	4	48 hrs.	7 days	280	190

* Interval of time between first exposure and the exposures to determine the LC_{50}.

Table IV

Weights of Lungs of Mice Exposed to Fluorine

(Mice were exposed to 30 ppm for 60 minutes; then, 4, 24 or 96 hours later, they were exposed for 15 minutes to the concentrations listed below. Weight expressed in grams.)

Time Between Exposures (hrs)	Conc. (ppm)	Time of Sacrifice: 2 hours Pre-Exposed (g)	2 hours Control (g)	14 days Pre-Exposed (g)	14 days Control (g)
4	285	0.66	0.97	0.49	0.48
	310	0.64	0.95	0.51	0.47
	410	0.60	0.89	0.50	0.55
	494	0.56	0.91	---	---
	570	0.53	0.76	---	---
24	179	0.67	0.80	0.47	0.38
	265	0.75	0.95	0.40	0.38
	359	0.78	1.08	0.40	0.48
	440	0.73	0.97	---	---
	614	0.79	1.20	---	---
96	183	0.83	0.82	0.52	0.53
	330	0.97	0.96	0.43	0.47
	447	0.91	1.05	---	---
	630	0.99	1.06	---	---

Note: The mean values of the weights of lungs of 200 normal mice (in groups of ten) ranged from 0.37 to 0.55 grams.

Table V

Weights of Kidneys of Mice Exposed to Fluorine

(Mice were exposed to 30 ppm for 60 minutes; then 4, 24 or 96 hours later, they were exposed for 15 minutes to the concentrations listed below. Weight expressed in grams.)

Time Between Exposures (hrs)	Conc. (ppm)	Time of Sacrifice: 2 hours Pre-Exposed (g)	2 hours Control (g)	14 days Pre-Exposed (g)	14 days Control (g)
4	285	0.95	1.09	0.88	0.86
	310	0.98	1.06	0.75	0.80
	410	1.07	1.08	0.85	0.80
	494	0.94	1.14	---	---
	570	1.08	1.22	---	---
24	179	0.85	1.02	0.76	0.62
	265	0.89	1.07	0.80	0.68
	359	0.98	1.05	0.75	0.65
	440	1.07	1.06	---	---
	614	1.07	1.07	---	---
96	183	1.00	1.02	0.68	0.70
	330	1.04	1.10	0.75	0.74
	447	1.04	1.04	---	---
	630	1.08	1.08	---	---

Note: The mean values of the weights of kidneys of 200 normal mice (in groups of ten) ranged from 0.62 to 0.75 grams.

Pulmonary Edema

Since the previous studies showed that fluorine in high concentrations, can damage the lungs, liver and kidneys, some experiments were started to determine the nature of these effects. While histological examination of the tissues reveals structural changes, other criteria also can reveal structural and functional changes. One method is to determine the weights of organs of the exposed animals.

An increase in the weight of the lung can be due to edema. Congestion in the lung also can cause an increase in the absolute weight of the fresh lung. Since the water content of blood or lung is about the same, the water content expressed as a percent of lung weight does not change if the increased lung weight is due to congestion. However, edema fluid is 95% water; therefore, the formation of edema is reflected by an increase in water content expressed as a percent of fresh lung weight.

The technique is that fresh lung is weighed, placed in a freezer (-20°C) overnight, transferred to an evacuated desiccator and reweighed 48 hours later. After the animal was sacrificed, the anterior rib cage was cut and laid over the head of the mouse. A preweighed ligature was tied around the trachea, close to its bifurcation. Excess trachea was removed and the whole lung was weighed immediately on glassine paper. The lungs were suspended on a rack, placed in a freezer overnight, and then the rack and lungs were transferred to an evacuated desiccator the next morning. After 48 hours the lungs were reweighed and the percent loss of weight was calculated.

A number of preliminary studies were conducted to obtain information on normal or control animals and to perfect the technique. For example, the influence of different methods of sacrificing the animal were compared--digital pressure on cervical spinal column, euthanasia with pentobarbital, euthanasia with ether, and anesthesia with exsanguination. Since there were no differences among these techniques, digital pressure on the cervical spinal column was selected. Data were collected using the whole lungs and using only the right lung. If the right lung were used, a preweighed ligature was tied around the hilus of the lungs before being separated. The rest of the technique was the same as described above. The results are shown in Table I. Possible differences between males and females also were compared. (There were none.)

After the techniques for determining the water content of the lungs had been successfully perfected in our laboratory, mice were exposed to fluorine. Using 10 mice per group, exposures were to 125 ppm for 15 minutes. One group of ten mice was sacrificed 24 hours after exposure. The lungs were weighed immediately after being removed from the animal and again after being dried. The percentage loss of water was calculated. The fresh lungs were heavier than lungs of control mice. The mean losses of water for each time of sacrifice were as follows: 24 hours -- 92.1%, 48 hours -- 82.7%, 5 days -- 81.7%. The values representing loss of water were all higher than the loss in control

animals (upper limit of normal range is 80.95%), indicating definite presence of edema.

In this experiment the formation of the most edema occurred within the first 24 hours and the amount of edema was reduced gradually over the next few days. It was still present, however, on the fifth day following this exposure.

Mice were also exposed for 15 minutes to a high concentration (176 ppm) to determine if more edema would result from exposure to a higher concentration. Twenty mice were exposed with ten of them being sacrificed 24 hours after exposure and the other ten being sacrificed 72 hours after exposure. Again the weights of the fresh lungs were heavier than normal. The mean water loss from the lungs was 92.7% 24 hours after the exposure and 92.0% 72 hours after the exposure. These figures indicate that the higher concentration of fluorine may have caused slightly more edema within 24 hours and more definitely that the edema lasted longer.

These studies were repeated to verify the fact that there was pulmonary edema in mice exposed to fluorine. During these latter experiments the technique of drying the lungs was changed slightly so that there was greater loss of weight, expressed as percent of wet weight, than in some of the previous studies. The mean loss of total weight (lung plus ligature) of 50 control mice was 82.1% with a standard deviation of 2.1%.

The mice in groups of 5 each were exposed to fluorine for 15 minutes at concentrations of about 100, 200, 300 and 450 ppm. They were sacrificed at 24, 48 and 72 hours after exposure and the water content of the whole lungs determined. The results are presented in Tables II, III and IV. The loss of weight averaged 94% to 97% indicating that edema was in the lungs at all three periods of sacrifice.

TABLE I

Water Content of Whole Lungs
and Right Lungs of Control Mice

Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
	Whole Lungs		
344.1	62.6	281.5	83
461.8	89.9	371.9	81
374.4	72.8	301.2	81
387.0	55.7	331.3	86
463.2	85.3	377.9	87
433.2	70.5	362.7	84
399.6	74.9	324.7	81
495.9	109.6	386.3	78
395.9	80.0	315.9	80
368.2	63.8	304.4	83
412.2*	73.3	355.8	82
	Right Lungs		
229.3	31.6	197.7	86
261.7	42.3	219.4	84
267.1	39.8	227.3	85
282.5	53.2	229.3	81
257.1	38.5	218.6	85
265.5	42.7	222.8	84
306.4	50.5	255.9	84
222.5	46.9	175.6	79
254.4	38.4	216.0	85
256.4	38.0	218.4	85
260.3*	42.2	218.1	84

* The numbers in this row are means.

TABLE II

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 24 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group I (100 ppm)				
1	472.9	18.2	454.2	96
2	448.7	19.6	429.1	96
3	382.7	17.0	365.7	95
4	540.4	22.8	517.6	96
5	448.3	20.3	428.0	95
Mean	458.6	19.6	438.9	96
1	530.0	22.6	507.4	96
2	470.5	11.9	458.6	95
3	783.9	13.1	770.8	98
4	623.7	12.9	610.8	98
5	591.6	23.5	568.1	96
Mean	599.9	16.8	583.1	97
Group II (200 ppm)				
1	393.8	14.1	379.7	97
2	514.0	15.4	498.6	97
3	692.9	15.0	677.9	97
4	522.7	17.2	505.5	96
5	567.7	14.1	553.6	97
Mean	538.2	15.1	523.0	96
1	476.0	17.3	458.7	96
2	562.9	16.7	546.2	97
3	537.2	15.5	521.7	97
4	587.9	13.9	574.0	97
5	522.3	14.8	507.5	97
Mean	537.2	15.6	521.6	97

TABLE II continued

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 24 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group III (300 ppm)				
1	511.9	24.3	487.6	95
2	520.6	28.5	492.1	94
3	544.2	18.1	526.1	96
4	538.7	19.7	519.0	96
5	487.9	14.1	473.8	97
Mean	520.7	20.9	499.7	95
1	577.3	14.7	562.6	97
2	551.9	22.5	529.4	95
3	587.2	13.9	573.3	97
4	505.5	13.1	492.4	97
5	527.5	17.6	509.9	96
Mean	549.8	16.3	533.5	96
Group IV (450 ppm)				
1	527.2	26.9	500.3	94
2	699.2	20.1	679.1	97
3	510.4	19.6	490.8	96
4	398.3	14.4	383.9	96
5	486.0	15.9	470.1	96
Mean	524.2	19.4	504.8	95
1	406.1	19.3	386.8	95
2	513.2	24.7	488.5	95
3	557.5	18.6	538.9	96
4	538.7	15.2	523.5	97
5	566.1	17.6	548.5	96
Mean	513.1	19.1	497.2	95

TABLE III

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 48 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group I (100 ppm)				
1	448.0	13.2	434.8	97
2	488.9	24.6	464.3	95
3	382.1	14.9	367.2	96
4	579.6	28.7	550.9	95
5	437.2	17.6	419.6	96
Mean	467.1	19.8	447.3	96
1	573.7	24.9	548.8	96
2	483.6	28.9	454.7	94
3	693.5	23.1	670.4	97
4	736.5	27.6	708.9	96
5	535.8	23.8	512.0	96
Mean	604.6	23.7	578.9	96
Group II (200 ppm)				
1	412.7	17.8	394.9	96
2	593.2	23.5	569.7	96
3	537.2	14.2	523.0	97
4	573.7	25.8	547.9	96
5	648.5	27.9	620.6	96
Mean	553.1	23.8	531.2	96
1	572.9	28.1	544.8	95
2	557.6	22.3	535.3	96
3	532.2	21.2	511.0	96
4	603.7	21.6	582.1	96
5	596.3	23.2	573.1	96
Mean	572.5	23.3	549.2	96

TABLE III continued

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 48 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group III (300 ppm)				
1	552.8	25.2	527.6	95
2	587.9	28.9	559.0	95
3	512.7	23.1	489.6	95
4	539.5	27.6	511.9	94
5	522.2	23.8	498.4	95
Mean	543.0	25.7	517.3	94
1	568.3	24.4	543.9	95
2	570.2	26.9	543.8	95
3	539.1	25.7	513.4	95
4	558.7	24.6	534.1	95
5	596.8	19.8	577.0	96
Mean	572.5	24.2	542.4	96
Group IV (450 ppm)				
1	504.8	18.6	486.2	96
2	533.6	28.1	595.5	94
3	564.1	29.4	534.7	94
4	528.1	22.6	505.5	95
5	500.9	17.9	483.0	96
Mean	543.0	25.3	502.9	95
1	542.7	24.5	518.2	95
2	567.5	29.8	537.8	94
3	583.1	22.2	560.9	96
4	592.2	23.9	568.3	95
5	554.6	26.3	528.3	95
Mean	544.6	25.3	542.7	95

TABLE IV

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 72 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group I (100 ppm)				
1	392.9	16.2	376.7	96
2	354.0	17.4	336.6	95
3	410.6	19.2	391.4	95
4	429.8	16.7	413.1	96
5	348.6	13.3	335.3	96
Mean	387.1	16.6	370.6	96
1	345.0	13.7	331.9	96
2	404.2	22.9	381.3	94
3	477.5	19.1	458.4	96
4	430.5	15.2	415.3	96
5	400.7	22.5	378.2	94
Mean	411.6	18.7	393.0	95
Group II (200 ppm)				
1	393.9	12.1	381.8	96
2	401.6	15.7	385.9	96
3	422.7	13.3	409.4	96
4	387.2	19.6	367.6	94
5	449.1	15.4	433.7	96
Mean	410.9	15.2	396.4	95
1	395.7	29.1	366.6	92
2	342.3	17.3	325.0	94
3	374.0	11.5	362.7	96
4	359.3	21.7	337.6	93
5	401.6	15.6	386.0	96
Mean	374.6	15.2	355.5	94

TABLE IV continued

Edema in Lungs of Mice
Exposed to Fluorine for 15 Minutes
and Sacrificed 72 Hours Later

Group and Conc.	Fresh Wt. (mg)	Dry Wt. (mg)	Loss of Wt. (mg)	Loss of Wt. (percent)
Group III (300 ppm)				
1	384.9	16.8	368.1	95
2	414.3	14.1	400.2	96
3	362.8	18.9	343.9	94
4	378.1	14.6	363.5	96
5	402.9	23.2	379.7	94
Mean	388.6	17.5	371.0	95
1	372.1	14.9	357.2	95
2	421.1	14.1	407.0	96
3	460.7	18.9	441.8	95
4	393.6	14.6	379.0	96
5	418.3	23.2	395.1	94
Mean	413.2	17.5	396.0	95
Group IV (450 ppm)				
1	517.2	19.4	497.8	96
2	506.3	27.9	478.4	94
3	489.1	15.3	473.8	96
4	527.7	12.6	515.1	97
5	513.3	24.7	486.6	95
Mean	510.3	20.0	490.3	95
1	576.2	25.8	550.4	95
2	501.2	16.1	485.1	96
3	537.7	11.7	526.0	97
4	498.8	15.9	482.9	96
5	516.9	14.5	502.4	97
Mean	522.1	16.8	509.3	96

EFFECTS ON SPECIFIC ENZYMES

Introduction

It has been reported in the literature that exposure to irritants causes in rats a decreased activity of alkaline phosphatase in the lungs and a reduction of succinic dehydrogenase activity in the lungs. Sulfhydryl and disulfide compounds protect against the effects of certain irritant compounds by maintaining the cellular sulfhydryl enzymes and their cofactors in an active, reduced state. Succinic dehydrogenase is the key sulfhydryl enzyme in the lung. Another report showed that inhalation of acrolein, ozone, nitrogen dioxide, sulfur dioxide or formaldehyde for 18 to 20 hours caused an increase in alkaline phosphatase in the liver of rats. Further investigations have shown that the action of these irritant materials very probably is due to a stress reaction. The pituitary-adrenal system is stimulated, which leads to hypersecretion of glucocorticoids which stimulate the synthesis of increased amounts of these enzyme proteins in the liver.

Our previous studies with fluorine, as well as most of the investigations of the toxicity of other compounds when inhaled, were confined to pathologic and physiologic effects on the mucous membranes of the lungs and respiratory passages. A few studies have been made on possible biochemical changes resulting from inhalation of irritants.

Since fluorine is an irritant, at least one biochemical test to measure its effect was succinic dehydrogenase. Succinic dehydrogenase (and glutathione) in the lung is decreased following a single exposure to another irritant, ozone. Tolerance to ozone (either by prior exposure to ozone itself or to oil mist) is reflected by a maintenance of succinic dehydrogenase and glutathione activities in the lungs.

It was felt therefore that more information regarding the biochemical changes produced by fluorine and their correlation with susceptibility of the animal was needed. Several enzyme systems were tested to determine if certain biochemical systems in the animal body were the target of fluorine.

The first objective was to determine if single, short-term exposures to fluorine caused biochemical changes similar to those seen following inhalation of other respiratory irritants. It was hoped that the information could be applied toward design of practicable tests which could be done in man to determine effects from exposure to fluorine.

The rat was selected as the experimental animal to be tested, partially because more background information with regard to enzyme changes as a result of inhalation of irritants was available. The lung, liver and kidney were selected for the enzyme studies because they were the target tissues which showed damage as a result of exposure to high concentrations of fluorine. The enzymes studied were succinic dehydrogenase, alkaline phosphatase and glutamic oxaloacetic transaminase. Each of these was studied in all three tissues.

Results

The succinic dehydrogenase activity of fresh lung, liver and kidney tissues was determined by modifications of the Method of Kun and Abood (Science 109: 144-146, 1949). Briefly, tissue homogenates in the presence of succinic dehydrogenase, reduce tetrazolium (colorless) to a red formoz an. The amount of color produced is proportional to the amount of succinic dehydrogenase. The test, therefore, is quantitative.

After a number of tests were conducted to determine the normal succinic dehydrogenase content of different tissues of experimental animals, the test was repeated on the tissues of animals which had been exposed to fluorine.

Alkaline phosphatase activity in the tissues was determined by modifications of the conventional method described in the handbook, "Bausch and Lomb, Spectronic 20."

Glutamic oxaloacetic transaminase activity in the tissues was determined by modifications of the method described by Sobel, et al (Am. J. Clin. Path. 26: 1477-1478, 1956).

While more recent methods for the determination of alkaline phosphatase and glutamic oxaloacetic transaminase in the serum are available, it should be emphasized that these tests were made on homogenates of tissue, not in serum.

A number of tests were conducted to determine the normal activity of these three enzymes in all three tissues. Then animals of the same age and sex were exposed to fluorine to determine its effect on the activities in these tissues.

In the first preliminary experiments the results indicated that exposure to fluorine caused an increase in the succinic dehydrogenase in the lungs. Rats were exposed to fluorine for 15 minutes at concentrations of 150 to 200 ppm. Groups of rats were sacrificed 1, 2 and 5 days after exposure and the succinic dehydrogenase content of the lungs determined. The mean value for succinic dehydrogenase in lungs from control rats was 0.674 units. The mean values for the lungs of the rats exposed to fluorine and sacrificed at 1, 2, and 5 days after exposure were 1.100, 1.336 and 1.290 units, respectively. These data suggested that at least certain exposures to fluorine caused an increase in succinic dehydrogenase content of the lungs of rats and that the increase was still apparent 5 days after exposure.

Additional experiments were conducted with six to ten animals per group exposed to fluorine. The concentration of fluorine was kept constant (at 100 ppm) for most of the studies. The exposure times were 15, 30 or 60 minutes. The times of sacrifice of the animals were 2, 24 or 96 hours after exposure.

These results indicated that the activity of succinic dehydrogenase (Table I) in the liver was unchanged. There was a slight increase in the activity of succinic dehydrogenase in the lungs. The change was apparent

in almost all of the animals, but was statistically significantly increased only in those sacrificed 24 hours after a single exposure to 100 ppm for 60 minutes. There was no significant change in succinic dehydrogenase activity in the kidney following a single exposure to fluorine at 100 ppm for 15 or 30 minutes, however, a single exposure to 100 ppm for 60 minutes caused a slight increase. Repeated exposures (100 ppm, 30 minutes each) caused a decrease of about 50% in the activity of succinic dehydrogenase in the kidney.

Alkaline phosphatase activity (Table II) was increased in the lungs and liver, but was unchanged in the kidney. This increase was apparent at 24 hours after exposure, but the activity was normal by 96 hours after exposure.

The activity of glutamic oxaloacetic transaminase (GOT) appeared to be significantly increased in the lungs when the animals were sacrificed 24 hours after exposure (Table III). Following exposure to 100 ppm for 60 minutes, the activity was increased 2 hours after exposure. At 24 hours the activity in the animals exposed for 30 minutes was not significantly increased. (This study was repeated.) The activity of GOT in both the liver and kidney appeared to be increased after a single exposure to fluorine at 100 ppm for 15, 30 or 60 minutes. The effect was quite apparent at 2 and 24 hours, but was not as apparent at 96 hours after exposure. Following three repeated exposures (24 hours apart), the activity in all three tissues was lower but not significantly lower.

TABLE I

Activity of Succinic Dehydrogenase in
Lungs, Liver and Kidneys of
Rats Exposed to 100 ppm Fluorine

(Activity expressed as mcg. triphenyltetrazolium chloride reduced.
Values are mean ± standard deviation of 6 animals/group.)

Exposure Time (minutes)	Sacrifice Time (hours)	Lung (mcg.)	Liver (mcg.)	Kidney (mcg.)
0	-	31.0 ± 7.5	338.0 ± 87.6	302.5 ± 47.3
15	2	56.0 ± 19.3	266.6 ± 55.7	353.6 ± 103.0
15	24	35.3 ± 20.4	320.0 ± 81.1	366.6 ± 73.8
30	2	46.5 ± 30.8	391.0 ± 101.6	317.3 ± 81.6
30	24	42.0 ± 12.7	327.0 ± 54.6	331.4 ± 81.6
60	2	47.0 ± 6.6	290.0 ± 126.0	422.6* ± 57.6
60	24	102.8* ± 32.4	430.0 ± 102.0	419.0* ± 63.2
60	96	42.6 ± 23.4	305.0 ± 31.0	347.5 ± 30.8
30**	24	37.0 ± 14.2	219.0 ± 37.2	149.5* ± 41.4

* Significantly different from control ($p = <0.05$)
** 30-minute exposures to 50 ppm every 24 hours for 3 days.

TABLE II

Activity of Alkaline Phosphatase in
Lungs, Liver and Kidneys of
Rats Exposed to 100 ppm Fluorine

(Activity expressed as micromoles of phenol. Values are mean ± standard deviation of 6 animals/group.)

Exposure Time (minutes)	Sacrifice Time (hours)	Lung (mcm.)	Liver (mcm.)	Kidney (mcm.)
0	-	0.303 ± 0.030	0.179 ± 0.034	0.725 ± 0.213
15	2	0.458* ± 0.132	0.170 ± 0.180	1.330* ± 0.254
15	24	0.610* ± 0.016	0.551* ± 0.055	0.831 ± 0.096
30	2	0.303 ± 0.031	0.164 ± 0.033	0.970 ± ***
30	24	0.393* ± 0.085	0.279* ± 0.079	0.866 ± 0.092
60	2	0.543* ± 0.121	0.210 ± 0.012	0.938 ± 0.087
60	24	0.336 ± 0.136	0.234* ± 0.036	0.998 ± 0.118
60	96	0.560* ± 0.092	0.176 ± 0.040	0.985 ± ***
30**	24	0.506* ± 0.142	0.105* ± 0.034	0.813 ± 0.182

* Significantly different from control ($p = <0.05$)
** 30-minute exposures to 50 ppm for 3 times, 24 hours apart.
*** Not calculated. Number of animals too few.

TABLE III

Activity of Glutamic Oxaloacetic Transaminase in
Lungs, Liver and Kidneys of
Rats Exposed to 100 ppm Fluorine

(Activity expressed as micrograms of pyruvic acid produced in 30 minutes. Values are mean ± standard deviation of 6 animals/group)

Exposure Time (minutes)	Sacrifice Time (hours)	Lung (mcg.)	Liver (mcg.)	Kidney (mcg.)
0	-	82.1 ± 47.8	216.6 ± 54.5	228.3 ± 39.8
15	2	98.5 ± 57.5	372.0* ± 83.6	339.7* ± 62.5
15	24	165.6* ± 39.6	400.0* ± 34.2	330.0* ± 84.6
30	2	39.8 ± 21.4	203.6 ± 33.5	274.6* ± 23.9
30	24	98.8 ± 21.2	348.8* ± 89.3	263.0* ± 42.4
60	2	164.6* ± 30.0	384.1* ± 68.0	382.0* ± 58.0
60	24	144.3* ± 11.5	427.4* ± 56.7	337.6* ± 79.8
60	96	106.8 ± 43.5	305.0 ± 77.5	290.6* ± 47.7
30**	24	54.7 ± 17.3	188.7 ± 94.4	174.3 ± 38.2

* Significantly different from controls ($p = <0.05$)
** 30-minute exposures to 50 ppm every 24 hours for 3 days.

These studies were continued to improve methodology, to obtain more data from control animals and to obtain additional results after exposures to fluorine. The concentration of fluorine was kept constant (about 100 ppm) and the exposure times were 15, 30 or 60 minutes.

Succinic Dehydrogenase

The activity of succinic dehydrogenase in tissues of control rats is presented in Table IV. The activity is expressed as micrograms of triphenyltetrazolium chloride reduced.

TABLE IV

Succinic Dehydrogenase

Control Rats

	Lung (mcg.)	Liver (mcg.)	Kidney (mcg.)
	-	170	260
	40	285	279
	33	270	260
	88	255	320
	33	147	150
	22	255	186
	18	232	202
	18	236	186
	25	182	158
	8	260	158
Mean	29 ± 23	229 ± 44	216 ± 18
	28	185	214
	43	170	218
	46	214	235
	24	170	173
	20	151	202
	46	218	226
Mean	35 ± 8	186 ± 21	211 ± 18

TABLE IV continued

Succinic Dehydrogenase

Control Rats

Lung (mcg.)	Liver (mcg.)	Kidney (mcg.)
45	435	390
32	390	373
32	360	215
20	200	280
20	437	282
30	380	282
23	407	227
25	373	244
36	490	303
36	262	310
37	237	305
27	310	252
40	250	262
	215	326
	325	
Mean 31 ± 7	338 ± 87	306 ± 47

The activity of succinic dehydrogenase was measured after exposure to 100 ppm fluorine for 15, 30 or 60 minutes. The animals were sacrificed at 2, 24 or 96 hours after exposure. Some animals also were exposed three times for 30 minutes to 50 ppm, every 48 hours after the last exposure. The activities of succinic dehydrogenase in the three tissues are presented in Tables V to VII and are summarized in Table VIII.

TABLE V

Succinic Dehydrogenase in Lungs
of Rats Exposed to 100 ppm Fluorine for 15 Minutes

Lungs		Liver		Kidney	
Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.
52	15	340	372	422	378
48	43	205	426	415	378
93	10	320	342	512	447
51	35	208	170	220	307
36	37	260	337	266	330
72		288		286	360
56*	35	267	320	354	367
(19)	(20)	(55)	(81)	(103)	(44)

* The numbers in this row indicate mean values.
Number in parentheses indicate standard deviation.

TABLE VI

Succinic Dehydrogenase in Lungs
of Rats Exposed to 100 ppm Fluorine for 30 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*
45	60	21	580	405	140	220	297	90
45	35	21	348	388	210	420	305	225
72	33	30	405	337	236	240	480	135
45	40	45	285	275	246	300	235	127
47	35	60	340	275	240	335	340	152
25	53	45	____	283	243	390	____	168
47**	42	37	392	327	219	318	331	150
(12)	(13)	(14)	(102)	(55)	(37)	(74)	(82)	(41)

* Exposed 3 times, every 48 hours.
** The numbers in this row indicate mean values.
Number in parentheses indicate standard deviation.

TABLE VII

Succinic Dehydrogenase in Lungs
of Rats Exposed to 100 ppm Fluorine for 60 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.
20	132	86	476	566	293	436	362	311
60	75	35	364	394	296	-	407	341
67	57	51	360	565	260	430	505	325
45	122	48	267	392	325	305	393	337
35	146	16	170	371	296	435	362	365
55	85	70	105	293	360	507	490	406
47*	103	43	290	430	305	423	419	348
(7)	(32)	(23)	(126)	(102)	(31)	(57)	(63)	(31)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE VIII

Summary of
Succinic Dehydrogenase Activity

(Activity expressed as mcg triphenyltetrazolium reduced)

ppm	minutes	sacrifice	Lung	Liver	Kidney
			31 ± 7	338 ± 87	302 ± 37
			-	327 ± 33	306 ± 47
			29 ± 23	229 ± 44	216 ± 18
			31 ± 8	338 ± 88	303 ± 47
100	15	2	56 ± 19*	267 ± 55	354 ± 103
		24	35 ± 20	320 ± 81	367 ± 44
100	30	2	47 ± 7*	392 ± 102	318 ± 74
		24	42 ± 13*	327 ± 55	331 ± 82
	30	24	52 ± 8*	228 ± 23	239 ± 25
100	60	2	47 ± 7*	290 ±126	423 ± 57
	60	2	48 ± 8*	295 ± 30	276 ± 28
		24	103 ± 32*	430 ± 102	419 ± 63
		96	43 ± 23	305 ± 31	348 ± 31
50**	30	24	37 ± 14	219 ± 37*	150 ± 41*

* Significantly different from concurrent control.
** Three exposures every 48 hours.

The mean activity of succinic dehydrogenase in lungs from control animals was about 30 mcg. The value of 29 ± 23 mcg was from one of the first groups, and the standard deviation was very high. This standard deviation was ignored in the statistical evaluation of effects after exposure to fluorine. In general, the activity was increased and the increase was apparent within two hours after exposure. The activity in animals sacrificed 96 hours after exposure appeared to be nearly normal. Three exposures, every 48 hours, did not change the activity significantly which indicated that some type of tolerance developed.

Although there was considerable variation in the activity of succinic dehydrogenase in homogenates of liver and kidneys, there did not appear to be a significant change caused by exposure to fluorine. If there were significant changes, the activity in both organs was lower after repeated exposures.

Glutamic Oxaloacetic Transaminase

The activity of glutamic oxaloacetic transaminase was determined in additional control animals at different times during the investigation. The concentrations of homogenates of tissue used in these studies varied from 0.5 to 10.0 percent. Some of the activities from control rats are presented in Table IX.

The activity in lungs, liver and kidneys of rats exposed to 100 ppm fluorine for 15, 30 or 60 minutes, and sacrificed 2, 24 or 96 hours later are presented in Tables X to XII. The activity after three intermittent exposures, 30 minutes each, also is included.

These results are summarized in Table XIII.

The activity of glutamic oxaloacetic transaminase was increased in homogenates of all three tissues at two and 24 hours after exposure but had returned to normal by 96 hours after exposure. The lower control values were from 0.5 percent homogenate of tissue. The values after 30 minutes of exposure also were on the same concentration, which is why they are significantly elevated; but do not appear to be as high as values after the other exposures (see Summary Table XIII.).

After three repeated exposures the activity in all three tissues was normal.

The activity in all of these tables is expressed as micrograms of pyruvic acid produced in thirty minutes.

TABLE IX

Glutamic Oxaloacetic Transaminase

Control Rats

(Activity expressed as mcg pyruvic acid)

	Lung (mcg.)	Liver (mcg.)	Kidney (mcg.)
	57	96	95
	72	100	102
	53	98	102
	50	95	95
	75	102	98
	80	105	102
Mean	65 ± 12	99 ± 5	99 ± 4
	95	262	130
	57	250	160
	135	295	190
	95	148	155
	78	108	185
	43	233	127
	78	220	150
	43	233	127
	78	220	150
	101	185	140
	50	265	155 ± 22
	75	200	190
	91		260
	67		270
	60		167
	97		218
	110		265
Mean	81 ± 24	217 ± 54	228 ± 40

TABLE X

Glutamic Oxaloacetic Transaminase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 15 Minutes

Lungs		Liver		Kidney	
Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.
38	116	352	375	345	330
38	244	225	550	335	380
103	149	463	550	380	330
192	177	353	550	232	242
68	154	380	550	372	252
152	154	463	550	375	442
99*	166	372	521	340	330
(58)	(40)	(84)	(36)	(63)	(84)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XI

Glutamic Oxaloacetic Transaminase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 30 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*
23	67	50	240	497	240	286	330	195
40	77	75	173	364	215	272	282	123
75	98	60	165	218	192	225	249	138
32	-	75	250	408	170	286	210	180
11	127	28	214	321	170	293	266	170
58	105	40	180	285	145	286	246	240
40**	95	55	204	349	189	275	263	174
(21)	(21)	(17)	(33)	(89)	(99)	(24)	(42)	(38)

* Exposed 3 times, every 30 minutes.
** The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XII

Glutamic Oxalcacetic Transaminase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 60 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.
155	140	138	303	467	297	376	296	298
138	143	69	302	387	227	289	482	268
130	155	183	437	-	381	350	215	260
200	127	114	448	462	435	460	334	380
200	161	77	352	336	249	447	352	139
-	140	60	463	485	241	370	338	265
146*	144	107	384	427	305	382	338	265
(30)	(12)	(44)	(68)	(57)	(77)	(58)	(80)	(48)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XIII

Summary of
Glutamic Oxaloacetic Transaminase
Activity

(Activity expressed as mcg pyruvic acid)

ppm	minutes	sacrifice	Lung	Liver	Kidney
			81 ± 24	217 ± 54	155 ± 22
			83 ± 18	185 ± 23	228 ± 40
			82 ± 48	217 ± 55	228 ± 40
			65 ± 12	99 ± 5	99 ± 4
100	15	2	99 ± 58	372 ± 84*	340 ± 63*
100	15	24	166 ± 40*	521 ± 36*	330 ± 84*
100	30	2	40 ± 21	204 ± 33*	275 ± 24*
100	30	24	95 ± 21*	349 ± 89*	263 ± 42*
100	60	2	146 ± 30*	384 ± 68*	382 ± 58*
100	60	24	144 ± 12*	427 ± 57*	338 ± 30*
100	60	96	107 ± 44	305 ± 77	265 ± 48
50**	30	24	55 ± 17	189 ± 99	174 ± 38

* Significantly different from concurrent control.
** Repeated exposures, 48 hours apart.

Alkaline Phosphatase

The activity of alkaline phosphatase in homogenates of tissue (not in serum) of control rats is presented in Table XIV. Again different concentrations of homogenates were used which explains the apparently considerable variation among groups.

The activities after exposure to fluorine are presented in Tables XV through XVIII.

These data were difficult to evaluate because of the marked variation between animals which resulted in high standard deviations. It appears that exposure to fluorine caused an increase in the activity of alkaline phosphatase in the lungs and liver, particularly at 24 hours after exposure. The activity in the kidneys appeared to be elevated only after 60 minutes exposure, but not at shorter exposures. Although the value after 15 minutes exposure with sacrifice at two hours was "statistically" higher, this probably was an artifact.

Repeated exposures did not cause an elevation.

The activities in all of these tables are expressed as millimicromoles of phenol produced.

TABLE XIV

Alkaline P' osphatase

Control Rats

(Activity expressed as millimicromoles of phenol)

Lung	Liver	Kidneys
98*	34**	170*
105	45	167
144	45	167
90	55	120
90	34	148
75	67	148
100 ± 12	47 ± 12	153 ± 20
64	150*	105**
80	160	84
84	250	86
99	180	40
80	140	60
100	210	60
81	300	53 ± 21
71	260	
80	350	
82 ± 11	180 ± 30	
31**	80*	
35	100	
30	110	
29	110	
32	110	
25	70	
30 ± 3	170	
	110 ± 30	

* 2.5% Homogenate
** 0.5% Homogenate

TABLE XV

Alkaline Phosphatase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 15 Minutes

Lungs		Liver		Kidney	
Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 2 hr.	Sac. 24 hr.
460	360	130	540	1400	880
255	610	135	615	1600	705
370	460	115	500	1300	910
580	675	275	165	990	700
430	720	170	150	1300	902
655	840	200	205	1390	860
458*	610	170	551	1330	831
(132)	(16)	(18)	(55)	(150)	(96)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XVI

Alkaline Phosphatase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 30 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*	Sac. 2 hr.	Sac. 24 hr.	Sac. 24 hr.*
1020	1010	350	275	345	120	705	970	750
-	820	350	170	410	140	830	880	710
725	1000	500	110	210	150	700	870	530
690	-	600	130	290	70	700	860	1120
815	1070	750	185	200	70	970	880	870
845	1020	490	115	220	80	850	750	900
819*	980	510	164	279	110	759	870	810
(115)	(90)	(10)	(33)	(78)	(30)	(83)	(40)	(180)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XVII

Alkaline Phosphatase
in Lungs, Liver and Kidneys of Rats
Exposed To 100 ppm Fluorine for 60 Minutes

Lungs			Liver			Kidney		
Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.	Sac. 2 hr.	Sac. 24 hr.	Sac. 96 hr.
720	320	605	200	240	170	800	830	980
560	385	680	-	245	220	970	1200	800
600	450	640	180	255	205	950	990	950
520	525	495	280	170	155	850	1000	990
330	195	410	210	280	200	1030	980	950
530	145	530	180	215	110	1030	980	960
543*	336	560	210	234	176	940	990	940
(121)	(136)	(92)	(12)	(36)	(40)	(90)	(120)	(81)

* The numbers in this row indicate mean values.
Numbers in parentheses indicate standard deviation.

TABLE XVIII

Summary of
Alkaline Phosphatase Activity

(Activity expressed as millimicromoles of phenol)

ppm	minutes	sacrifice	Lung	Liver	Kidney
			303 ± 30	179 ± 34	725 ± 213
			820 ± 110	180 ± 30	530 ± 210
			300 ± 30	110 ± 30	-
			100 ± 12	47 ± 12	153 ± 20
100	15	2	458 ± 132*	170 ± 18	1330 ± 25*
100	15	24	610 ± 16*	551 ± 55*	831 ± 96
100	30	2	819 ± 155*	164 ± 33	759 ± 83
100	30	24	980 ± 90*	279 ± 78*	870 ± 90
		24	510 ± 100	110 ± 30	810 ± 180
100	60	2	543 ± 121*	210 ± 12	940 ± 90*
100	60	24	336 ± 136	234 ± 36*	990 ± 120*
100	60	96	560 ± 92*	176 ± 40	940 ± 81*
50**		24	510 ± 10	110 ± 3	810 ± 180

* Significantly different from concurrent control.
** Repeated exposures, 48 hours apart.

Summary

These results from studies of the activity of certain enzymes in the lung, liver and kidney indicated that there was some increase in activity. In the lung, the activities of succinic dehydrogenase, alkaline phosphatase and glutamic oxaloacetic transaminase were increased. In the liver, alkaline phosphatase and glutamic oxaloacetic transaminase were increased, but succinic dehydrogenase was unchanged. In the kidney, glutamic oxaloacetic transaminase was increased, while succinic dehydrogenase was unchanged. Alkaline phosphatase in the kidney appeared to be unchanged after 15 or 30 minutes of exposure, but appeared to be increased after exposures of 60 minutes.

The activity of succinic dehydrogenase in the lungs is in agreement with and offers at least a partial explanation of the apparent tolerance from previous exposures to fluorine.

INFLUENCE OF AGE OF ANIMAL

The age of an animal is very important with regard to response to a chemical. The very young and/or very old animals often are more susceptible than normal, adult animals. Sometimes the young are more susceptible because certain enzyme systems are not fully developed. The old may be more susceptible because of tissue changes which result from ageing. The ageing lung, for example, shows a reduced vital capacity, reduced total lung capacity, increased residual volume, loss of elastic forces, emphysema, etc.

Accidental exposure to fluorine from a launch vehicle could occur in people of all ages. Therefore, the effects of fluorine in animals of different ages were determined.

The lethal effects of fluorine were measured in weanling, young adult and rather old mice. The three different ages of the mice were 21, 50 and 365 days. The LC_{50} following 15 minutes of exposure was determined. Ten mice were exposed at each concentration and five or six concentrations were used to determine the LC_{50} of each age group. The LC_{50}'s were 530 ppm--21 days old, 250 ppm--50 days old, 450 ppm--365 days old. These data indicate that neither the very young (21 day old) mouse nor the old (365 day old) mouse is more susceptible to fluorine than a young adult animal.

Coagulation of Blood

Introduction

In some of the previous experiments it was noted that the blood from animals exposed to high concentrations of fluorine did not appear to coagulate as rapidly as blood from the normal animal. It is well known that fluorides are anticoagulants and it was felt that enough fluoride might be absorbed to inhibit clotting. Therefore the coagulation time and bleeding time in rats were determined before and after exposure to fluorine.

Methods

Young adult (three month old) albino female rats were used. Bleeding time was determined by the method of Dukes which is a measure of the time necessary for blood to clot from a small laceration in the skin. The end-point is determined when the blood clots sufficiently so that no stain appears when touched with filter paper. The coagulation time is the time it takes for the blood to clot after it has been shed. The blood was drawn into a glass capillary tube and clotting determined by breaking the tube.

Both coagulation and bleeding times were determined on each of 40 rats before exposure. Then groups of ten rats were exposed as follows:

Group A - 60 minutes - 100 ppm

Group B - 15 minutes - 203 ppm

Group C - 15 minutes - 165 ppm

Group D - 60 minutes - 77 ppm

The coagulation and bleeding times of Groups A and C were measured 2, 5 and 14 days after exposure, while those of Groups B and D were measured 1, 3 and 7 days after exposure.

Results

The results of these tests are presented in Tables III to VI. Six control groups of 10 rats each also were used for comparison. The coagulation times are presented in Table I, and the bleeding times are presented in Table II. Coagulation and bleeding times after exposure were all conducted in the morning.

There was a slight increase in coagulation time of rats exposed to 100 ppm fluorine for 60 minutes. The bleeding time, however, was unchanged. Neither the bleeding time nor coagulation time was significantly changed following exposure to 203 ppm for 15 minutes.

Although the absorption of sufficient quantities of fluoride can cause an increase in bleeding time and coagulation time, these exposures to fluorine apparently were not severe enough to cause absorption of the amount of fluoride necessary to prolong the clotting time of the blood.

TABLE I

Coagulation Time of Control Rats

(Times Expressed in Seconds)

Rat Number	Groups E	F	G	H	I	J
1	240	150	180	180	210	210
2	210	180	210	210	210	210
3	210	150	180	210	210	240
4	180	210	210	180	210	180
5	180	180	180	150	240	210
6	240	150	180	150	210	210
7	270	150	240	120	210	150
8	270	150	240	210	240	240
9	240	150	210	150	240	180
10	240	210	210	240	210	210
Mean	228	168	204	180	219	204
S. D.	37	25	25	37	15	28

TABLE II

Bleeding Time of Control Rats

(Times Expressed in Seconds)

Rat Number	Groups E	F	G	H	I	J
1	135	136	144	139	100	133
2	160	133	131	135	118	99
3	136	112	119	164	107	105
4	109	115	90	99	116	120
5	117	85	120	111	112	77
6	85	116	129	84	97	107
7	108	85	132	86	159	92
8	119	76	166	151	68	169
9	113	104	88	92	111	84
10	146	100	110	125	135	94
Mean	123	106	123	119	112	108
S.D.	22	20	24	28	24	27

TABLE III

Coagulation Time and Bleeding Time
of Rats Exposed to Fluorine at 100 ppm for 60 Minutes

(Times Expressed in Seconds)

Rat Number	Coagulation Time				Bleeding Time			
	Control	2 Days	5 Days	14 Days	Control	2 Days	5 Days	14 Days
1A	150	210	210	210	113	109	82	92
2A	180	270	180	240	90	175	74	100
3A	150	240	240	210	92	90	108	128
4A	150	150	150	180	82	96	53	81
5A	180	210	190	210	102	105	84	112
6A	210	270	150	180	106	95	105	128
7A	180	240	210	210	92	81	102	131
8A	210	240	210	240	97	99	71	104
9A	210	270	210	210	86	102	83	110
10A	240	210	150	240	109	102	79	124
Mean	186	233	190	213	97	105	84	111

TABLE IV

Coagulation Time and Bleeding Time
of Rats Exposed to Fluorine at 203 ppm for 15 Minutes

(Times Expressed in Seconds)

Rat Number	Coagulation Time				Bleeding Time			
	Control	1 Day	3 Days	7 Days	Control	1 Day	3 Days	7 Days
1B	210	330	210	210	109	143	137	91
2B	270	60	180	240	140	63	82	109
3B	270	240	270	240	91	77	119	94
4B	270	240	240	210	100	77	60	114
5B	240	300	270	270	79	119	109	131
6B	240	300	150	240	117	98	108	112
7B	210	270	180	240	86	89	92	161
8B	240	240	150	270	106	125	78	118
9B	240	270	210	180	115	92	72	97
10B	270	240	270	240	81	81	190	91
Mean	246	249	213	234	102	96	105	112

TABLE V

Coagulation Time and Bleeding Time
of Rats Exposed to Fluorine at 165 ppm for 15 Minutes

(Times Expressed in Seconds)

Rat Number	Coagulation Time				Bleeding Time			
	Control	2 Days	5 Days	14 Days	Control	2 Days	5 Days	14 Days
1C	240	240	240	180	118	103	120	95
2C	300	240	180	150	159	124	104	83
3C	240	240	270	240	121	158	147	129
4C	240	180	270	150	116	76	116	88
5C	240	210	240	240	121	108	134	124
6C	180	180	210	210	86	74	97	115
7C	240	150	240	300	121	79	102	158
8C	210	210	150	180	103	103	99	80
9C	270	240	240	390	161	134	163	200
10C	210	180	300	240	112	125	102	113
Mean	237	207	234	228	122	108	118	119

TABLE VI

Coagulation Time and Bleeding Time
of Rats Exposed to Fluorine at 77 ppm for 60 Minutes

(Times Expressed in Seconds)

Rat Number	Coagulation Time				Bleeding Time			
	Control	1 Day	3 Days	7 Days	Control	1 Day	3 Days	7 Days
1D	180	180	210	210	112	79	113	101
2D	180	240	240	210	100	86	100	79
3D	210	210	240	180	87	140	128	107
4D	180	210	270	180	80	75	188	92
5D	180	180	150	240	95	90	80	77
6D	240	240	270	210	106	92	86	84
7D	210	270	210	210	97	85	86	104
8D	150	240	180	150	66	110	66	76
9D	300	210	240	180	107	99	148	94
10D	270	210	240	180	188	90	193	76
Mean	210	219	225	195	104	95	119	89

Effects of Ascorbic Acid on Mortality and Pulmonary Edema Produced by Fluorine

Introduction

These studies were designed to determine if ascorbic acid (Vitamin C) protected mice against effects of fluorine.

Experimental Investigation

There were 20 mice per group exposed to fluorine. Ten mice in each group served as controls. The other 10 mice in each group were injected intraperitoneally with ascorbic acid (500 mg/kg) before or after being exposed to fluorine according to the following schedule:

Three Groups - ascorbic acid 4 hours before fluorine
Three Groups - ascorbic acid 30 to 60 minutes before fluorine
Three Groups - ascorbic acid 5 to 10 minutes after fluorine

All mice were exposed to fluorine for 15 minutes. The concentration of fluorine ranged from 0.129 to 1.470 mg/l. These concentrations were selected to cover those which cause no mortality to those which cause 100% mortality within a few hours. Survivors were sacrificed 48 hours after exposure.

The wet weight of the lungs of all mice was recorded. The amount of edema in the lungs was determined according to the methods described previously.

Results

The results are presented in Tables I through IX. The wet weight of the lungs (after blotting lightly with filter paper), the dry weight of the lungs (after freezing and being in a dessicator), the loss in weight after drying and the per cent loss in weight are included. Ordinarily by this technique, it is considered that the normal mean loss in weight is 78.4 per cent with a standard deviation of ± 1.02 per cent. Any loss greater than 80 or 81 per cent is considered indicative of edema. Edema fluid is 94.9 per cent water while blood is 78.5 per cent water.

With some of these animals the loss in weight was not as much as had been found previously. However the point of emphasis in these experiments was any difference between animals treated with ascorbic acid and those untreated.

The results are summarized in Table X where the concentrations of fluorine, time of treatment with ascorbic acid relative to exposure, per cent mortality and range of loss of weight of lungs after drying are presented.

Summary

There was a slight indication of protection from the lethal effects in two of the nine groups. In one of the groups exposed to 0.129 mg/l and injected with ascorbic acid 60 minutes before exposure, none of the treated animals died, while there was 20% mortality in control animals. In one of the groups injected four hours before exposure to 0.520 mg/l, there was 70% mortality while all of the controls died (and died in a shorter period of time after exposure).

There was also a slight indication of protection against edema in the group treated 45 minutes before exposure to 1.470 mg/l (a concentration lethal within a few minutes to a few hours). The loss of weight of the lungs of control (exposed) mice ranged from 82 to 87%, while that of treated mice ranged from 77 to 81% indicating less edema in the lungs of treated mice.

In the other six groups of mice there was no significant difference of protection by ascorbic acid, as used in these studies, against death or the edema produced by exposure to fluorine for 15 minutes.

TABLE I

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.430 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	296	82	214	72
	2	293	84	209	71
	3	299	85	214	72
	4	210	62	152	72
	5	308	90	218	71
	6	273	81	192	70
	7	250	69	181	73
	8	330	92	238	72
	9	279	77	202	72
	10	593	94	479	81
	Mean				
Treated	1	275	68	207	75
	2	221	59	162	73
	3	256	68	188	74
	4	256	66	190	77
	5	203	53	150	74
	6	283	70	213	75
	7	269	67	202	75
	8	300	85	215	72
	9	451	114	347	77
	10	519	91	427	82
	Mean				

* Injected with ascorbic acid 5 minutes after fluorine

TABLE II

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.220 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	298	79	219	74
	2	361	90	271	75
	3	342	83	259	76
	4	279	75	204	73
	5	423	99	324	77
	6	279	72	207	74
	7	335	80	255	76
	8	316	86	230	73
	9	206	51	155	75
	10	239	61	178	75
	Mean				
Treated	1	181	41	140	77
	2	277	62	215	75
	3	268	65	203	76
	4	257	54	203	79
	5	201	53	152	76
	6	208	50	158	76
	7	184	45	139	76
	8	272	64	208	77
	9	211	53	158	75
	10	281	66	215	77
	Mean				

* Injected with ascorbic acid 30 minutes before fluorine

TABLE III

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.338 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	231	69	162	70
	2	330	63	267	81
	3	307	88	219	72
	4	276	74	202	73
	5	317	92	225	71
	6	312	90	222	71
	7	320	90	230	72
	8	333	82	252	76
	9	215	62	153	71
	10	735	143	592	81
	Mean				
Treated	1	314	80	234	75
	2	343	89	254	74
	3	277	74	203	73
	4	366	96	270	74
	5	358	81	277	77
	6	236	66	170	72
	7	303	81	222	73
	8	238	53	185	77
	9	887	191	698	80
	10	583	139	444	76
	Mean				

* Injected with ascorbic acid 10 minutes after fluorine

TABLE IV

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 1.470 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	558	96	462	82
	2	569	76	493	87
	3	355	90	265	75
	4	446	62	384	86
	5	561	89	472	84
	6	567	86	481	85
	7	645	84	561	87
	8	531	98	433	82
	9	742	185	557	75
	10	492	118	374	76
	Mean				
Treated	1	466	111	355	76
	2	407	100	307	76
	3	328	79	249	77
	4	426	79	347	81
	5	268	63	205	77
	6	404	79	325	80
	7	387	101	286	73
	8	474	117	357	75
	9	491	115	376	76
	10	460	113	347	75
	Mean				

* Injected with ascorbic acid 45 minutes before fluorine

TABLE V

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.374 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	348	91	257	74
	2	323	86	247	76
	3	357	84	273	77
	4	514	111	403	79
	5	430	110	330	77
	6	430	104	326	76
	7	340	81	259	77
	8	277	65	208	75
	9	290	76	214	74
	10	317	76	241	76
	Mean				
Treated	1	406	95	311	77
	2	231	60	171	74
	3	310	75	235	77
	4	258	62	196	76
	5	312	86	226	72
	6	364	91	275	75
	7	358	92	266	74
	8	226	58	168	75
	9	185	56	129	70
	10	298	69	229	77
	Mean				

* Injected with ascorbic acid 4 hours before fluorine

TABLE VI

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.129 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	276	76	200	73
	2	277	74	202	73
	3	336	89	247	74
	4	356	93	263	74
	5	304	78	226	74
	6	238	66	172	73
	7	232	63	169	73
	8	314	84	230	73
	9	393	89	304	77
	10	310	77	233	75
	Mean				
Treated	1	304	82	222	73
	2	204	54	150	74
	3	236	59	177	75
	4	242	67	175	73
	5	251	69	182	72
	6	200	49	151	76
	7	197	58	141	72
	8	246	62	184	75
	9	171	49	122	71
	10	320	94	226	71
	Mean				

* Injected with ascorbic acid 60 minutes before fluorine

TABLE VII

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.520 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	452	94	358	79
	2	438	109	329	75
	3	444	80	364	82
	4	307	70	237	77
	5	432	102	330	76
	6	329	77	252	77
	7	454	113	341	75
	8	584	135	469	80
	9	463	105	358	77
	10	493	105	388	79
	Mean				
Treated	1	542	123	419	78
	2	529	84	445	84
	3	575	88	487	85
	4	312	68	244	78
	5	328	64	264	81
	6	512	109	403	79
	7	411	98	313	76
	8	277	80	197	71
	9	238	64	174	73
	10	350	97	253	72
	Mean				

* Injected with ascorbic acid 4 hours before fluorine

TABLE VIII

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.259 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	233	63	170	73
	2	317	80	237	75
	3	272	68	204	75
	4	328	77	251	77
	5	242	62	180	75
	6	417	115	302	73
	7	313	83	230	74
	8	194	47	147	76
	9	236	61	175	74
	10	335	79	256	77
	Mean				
Treated	1	285	64	221	78
	2	367	84	284	80
	3	311	74	237	76
	4	299	65	234	78
	5	241	53	188	78
	6	274	62	212	77
	7	232	54	178	77
	8	291	65	226	78
	9	324	66	258	80
	10	256	55	201	79
	Mean				

* Injected with ascorbic acid 4 hours before fluorine

TABLE IX

Fresh and Dry Weight of Lungs of Mice
Injected With Ascorbic Acid
and Exposed to Fluorine

Fluorine Concentration - 0.339 mg/l*

Group	Animal Number	Wet Weight (mg)	Dry Weight (mg)	Weight Loss (mg)	Per Cent Loss
Control	1	261	68	193	74
	2	309	79	230	75
	3	240	65	175	73
	4	151	37	114	76
	5	219	51	168	77
	6	210	55	165	79
	7	252	60	192	76
	8	266	66	200	75
	9	530	110	420	79
	10	458	91	367	80
	Mean				
Treated	1	277	69	208	75
	2	273	75	198	73
	3	230	66	164	71
	4	304	76	228	75
	5	333	83	250	75
	6	305	92	213	70
	7	342	84	258	75
	8	394	89	305	77
	9	340	80	260	76
	10	335	80	255	76
	Mean				

* Injected with ascorbic acid 10 minutes after fluorine

TABLE X

Summary of Effects of Ascorbic Acid On
Mortality and Pulmonary Edema
Produced by Fluorine in Mice

Fluorine Concentration (mg/l)	Time of Treatment	Per Cent Mortality		Range of Weight Loss of Lungs	
		Treated	Control	Treated	Control
0.220	30 minutes before	0	0	75-79	73-76
1.470	45 minutes before	100	100	73-81	75-87
0.129	60 minutes before	0	20	71-75	73-77
0.374	4 hours before	10	0	70-77	74-79
0.520	4 hours before	70	100	72-85	75-82
0.259	4 hours before	10	0	76-80	73-77
0.430	5 minutes after	30	20	72-80	70-73
0.338	10 minutes after	20	10	67-77	70-81
0.339	10 minutes after	33	30	71-77	73-80

Clinical Chemistry Following Exposure To Fluroine

Introduction

These studies were designed to measure certain clinical chemical parameters in the blood of rats and dogs and to determine if changes were caused by exposure to fluorine. The tests were selected particularly to reflect changes in protein, damage to the liver or kidneys and/or stress in general.

The tests selected were blood urea nitrogen, blood glucose, serum alkaline phosphatase, creatinine, uric acid, total protein and albumin. Methods of analysis were those for the Technicon Auto-Analyzer.

Abnormal changes in blood glucose reflect stress on the animal. Creatinine levels in the blood reflect nephrosis and impairment of kidney function. Uric acid, an end-product of protein metabolism, and urea nitrogen reflect nephritis and damage to tissues by a change in the proteins. Total protein, albumin, globulins and albumin to globulins ratio also could reflect changes caused by a pulmonary irritant such as fluorine. Changes in alkaline phosphatase indicate malfunction in the liver.

These studies were not designed to detect changes caused by high concentrations of fluorine, but were to detect minor changes if caused by lower concentrations. It was felt that if a certain parameter were changed significantly and could be correlated with degree of exposure, such a

clinical test could be performed on man. This would serve as a valuable index of exposure and as a means of predicting potentially harmful effects on the health of a man.

Methods

Young adult albino rats and young adult beagle dogs were exposed for 5 or 15 minutes to concentrations ranging from 0.082 to 0.310 mg/l which are equivalent to 29 to 200 ppm. Some animals were exposed once, while others were exposed three or four times to the same concentration of fluorine. Blood samples were withdrawn one to 14 days after exposure for analyses on the Auto-Analyzer as enumerated previously.

Dogs were exposed individually in the chamber. Therefore the concentration during exposure of each dog was slightly different. Rats were exposed in groups of five. There were 15 rats at each concentration. Subgroups of 5 rats each were sacrificed at three different intervals of time after exposure ranging from one to seven days.

Results

Rats - Single Exposures

The ranges of mean values in control or normal animals are included in Table I. It should be emphasized that samples of blood from the dogs were withdrawn for analysis before exposure. Therefore, the values for each dog can be compared before and after exposure as well as being compared to normal values in the dog.

The results of clinical chemical determinations after single exposures of rats for 5 minutes to concentrations of 0.082, 0.173 or 0.247 mg/l (29, 112 or 160 ppm) or for 15 minutes to concentrations of 0.116, 0.150 or 0.173 mg/l (75, 97 or 112 ppm) are tabulated as follows:

Table II	-	Blood Glucose
Table III	-	Blood Urea Nitrogen
Table IV	-	Uric Acid
Table V	-	Creatinine
Table VI	-	Serum Alkaline Phosphatase
Table VII	-	Total Protein
Table VIII	-	Albumin

Although not statistically significant, there was a slight increase of blood glucose in rats exposed to 0.173 ppm for 15 minutes at the 24 and 72 hour sacrifices and in those exposed to 0.247 mg/l for 5 minutes at the 24 and 48 hour sacrifices.

In some of the rats the concentrations of Blood Urea Nitrogen and activity of Alkaline Phosphatase appeared to be increased after exposure to the higher concentrations for 15 minutes. These values were not much higher than controls, but indicated a possible trend of an effect.

The other parameters were not significantly increased. Since neither total protein or albumin were not changed, the concentrations of globulins and the albumin to globulin ratios are not presented.

TABLE I

Normal Clinical Chemistry Values in
Dogs and Rats

Test	Range of Normal Values (mg %) Dog	Rat
Blood Urea Nitrogen	4.2-20.2	13.1-19.3
Fasting Blood Glucose	70-80	103-220
Uric Acid	0.38-1.12	1.44-3.36
Creatinine	0.88-1.25	0.80-1.20
Alkaline Phosphatase	4.27-13.80*	12.1-114.2*
Total Protein	3.86-7.32	6.05-6.73
Albumin	1.88-3.56	2.80-5.00

* King-Armstrong Units

TABLE II

Glucose In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	140	124	118	134	128	128 ± 9
	0.082	48	114	145	152	124	164	140 ± 21
	0.082	96	112	118	130	148	138	129 ± 13
	0.173	24	124	130	110	155	155	135 ± 19
	0.173	48	190	157	166	156	206	175 ± 21
	0.173	96	72	148	167	170	-	137 ± 32
	0.247	24	215	164	160	168	174	174 ± 24
	0.247	48	155	159	164	178	158	163 ± 10
	0.247	96	143	168	160	227	186	177 ± 50
15	0.116	24	136	140	136	162	117	138 ± 19
	0.116	72	181	118	194	156	144	159 ± 33
	0.116	168	136	184	122	108	140	138 ± 28
	0.150	24	169	133	162	124	155	153 ± 19
	0.150	72	128	134	206	244	170	176 ± 50
	0.150	168	174	116	120	156	161	145 ± 25
	0.173	24	228	136	145	170	152	186 ± 37
	0.173	72	172	122	212	208	208	184 ± 39
	0.173	168	116	130	114	128	202	138 ± 41

TABLE III

Blood Urea Nitrogen (BUN)
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	29.0	22.0	24.5	27.0	22.0	24.9 ± 3.0
	0.082	48	23.5	23.0	23.0	27.0	31.5	25.6 ± 3.6
	0.082	96	19.0	13.4	20.2	24.5	19.8	19.4 ± 4.8
	0.173	24	23.0	18.0	22.5	23.5	20.5	21.5 ± 2.4
	0.173	48	23.0	25.5	23.0	28.5	27.5	25.5 ± 2.4
	0.173	96	14.8	10.0	14.8	16.0	-	13.9 ± 2.6
	0.247	24	25.0	20.5	19.5	23.5	17.0	21.1 ± 3.4
	0.247	48	24.0	22.0	25.5	23.0	25.5	24.0 ± 1.5
	0.247	96	18.5	11.2	14.5	13.2	17.0	14.5 ± 3.1
15	0.116	24	30.0	27.0	28.5	34.0	29.0	29.7 ± 3.0
	0.116	72	24.0	23.0	21.5	22.0	26.0	23.3 ± 1.7
	0.116	168	25.0	24.5	22.0	24.0	25.8	24.3 ± 1.6
	0.150	24	20.5	26.5	21.0	24.5	26.5	23.8 ± 2.4
	0.150	72	18.0	19.5	18.0	21.0	21.5	19.6 ± 1.5
	0.150	168	19.8	22.5	20.0	17.2	20.5	20.0 ± 1.1
	0.173	24	24.0	19.5	23.5	27.0	23.0	23.4 ± 3.2
	0.173	72	21.5	23.0	17.0	15.5	18.5	19.1 ± 3.2
	0.173	168	24.0	24.0	21.0	25.2	22.0	23.2 ± 1.8

TABLE IV

Uric Acid In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	2.80	1.45	1.35	1.45	2.00	1.83 ± 0.62
	0.082	48	1.65	2.50	1.50	1.95	2.10	1.94 ± 0.43
	0.082	96	1.95	2.95	2.30	2.55	2.45	2.40 ± 0.48
	0.173	24	2.40	2.70	1.70	2.00	2.55	2.27 ± 0.43
	0.173	48	2.80	1.90	2.45	1.80	2.30	2.25 ± 0.43
	0.173	96	2.75	1.60	3.25	1.85	-	2.36 ± 0.68
	0.247	24	1.65	2.10	2.80	3.35	1.35	1.85 ± 0.86
	0.247	48	2.60	2.05	2.50	2.70	2.80	2.53 ± 0.33
	0.247	96	1.35	1.65	1.80	3.90	5.25	2.79 ± 0.90
15	0.116	24	1.75	2.70	2.10	2.90	2.20	2.33 ± 0.49
	0.116	72	3.55	2.35	4.40	2.50	1.85	3.93 ± 1.12
	0.116	168	1.25	1.80	1.95	1.85	4.30	2.23 ± 1.10
	0.150	24	1.50	1.60	1.70	1.55	2.85	1.84 ± 0.58
	0.150	72	2.45	2.45	3.25	4.70	2.40	3.85 ± 1.02
	0.150	168	2.30	2.15	2.30	1.60	1.65	2.00 ± 0.36
	0.173	24	1.85	1.40	1.70	1.80	1.30	1.61 ± 0.24
	0.173	72	1.75	-	4.60	3.95	2.50	2.56 ± 0.84
	0.173	168	1.40	1.45	2.15	3.20	3.00	2.24 ± 0.77

TABLE V

Creatinine In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	0.85	1.00	0.90	1.00	1.00	0.95 ± 0.06
	0.082	48	0.90	0.90	0.85	1.00	1.15	0.96 ± 0.13
	0.082	96	1.05	1.05	1.20	1.00	1.00	1.06 ± 0.09
	0.173	24	0.80	1.05	1.00	1.00	0.95	0.96 ± 0.11
	0.173	48	1.00	0.95	0.80	0.95	1.10	0.98 ± 0.13
	0.173	96	0.95	1.10	1.10	1.05	-	1.05 ± 0.06
	0.247	24	1.00	0.90	1.00	1.00	0.90	0.96 ± 0.04
	0.247	48	0.90	0.85	0.80	1.00	0.90	0.89 ± 0.09
	0.247	96	0.90	0.95	0.90	1.10	1.20	1.01 ± 0.13
15	0.116	24	1.05	1.30	1.20	1.30	1.35	1.24 ± 0.13
	0.116	72	1.10	1.10	1.15	1.10	1.00	1.09 ± 0.06
	0.116	168	1.00	1.10	1.00	0.95	1.10	1.03 ± 0.06
	0.150	24	0.70	0.95	0.85	1.05	1.10	0.93 ± 0.15
	0.150	72	0.95	1.00	1.10	1.15	1.10	1.06 ± 0.09
	0.150	168	0.90	0.85	0.85	0.80	0.85	0.83 ± 0.04
	0.173	24	1.10	0.95	1.10	0.90	0.95	1.00 ± 0.09
	0.173	72	1.00	-	1.00	1.00	0.95	0.99 ± 0.02
	0.173	168	0.80	0.85	0.80	0.80	0.90	0.83 ± 0.04

TABLE VI

Alkaline Phosphatase In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in King-Armstrong Units) 1	2	3	4	5	Mean ± SD
5	0.082	24	49.1	65.5	63.4	69.6	62.2	62.0 ± 9.1
	0.082	48	110.6	78.7	65.2	78.7	59.0	78.4 ± 22.1
	0.082	96	52.5	76.2	20.0	70.0	64.3	56.6 ± 23.7
	0.173	24	97.6	62.2	39.0	151.3	64.7	83.0 ± 49.5
	0.173	48	88.6	91.0	43.0	97.1	63.9	66.7 ± 14.3
	0.173	96	18.8	21.2	20.0	58.8	-	29.7 ± 17.2
	0.247	24	48.7	87.8	96.3	59.7	73.2	73.1 ± 20.4
	0.247	48	41.8	59.0	43.0	43.0	67.7	50.9 ± 10.7
	0.247	96	53.7	55.0	55.0	38.8	29.9	46.5 ± 10.8
15	0.116	24	80.6	69.4	73.2	56.1	79.2	71.7 ± 10.5
	0.116	72	61.4	103.6	84.8	27.3	67.7	69.0 ± 32.6
	0.116	168	66.4	35.1	113.5	103.1	64.5	76.5 ± 33.4
	0.150	24	87.8	81.7	51.2	42.7	48.7	62.4 ± 19.3
	0.150	72	71.2	68.8	65.2	36.9	65.2	61.5 ± 15.2
	0.150	168	87.3	66.4	76.6	95.7	84.8	82.2 ± 12.3
	0.173	24	63.3	114.6	97.5	113.3	95.2	96.8 ± 22.0
	0.173	72	41.8	106.8	63.9	54.1	91.0	71.5 ± 27.9
	0.173	168	120.5	161.0	60.7	71.5	109.0	104.5 ± 43.0

TABLE VII

Total Protein In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	5.78	6.00	6.50	6.37	5.80	6.09 ± 0.30
	0.082	48	6.78	5.35	5.95	6.25	5.80	6.26 ± 0.63
	0.082	96	6.58	6.40	6.35	6.45	6.05	6.37 ± 0.23
	0.173	24	6.72	7.54	8.08	7.85	7.70	7.58 ± 1.04
	0.173	48	6.10	5.75	6.12	6.30	6.25	6.10 ± 0.28
	0.173	96	6.65	5.95	6.15	6.60	-	6.34 ± 0.30
	0.247	24	7.85	7.85	8.25	7.80	7.40	7.83 ± 0.36
	0.247	48	5.97	6.60	6.25	6.30	5.75	6.17 ± 0.36
	0.247	96	5.60	5.65	5.80	5.95	6.13	5.83 ± 0.23
15	0.116	24	7.27	6.85	7.37	8.28	7.50	7.55 ± 1.02
	0.116	72	7.00	6.80	6.50	6.80	6.60	6.75 ± 0.22
	0.116	168	5.63	6.50	6.52	6.25	6.00	6.18 ± 0.36
	0.150	24	8.25	7.80	7.20	8.15	8.52	7.98 ± 0.45
	0.150	72	5.90	6.30	6.05	6.01	6.30	6.11 ± 0.17
	0.150	168	5.85	6.30	6.12	5.80	5.78	5.97 ± 0.22
	0.173	24	7.55	7.35	7.46	8.05	7.70	7.62 ± 0.30
	0.173	72	6.20	6.70	6.30	6.40	6.40	6.40 ± 0.22
	0.173	168	5.65	6.15	7.20	6.00	5.65	6.13 ± 0.66

TABLE VIII

Albumin In Blood
of Rats Exposed To Fluorine

Single Exposure

Exposure Time (min.)	Conc. (mg/l)	Sac. After Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
5	0.082	24	3.65	4.00	4.05	5.42	3.17	4.06 ± 1.00
	0.082	48	3.10	3.60	3.37	3.10	4.65	3.56 ± 0.68
	0.082	96	4.70	3.83	3.52	3.55	3.45	3.91 ± 0.55
	0.173	24	2.68	3.85	4.00	4.23	4.20	3.79 ± 0.58
	0.173	48	4.40	2.90	3.92	3.00	3.15	3.47 ± 0.63
	0.173	96	2.75	3.40	3.93	3.70	-	3.45 ± 0.52
	0.247	24	3.75	4.70	4.05	4.15	5.25	4.38 ± 0.63
	0.247	48	3.20	3.22	3.05	3.15	2.85	3.09 ± 0.20
	0.247	96	2.93	3.12	2.95	2.85	3.30	3.03 ± 0.20
15	0.116	24	5.23	4.38	4.68	5.42	3.95	4.73 ± 0.72
	0.116	72	3.50	3.90	4.70	4.40	3.70	4.04 ± 0.53
	0.116	168	3.75	3.45	3.80	4.05	3.92	3.79 ± 0.26
	0.150	24	6.15	3.15	3.13	3.38	3.98	3.96 ± 1.32
	0.150	72	3.60	3.20	3.30	5.20	3.65	3.79 ± 0.70
	0.150	168	3.68	2.68	3.35	3.12	3.05	3.18 ± 0.43
	0.173	24	3.70	4.65	4.15	3.92	4.85	4.25 ± 0.40
	0.173	72	3.60	2.70	3.10	3.10	3.50	3.20 ± 0.39
	0.173	168	3.80	3.00	3.72	3.03	2.75	3.26 ± 0.43

Rats - Repeated Exposures

The results of clinical determinations in the blood of rats exposed three times (15 minutes each exposure and 48 hours between exposures) to concentrations of 0.111 or 0.170 mg/l (72 or 110 ppm) are tabulated as follows:

Table IX	-	Blood Glucose
Table X	-	Blood Urea Nitrogen
Table XI	-	Uric Acid
Table XII	-	Creatinine
Table XIII	-	Serum Alkaline Phosphatase
Table XIV	-	Total Protein
Table XV	-	Albumin

Glucose was slightly elevated in some animals at the 24 hour sacrifice. Creatinine appeared to be slightly lower in these animals. Alkaline phosphatase was elevated at the 48 and 72 hour sacrifice after exposures to a concentration of 0.170 ppm, but the effect had disappeared one week after the last exposure.

Values of other parameters were not changed significantly.

TABLE IX

Glucose In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	142	204	151	133	200	166 ± 30
0.111	72	158	191	146	157	184	167 ± 19
0.111	168	113	106	130	148	134	126 ± 17
0.170	48	160	156	168	190	295	194 ± 60
0.170	72	130	118	94	130	173	129 ± 15
0.170	168	109	118	109	138	158	126 ± 21

TABLE X

Blood Urea Nitrogen (BUN)
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	16.0	19.0	18.0	17.0	18.2	17.6 ± 1.3
0.111	72	18.5	18.5	16.0	17.0	17.5	17.5 ± 1.1
0.111	168	13.0	13.5	16.5	18.0	18.5	15.9 ± 2.4
0.170	48	17.0	20.1	18.0	15.8	15.0	17.2 ± 2.2
0.170	72	13.5	16.0	17.0	18.5	16.5	16.3 ± 2.1
0.170	168	16.5	14.5	15.5	21.0	18.0	17.1 ± 2.8

TABLE XI

Uric Acid In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	1.70	5.20	1.27	1.95	2.40	2.50 ± 1.42
0.111	72	1.70	2.32	1.80	1.60	1.85	1.85 ± 0.32
0.111	168	1.15	1.20	1.30	2.80	1.85	1.66 ± 0.69
0.170	48	1.45	1.75	2.80	3.40	5.20	2.92 ± 0.84
0.170	72	1.15	1.12	1.75	1.40	4.20	1.92 ± 1.35
0.170	168	1.30	1.55	1.20	1.60	1.90	1.51 ± 0.31

TABLE XII

Creatinine In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	0.60	0.80	0.55	0.65	0.70	0.66 ± 0.11
0.111	72	0.75	0.65	0.60	0.65	0.75	0.68 ± 0.06
0.111	168	0.75	0.78	0.80	0.80	0.90	0.81 ± 0.06
0.170	48	0.70	0.80	0.75	0.80	0.95	0.80 ± 0.09
0.170	72	0.60	0.65	0.70	0.65	0.75	0.67 ± 0.06
0.170	168	0.75	0.75	0.80	0.75	0.75	0.76 ± 0.02

TABLE XIII

Alkaline Phosphatase In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in King-Armstrong Units) 1	2	3	4	5	Mean ± SD
0.111	48	55.6	98.2	111.8	123.2	99.8	97.7 ± 29.2
0.111	72	38.2	104.8	49.3	99.8	85.1	75.4 ± 28.2
0.111	168	28.4	29.6	34.6	43.1	35.7	34.3 ± 6.5
0.170	48	76.8	108.0	109.2	59.3	93.7	89.4 ± 21.4
0.170	72	46.9	134.3	93.6	88.8	90.0	90.7 ± 37.8
0.170	168	24.7	28.4	39.5	34.6	45.6	34.6 ± 6.5

TABLE XIV

Total Protein In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	5.42	6.00	6.00	6.10	5.82	5.87 ± 0.29
0.111	72	5.25	6.10	5.65	6.37	6.85	6.04 ± 0.70
0.111	168	5.75	5.95	6.18	6.60	6.10	6.12 ± 0.36
0.170	48	6.00	6.10	5.65	6.30	5.95	6.00 ± 0.32
0.170	72	6.20	5.85	6.28	6.00	6.00	6.07 ± 0.18
0.170	168	5.80	5.95	5.85	6.00	6.45	6.01 ± 0.28

TABLE XV

Albumin In Blood
of Rats Exposed To Fluorine

Three Repeated Exposures - 15 Minutes Each

Conc. (mg/l)	Sac. After Last Exposure (hours)	Animal Number (Concentration in mg per cent) 1	2	3	4	5	Mean ± SD
0.111	48	2.70	2.92	3.00	2.75	3.25	2.92 ± 0.24
0.111	72	2.92	3.00	2.75	3.23	2.90	2.98 ± 0.21
0.111	168	3.00	2.90	2.95	2.35	3.12	2.86 ± 0.08
0.170	48	3.43	3.50	2.95	3.47	3.00	3.27 ± 0.24
0.170	72	3.00	3.50	3.05	3.00	3.52	3.21 ± 0.23
0.170	168	2.68	3.45	3.00	2.90	3.00	3.01 ± 0.34

Dogs - Single Exposure

The results of clinical chemical determinations in dogs exposed once for 5 or 15 minutes to concentrations ranging from 0.116 to 0.310 mg/l (75 to 200 ppm) are tabulated as follows:

Table XVI	-	Blood Glucose
Table XVII	-	Blood Urea Nitrogen
Table XVIII	-	Uric Acid
Table XIX	-	Creatinine
Table XX	-	Serum Alkaline Phosphatase
Table XXI	-	Total Protein
Table XXII	-	Albumin

There was an indication of a slight increase in blood glucose on the first, second or third day after exposures to the higher concentrations. None of the other parameters were changed significantly. It should be emphasized that these concentrations were rather low when compared to lethal effects but were in the range where emergency limits have been suggested.

TABLE XVI

Glucose In Blood
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in mg per cent) 0	1	2	3	4	7	9	11	14
001	5	0.116	75	78	88	90	88	100	64	80	80
019	5	0.148	85	108	97	88	104	94	105	76	88
011	5	0.150	76	92	96	92	90	100	102	72	96
065	5	0.298	84	84	110	100	108	104	103	74	99
022	5	0.310	82	98	102	116	-	98	-	-	-
15	15	0.128	88	92	93	84	98	86	103	62	75
032	15	0.147	88	142	126	114	108	112	104	82	103
16	15	0.256	75	91	84	82	92	80	90	63	73

TABLE XVII

Blood Urea Nitrogen (BUN)
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in mg per cent) 0	1	2	3	4	7	9	11	14
001	5	0.116	-	11.5	12.5	12.0	10.5	10.5	10.0	10.0	10.0
019	5	0.148	12.5	14.5	9.5	10.5	12.2	10.5	10.2	9.5	9.0
011	5	0.150	16.5	17.0	20.0	11.5	17.0	14.5	15.8	14.0	9.0
065	5	0.298	13.5	16.0	14.5	12.5	13.0	16.5	12.0	10.5	9.8
022	5	0.310	15.0	15.5	13.0	9.5	-	17.0	-	-	-
15	15	0.128	18.0	10.5	10.5	7.5	13.2	16.0	16.0	14.0	12.5
032	15	0.147	16.5	13.5	14.5	11.5	14.3	13.4	12.2	15.0	11.5
16	15	0.256	17.5	8.5	11.0	8.5	13.0	13.8	9.2	11.0	9.2

TABLE XVIII

Uric Acid In Blood
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in mg per cent) 0	1	2	3	4	7	9	11	14
001	5	0.116	-	0.55	0.50	-	0.48	0.35	0.55	0.40	0.40
019	5	0.148	0.53	0.50	0.70	0.48	0.45	0.45	0.32	0.55	0.45
011	5	0.150	0.85	0.55	0.68	0.50	0.60	0.48	-	0.50	0.35
065	5	0.298	0.38	0.50	0.70	0.48	0.50	0.60	0.32	0.55	0.45
022	5	0.310	0.50	0.55	0.52	0.72	-	0.53	-	-	-
15	15	0.128	1.15	0.55	0.80	0.52	0.65	0.50	0.35	0.65	0.40
032	15	0.147	0.50	0.73	0.65	0.42	0.70	0.55	-	0.50	0.40
16	15	0.256	0.28	0.50	0.55	0.52	0.40	0.42	0.35	0.45	0.40

TABLE XIX

Creatinine In Blood
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in mg per cent) 0	1	2	3	4	7	9	11	14
001	5	0.116	0.91	0.98	1.10	1.15	1.20	0.90	0.90	1.03	0.90
019	5	0.148	0.84	0.90	0.80	0.80	1.00	1.05	0.85	0.92	0.95
011	5	0.150	1.10	1.12	1.10	1.00	1.20	1.28	-	1.00	1.05
065	5	0.298	1.10	0.80	0.92	0.92	1.18	1.00	0.95	0.95	1.00
022	5	0.310	1.05	1.10	1.05	0.85	-	1.23	-	-	-
15	15	0.128	0.88	0.95	1.00	0.85	1.00	1.00	0.85	0.85	0.90
032	15	0.147	1.15	1.12	1.05	1.03	1.20	1.20	-	1.10	1.00
16	15	0.256	1.10	0.98	1.00	0.90	1.25	1.25	1.10	1.00	1.00

TABLE XX

Alkaline Phosphatase In Serum
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in King-Armstrong Units) 0	1	2	3	4	7	9	11	14
001	5	0.116	10.00	10.06	10.00	9.46	8.00	9.04	8.00	7.64	8.43
019	5	0.148	8.04	7.32	5.66	5.78	6.62	7.53	5.86	6.29	5.82
011	5	0.150	4.27	5.36	5.28	5.41	6.02	5.56	5.25	4.57	4.22
065	5	0.298	12.30	11.70	10.81	11.53	13.24	12.98	10.37	11.46	11.02
022	5	0.310	7.68	7.82	7.23	6.64	-	7.85	-	-	-
15	15	0.128	13.80	13.92	14.13	14.26	17.25	17.12	16.25	14.79	12.15
032	15	0.147	5.73	7.44	6.14	7.62	9.18	9.96	8.50	7.51	6.82
16	15	0.256	5.00	8.29	7.62	7.37	9.00	8.30	7.63	7.27	6.94

TABLE XXI

Total Protein In Blood
of Dogs Exposed To Fluorine

Single Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After Exposure in Days (Concentration in mg per cent) 0	1	2	3	4	7	9	11	14
001	5	0.116	6.3	6.6	5.9	6.0	6.1	5.9	5.8	-	-
019	5	0.148	6.2	6.6	5.9	6.8	5.8	6.3	6.0	-	-
011	5	0.150	5.9	6.2	5.8	5.9	5.4	5.4	5.9	-	-
065	5	0.298	6.1	6.3	6.5	6.5	5.7	5.9	6.0	-	-
022	5	0.310	6.4	6.7	6.2	6.8	-	6.1	-	-	-
15	15	0.128	5.7	6.0	5.7	5.9	5.6	5.5	5.6	-	-
032	15	0.147	6.1	6.7	6.8	6.9	5.9	6.6	6.2	-	-
16	15	0.256	5.5	5.6	5.4	5.6	5.1	5.4	5.5	-	-

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Dogs - Repeated Exposures

Four dogs were exposed repeatedly, four times at 48 hour intervals, for five or 15 minutes to concentrations ranging from 0.085 to 0.152 mg/l (55 to 98 ppm). Blood samples for the clinical chemical tests were withdrawn before the first exposure and 24 hours after this exposure. Monitoring continued during the week of exposures and for one week after the fourth exposure. The results are presented in the tables listed as follows:

Table XXIII	-	Blood Glucose
Table XXIV	-	Blood Urea Nitrogen
Table XXV	-	Uric Acid
Table XXVI	-	Creatinine
Table XXVII	-	Serum Alkaline Phosphatase
Table XXVIII	-	Total Protein
Table XXIX	-	Albumin

Because of a malfunction in the valving system used for introduction of the fluroine into the chamber, the concentration became extremely high during the third exposure and dog number 010 died. The actual concentration was not measured. Therefore no analytical values for this dog were available after Day 4. The values reported for Day 4 were from blood drawn before exposure.

Alkaline phosphatase was higher in dog number 005 on Days 7, 9, 11 and 14. This dog received the highest exposures repeatedly which might indicate some change related to the fluorine. None of the values, however, were much higher than found normally in the beagle dog. These three values in one dog do not offer conclusive evidence of an effect.

The other parameters were not changed significantly in these dogs even after repeated exposures to fluorine.

TABLE XXIII

Glucose In Blood
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	86	100	112	98	106	108	104	80	91
064	5	0.106-0.120	81	104	102	92	102	102	78	82	101
010	15	0.060-0.148	84	81	108	98	80	-	-	-	-
005	15	0.060-0.152	73	96	112	96	100	96	100	60	73

* Exposed for the second, third or fourth time on this day.

TABLE XXIV

Blood Urea Nitrogen (BUN)
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	12.0	14.0	10.0	8.5	12.0	12.5	9.0	11.0	10.0
064	5	0.106-0.120	14.5	18.5	15.0	9.0	16.8	21.2	14.5	15.0	12.2
010	15	0.060-0.148	15.0	14.0	15.5	7.0	11.3	-	-	-	-
005	15	0.060-0.152	10.0	13.0	11.0	8.5	13.0	12.8	7.5	9.5	11.5

* Exposed for the second, third or fourth time on this day.

TABLE XXV

Uric Acid In Blood of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	0.55	0.65	0.65	0.55	0.48	0.55	0.40	0.70	0.45
064	5	0.106-0.120	0.45	0.60	0.60	0.45	0.45	0.55	0.40	0.55	0.40
010	15	0.060-0.148	0.40	0.53	0.55	0.55	0.55	-	-	-	-
005	15	0.060-0.152	0.65	0.71	0.70	-	0.55	0.60	0.50	0.82	0.70

* Exposed for the second, third or fourth time on this day.

TABLE XXVI

Creatinine In Blood
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	1.00	1.00	1.05	0.80	1.32	1.15	1.00	1.05	0.10
064	5	0.106-0.120	1.05	1.10	0.95	0.90	1.20	1.15	0.90	0.95	1.05
010	15	0.060-0.148	1.25	1.00	1.10	1.05	1.35	-	-	-	-
005	15	0.060-0.152	1.05	0.95	1.05	-	1.10	1.25	0.80	0.85	0.90

* Exposed for the second, third or fourth time on this day.

TABLE XXVII

Alkaline Phosphatase In Serum
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in King-Armstrong Units) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	12.44	11.02	11.05	10.57	10.45	10.47	10.12	8.88	8.56
064	5	0.106-0.120	9.50	8.78	7.12	7.86	8.24	8.67	10.75	8.38	7.43
010	15	0.060-0.148	13.40	14.02	11.30	12.78	14.00	-	-	-	-
005	15	0.060-0.152	13.05	11.20	11.78	11.91	12.62	17.05	22.0	27.80	22.80

* Exposed for the second, third or fourth time on this day.

TABLE XXVIII

Total Protein In Blood
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	6.9	7.4	7.1	7.9	6.7	7.1	7.3	-	-
064	5	0.106-0.120	6.5	6.7	6.1	7.2	6.0	6.7	6.0	-	-
010	15	0.060-0.148	6.5	7.0	6.0	6.8	6.5	-	-	-	-
005	15	0.060-0.152	6.2	6.3	6.5	6.6	5.7	6.1	6.1	-	-

* Exposed for the second, third or fourth time on this day.

TABLE XXIX

Albumin In Blood
of Dogs Exposed To Fluorine

Repeated Exposures

I. D. No. of Dog	Exposure Time	Conc. (mg/l)	Time After First Exposure in Days (Concentration in mg per cent) 0	1	2*	3	4*	7*	9	11	14
021	5	0.085-0.104	2.0	2.1	2.1	2.6	2.0	2.1	2.1	-	-
064	5	0.106-0.120	1.7	2.2	2.1	2.1	2.0	2.1	1.7	-	-
010	15	0.060-0.148	2.2	2.3	2.3	2.5	2.4	-	-	-	-
005	15	0.060-0.152	1.9	2.1	2.2	2.3	2.1	2.2	1.8	-	-

* Exposed for the second, third or fourth time on this day.

Summary of
Clinical Chemistry After Exposures To Fluorine

Blood was withdrawn from dogs and rats after single or repeated exposure to fluroine for clinical chemistry tests. Analyses were made for blood urea nitrogen, blood glucose, creatinine, uric acid, alkaline phosphatase, total protein and albumin.

In rats the blood urea nitrogen and serum alkaline phosphatase levels were increased following exposure to fluorine. There was an indication of a slight increase in fasting blood glucose after exposure to rather high concentrations of fluroine. The creatinine, uric acid, total protein or albumin did not appear to be changed significantly.

In dogs the blood glucose appeared to be slightly elevated, but none of the other parameters measured appeared to be changed significantly. It should be emphasized that these dogs were exposed to rather low concentrations of fluorine.

FLUORIDE CONCENTRATION IN SOFT TISSUES - LUNG, LIVER AND KIDNEY

The storage of fluoride in the lungs, liver and kidneys of rats and dogs exposed to fluorine for five or 15 minutes was measured to determine the concentration in tissue in relation to concentration in air, to determine excretion rates and/or accumulation.

Preparation of Tissue Samples:

The whole organs (lung, liver, kidney) were stored in the freezer in tightly closed containers until time of analysis. A 1.0% homogenate with de-ionized water was prepared of the tissue using an all-glass homogenizer.

Preparation of Urine Samples:

Prior to fluoride analysis, the sample was acidified with 60% perchloric acid and then treated with a solution of 60% silver perchlorate in order to remove the chloride ions present in the urine. The clear filtrate was used for the analysis. In case of high fluoride concentration, a 1:10 dilution of the filtrate with de-ionized water was used in the determination of fluorides.

Reference: H. A. Derner, Automated Determination of Fluoride in Urine, A.I.H.A.J. 28, 357, 1967.

Analytical Method:

Colorimetric, semi-automated, analysis of fluoride using the TECHNICON Auto-Analyzer.

Reference: R. H. Mandl, L. H. Weinstein, J. S. Jacobson, D. C. McCune, and A. E. Hitchcock, presented at the Technicon Symposium "Automation in Analytical Chemistry", New York, N. Y., Sept. 8, 1965.

Rats

The concentrations of fluoride in tissues of control rats are presented in Table I. The mean concentrations and standard deviations of the means were 3.43 ± 0.77 ppm in the lung, 3.36 ± 0.26 ppm in the liver and 3.43 ± 0.80 ppm in the kidneys. It is note-worthy that there was no difference in the concentration in all three of these tissues.

The concentrations in tissues of rats following a single exposure for five or 15 minutes are presented in Table II and are summarized in Table III. These rats were exposed in groups of 15 each. Then five rats in each group were sacrificed after exposure at various intervals of time ranging from 24 to 168 hours. A number of the mean concentrations were significantly higher than the concentrations in control rats.

If the concentrations in the tissues 24 hours after sacrifice are compared to the concentrations in air or total exposure (expressed as concentration x time), it is apparent that there is poor correlation. For example, the results were:

Exposure Time (minutes)	Concentration in Air (mg/l)	Concentration in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
5	0.082	8.8	7.6	7.1
5	0.173	4.9	6.4	5.8
5	0.247	6.0	6.8	6.3
15	0.116	8.2	5.4	7.1
15	0.150	7.1	6.8	7.7
15	0.173	8.4	6.5	3.7

The concentrations in tissues were not higher as the concentrations in air increased. The probable explanation for these results is that the fluoride is excreted rapidly. If samples of tissues, particularly lungs, had been taken sooner after exposure, such as four hours, the concentration might have been more closely correlated to exposure.

When the tissue concentration is viewed with regard to time after exposure, it is apparent the fluoride was significantly increased at 24 hours after exposure. In general there was no significantly increased fluoride in the lungs at 48 or more hours after exposure. Fluoride levels in the liver and kidneys were slightly higher than in control animals as long as 168 hours after exposure. These indicate, probably, a slow excretion of fluoride.

Repeated Exposures

Groups of 15 rats were exposed three times (48 hours apart) to fluorine at concentrations of 0.111 or 0.170 mg/l. At 48, 72 and 96 hours after exposure groups of five rats each were sacrificed. Concentrations of fluoride in lungs, liver and kidneys are presented in Table IV and the means are summarized in Table V. Although both means in the liver at 72 hours and the means of the lungs of rats exposed to 0.170 mg/l and sacrificed at 72 and 96 hours after exposure were "statistically" significantly higher than the means of these tissues from control rats, it is very doubtful that these are real differences.

These data indicate that repeated exposures, at least 48 hours apart, do not cause accumulation of fluoride in these tissues. The results offer a very good explanation of why our previous results showed no more effect from such repeated exposures than from a single exposure to the same concentration.

TABLE I

Fluoride Concentration in Tissue of Control Rats

Identification Number	Concentration in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-3514	3.0	-	3.5
D-3515	4.0	-	4.0
D-3516	5.0	-	4.0
D-3517	4.7	-	3.5
D-3518	5.0	-	3.0
D-3519	4.0	-	2.5
D-3526	3.0	3.0	6.2
D-3527	2.3	4.3	3.0
D-3528	3.0	3.0	2.5
D-3529	3.0	3.0	3.7
D-3601	3.0	3.5	3.0
D-3602	3.0	-	3.0
D-3603	3.0	-	3.0
D-3604	3.0	-	3.5
D-3605	2.5	-	3.0
Mean	3.43	3.36	3.43
S.D.	±0.77	±0.26	±0.80

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure To Fluorine For Five Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2474	0.082	11.0	12.5	7.3
		8.5	3.8	6.7
		11.0	5.5	7.7
		7.3	6.7	6.7
		6.0	7.3	7.3
	Mean	8.8	7.6	7.1
D-2480	0.082	3.5	4.0	4.0
		4.0	3.5	3.5
		4.5	3.5	7.2
		4.5	3.5	5.2
		4.0	2.8	5.2
	Mean	4.1	3.5	5.0
D-2489	0.082	3.8	8.3	6.2
		3.0	5.2	7.0
		3.0	3.8	5.5
		3.0	5.2	7.6
		2.2	6.2	3.8
	Mean	3.0	5.7	6.0

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure to Fluorine For Five Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2476	0.173	6.5	7.8	6.0
		4.5	5.2	6.5
		4.0	5.2	6.0
		4.5	7.8	5.2
		5.2	6.0	5.2
	Mean	4.9	6.4	5.8
D-2481	0.173	4.8	4.2	4.2
		4.8	4.2	5.5
		3.5	5.5	6.0
		4.2	3.5	5.5
		4.8	4.2	4.8
	Mean	4.4	4.3	5.2
D-2490	0.173	5.5	6.0	7.5
		4.8	5.5	7.5
		4.8	4.8	8.0
		4.8	4.2	6.0
	Mean	5.0	5.1	7.3

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure To Fluorine For Five Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2478	0.247	4.5	7.8	5.5
		5.2	8.4	4.8
		5.2	6.0	6.0
		6.5	6.5	8.5
		8.4	5.2	6.5
	Mean	6.0	6.8	6.3
D-2482	0.247	4.0	5.0	4.0
		4.0	7.0	4.5
		4.5	4.5	8.0
		3.5	4.0	6.2
		3.5	4.5	5.6
	Mean	3.9	5.0	5.7
D-2491	0.247	3.5	4.8	4.8
		10.0	3.5	4.2
		4.2	3.5	4.2
		3.5	9.0	4.2
		4.8	7.5	4.2
	Mean	5.2	5.7	4.3

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure to Fluorine For Fifteen Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2475	0.116	4.8	4.5	6.0
		15.5	4.0	9.1
		10.0	7.3	7.0
		6.0	5.2	7.0
		4.5	6.0	6.5
	Mean	8.2	5.4	7.1
D-2486	0.116	4.2	6.3	6.3
		3.5	5.1	6.3
		4.2	6.3	6.3
		5.0	6.3	4.6
		4.2	5.1	3.5
	Mean	4.2	5.8	5.4
D-2494	0.116	2.6	4.8	4.8
		4.8	5.5	5.5
		5.5	5.5	6.0
		2.6	4.8	5.5
		2.0	4.8	6.0
	Mean	3.5	5.1	5.6

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure to Fluorine For Fifteen Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2477	0.150	6.5	7.0	7.0
		12.0	10.5	8.4
		6.5	6.0	7.0
		6.5	5.2	7.0
		4.0	5.2	9.1
	Mean	7.1	6.8	7.7
D-2487	0.150	4.6	5.1	4.0
		3.5	2.8	3.5
		4.6	4.6	4.6
		6.3	3.5	7.0
		4.6	2.8	5.1
	Mean	4.7	3.8	4.8
D-2495	0.150	2.0	3.5	4.8
		2.0	4.2	4.8
		4.5	7.5	3.5
		2.0	4.8	6.0
		2.0	5.5	3.5
	Mean	2.5	5.1	4.5

TABLE II

Concentration of Fluoride in Tissue of Rats
After a Single Exposure to Fluorine For Fifteen Minutes

I. D. Number of Animals	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2479	0.173	8.5	5.8	4.8
		8.0	6.5	5.2
		10.4	6.5	2.3
		8.5	7.2	3.0
		6.5	6.5	3.0
	Mean	8.4	6.5	3.7
D-2488	0.173	6.7	6.0	5.0
		8.0	5.6	6.0
		8.0	4.0	5.6
		4.5	4.0	7.3
		6.0	4.5	6.7
	Mean	6.6	4.8	6.1
D-2496	0.173	2.6	4.2	3.5
		2.6	2.6	4.8
		3.5	4.5	4.2
		2.6	3.5	4.2
		2.6	3.5	4.2
	Mean	2.8	3.7	4.2

TABLE III

Summary of Fluoride Concentrations in Tissues
of Rats Exposed to Fluorine

Single Exposures

Identification Number	Time of Sacrifice (hours)	Concentration (mg/l)	Number of Rats in Group	Time of Each Exposure (minutes)	Mean Concentration in Tissues Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2475	24	0.116	5	15	8.2*	5.4*	7.1*
D-2486	72	0.116	5	15	4.2	5.8*	5.4*
D-2494	168	0.116	5	15	3.5	5.1*	5.6*
D-2477	24	0.150	5	15	7.1*	6.8*	7.7*
D-2487	72	0.150	5	15	4.7	3.8	4.8
D-2495	168	0.150	5	15	2.5	5.1*	4.5
D-2479	24	0.173	5	15	8.4*	6.5*	3.7
D-2488	72	0.173	5	15	6.6*	4.8*	6.1*
D-2496	168	0.173	5	15	2.8	3.7	4.2

* Significantly Different from Control Rats ($p < 0.05$)

TABLE III

Summary of Fluoride Concentrations in Tissues
of Rats Exposed to Fluorine

Single Exposures

Identification Number	Time of Sacrifice (hours)	Concentration (mg/l)	Number of Rats in Group	Time of Each Exposure (minutes)	Mean Concentration in Tissues Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2474	24	0.082	5	5	8.8*	7.6*	7.1*
D-2480	48	0.082	5	5	4.1	3.5	5.0
D-2489	96	0.082	5	5	3.0	5.7*	6.0*
D-2476	24	0.173	5	5	4.9*	6.4*	5.8*
D-2481	48	0.173	5	5	4.5	4.2	5.3*
D-2490	96	0.173	5	5	5.0	5.1*	7.3*
D-2478	24	0.247	5	5	6.0*	6.8*	6.3*
D-2482	48	0.247	5	5	3.9	5.0*	5.7*
D-2491	96	0.247	5	5	5.2	5.7*	4.3

* Significantly Different from Control Rats ($p < 0.05$)

TABLE IV

Concentration of Fluoride in Tissues of Rats
After Three Exposures to Fluorine for Fifteen Minutes

Repeated Exposures

Identification Number	Time of Sacrifice (hours)	Concentration (mg/l)	Number of Rats in Group	Time of Each Exposure (minutes)	Mean Concentration in Tissues Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2498	48	0.111	5	15	3.3	2.0	2.7
					6.6	1.5	3.3
					2.7	2.0	2.7
					4.0	4.0	3.3
					6.0	2.7	2.7
D-2500	72	0.111	5	15	3.5	3.5	5.0
					4.2	3.5	5.7
					5.0	7.0	4.2
					6.5	6.5	4.2
					5.7	5.7	4.2
D-2502	96	0.111	5	15	3.8	3.6	3.0
					3.2	2.4	3.0
					2.5	3.0	3.0
					2.5	2.4	2.4
					2.5	3.0	3.0

TABLE IV

Concentration of Fluoride in Tissues of Rats
After Three Exposures to Fluorine for Fifteen Minutes

Repeated Exposures

Identification Number	Time of Sacrifice (hours)	Concentration (mg/l)	Number of Rats in Group	Time of Each Exposure (minutes)	Mean Concentration in Tissues Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2497	48	0.170	5	15	3.3	2.0	2.7
					6.6	1.5	3.3
					2.7	2.0	2.7
					4.0	4.0	3.3
					6.0	2.7	2.7
D-2499	72	0.170	5	15	5.0	4.2	5.0
					5.7	5.0	4.2
					7.0	3.5	4.2
					6.5	3.5	4.2
					5.0	4.2	4.2
D-2501	96	0.170	5	15	3.8	3.8	2.5
					5.5	2.5	2.5
					5.5	2.0	2.5
					5.5	3.2	2.0
					7.6	2.5	2.0

TABLE V

Summary of Fluoride Concentrations in Tissues
of Rats Exposed Three Times To Fluorine

Repeated Exposures

Identification Number	Time of Sacrifice (hours)	Concentration (mg/l)	Number of Rats in Group	Time of Each Exposure (minutes)	Mean Concentration in Tissues Lung (ppm)	Liver (ppm)	Kidney (ppm)
D-2498	48	0.111	5	15	3.2	3.7	4.0
D-2500	72	0.111	5	15	5.0	5.2*	4.7
D-2502	96	0.111	5	15	2.9	2.9	2.9
D-2497	48	0.170	5	15	4.5	2.4	2.9
D-2499	72	0.170	5	15	5.8*	4.1*	4.4
D-2501	96	0.170	5	15	5.6*	2.8	2.3

* Significantly Different from Control Rats ($p < 0.05$)

Dogs

Single Exposure

Dogs were exposed to fluorine and were sacrificed with the tissues (lungs, liver and kidneys) being analyzed for fluoride. The results are presented in Table VI. It should be emphasized that these dogs were not sacrificed until 17 days after exposure.

The concentrations were not higher than those in control dogs with the exception of dog number 64. This dog had much more fluoride in the lungs than control dogs, however the dog died in the chamber during exposure. Although the mean concentration of fluorine in air during the exposure was 0.302 mg/l (193 ppm), the peak concentration was much higher due to a malfunction of the system. Unfortunately, the peak concentration could not be measured.

Repeated Exposures

Dogs were exposed for four times at 48-hour intervals to fluorine and were sacrificed 10 days after the last exposure. The concentrations in lungs, liver and kidneys are presented in Table The concentrations in all three tissues of three of the dogs were essentially the same as those in control animals, indicating that there was no accumulation of fluoride in these soft tissues.

In dog number 010 the levels of fluoride in the lungs and liver were much higher than in the same tissues of control dogs. It should be emphasized, however, that the mean concentration of fluorine in the

air is a mean value which does not reflect the peak concentration in the chamber which occurred as a malfunction of the introduction system. The peak concentration was not determined but caused the death of the animal.

The tissue concentrations, particularly in the lungs, of dogs 010 and 64 which died from exposure to fluorine revealed however that excess fluoride can be detected when exposure to fluorine is high enough to cause death.

The results are presented in Table VII.

TABLE VI

Fluoride Concentration in Dog Tissues
Following A Single Exposure To Fluorine

Tattoo Number	Exposure Time (minutes)	Concentration (mg/l)	Fluoride in Tissues Mean Concentration Lung (ppm)	Liver (ppm)	Kidney (ppm)
019	5	0.148	1.3	1.3	2.0
011	5	0.150	1.0	2.2	2.9
065	5	0.298	1.8	2.2	2.2
022	5	0.310	1.8	3.3	2.9
001	15	0.116	4.5	2.2	3.5
15	15	0.128	6.5	1.6	1.2
032	15	0.147	1.8	2.2	4.0
16	15	0.250	3.3	1.8	4.0
64*	15	0.302	19.5**	4.0	3.7

* Dog died in chamber
** Significantly higher than concentration in control dogs

TABLE VII

Fluoride Concentration in Dog Tissues
Following Four Exposures To Fluorine

Tattoo Number	Exposure Time (minutes)	Concentration (mg/l)	Fluoride in Tissues Mean Concentration Lung (ppm)	Liver (ppm)	Kidney (ppm)
021	5	0.094	1.8	2.2	2.9
064	5	0.112	3.3	3.3	3.3
005	15	0.116	2.5	1.0	1.5
010*	15	0.120	12.5**	14.0**	2.7

* Dog found dead after third exposure
** Significantly higher than concentration in control dogs

Summary of
Fluoride Concentration in Soft Tissue

The storage of fluoride in the lungs, liver and kidneys of rats and dogs exposed to fluorine for 5 or 15 minutes was measured to determine the concentration in tissue in relation to concentration in air. The concentrations in tissue also were determined to reflect rates of excretion and/or accumulation. The animals were sacrificed at 1 to 16 days after single or repeated exposures.

At 24 hours after exposure there was poor correlation between the amount of fluoride in rat tissues and the amount of fluorine in the air of the chamber. At the concentrations used in the chamber, the concentrations of fluoride in all three tissues were higher than the concentrations in tissues from control rats. At 48 or more hours after exposure the fluoride in the lungs was not higher than controls. The fluoride in liver and kidneys was slightly higher than fluoride in control animals as long as one week after exposure.

Dogs sacrificed at 16 days after a single exposure had no more fluoride in the lungs, liver or kidneys than control dogs.

Repeated exposures of dogs and rats for 3 or 4 times (48 hours apart) caused no more fluoride in these tissues than did a single exposure to the same concentration of fluorine.

These data indicate that fluoride is found in the lungs, liver and kidneys soon after exposure to fluorine, and that it disappears more

rapidly from the lungs than from the liver or kidney. The fluoride does not accumulate in these tissues even after intermittent, repeated exposures. This offers some explanation of our previously reported results which showed no more effect from exposures repeated every 48 hours than from a single exposure to the same concentration.

Fluoride in Urine Following Exposures to Fluorine

Introduction

It is known that fluoride is excreted in the urine following exposure to various fluorides. The measurement of the urinary excretion of fluoride, however, had never been done following short-term exposures to fluorine.

These studies were designed primarily to determine if urinary excretion of fluoride could be correlated with the degree of exposure to fluorine and the effects from such exposures.

The obvious reason for such a correlation was to be able, hopefully, to measure the urinary fluoride of men who might be exposed accidentally and to predict any potential hazard from such exposures. Urine was collected from both dogs and rats after a single exposure to fluorine and after repeated exposures. The animals were exposed for five or 15 minutes.

Results

The urinary fluoride concentrations of control animals (not exposed to fluorine) are presented in Table I. The mean values with standard deviation of the mean were 4.42 ± 0.38 mg/l in dogs. In rats which had food and water during the period of collection of urine the mean and standard deviation were 7.74 ± 0.95 mg/l. In rats which had water only during the collection period the mean with standard deviation was 2.32 ± 0.45. It is obvious that there is a difference in the concentration of urinary fluoride if food is ingested during the collection. Apparently the fluoride in the food caused the increased excretion.

Fluoride Urine - Rats

Single Exposures

Rats were exposed for five minutes to concentrations of 0.082, 0.173 and 0.247 mg/l or for 15 minutes to concentrations of 0.116, 0.150 and 0.173 mg/l. After exposure they were placed into metabolism cages for collection of urine to be analyzed for fluoride concentration.

The concentrations of fluoride in urine for each 24-hour interval for four days after exposure are presented in Table II.

When the concentration of fluoride in the urine of rats exposed to fluorine is compared to the concentration in the urine of control rats, it is obvious that the differences are significant. Exposure to fluorine increased the concentration of fluoride in the urine of these rats.

The concentration of fluoride in the urine during each subsequent 24-hour period (after exposure) decreased at about the same rate. Even at the fourth day after exposure the concentration had not returned to normal which indicated that excretion was not complete.

If the exposure times are considered separately, it appears that the concentration of fluoride in urine excreted during the first 24 hours after exposure might be correlated with the concentration of fluoride in the air during exposure. This is a poor correlation, however.

If the exposure time was disregarded and all exposures expressed as mg/l/min there was no correlation between rate of exposure and concentration of fluoride in the urine.

Repeated Exposures

One group of 15 rats was exposed every other day to concentrations of fluorine in air of 0.314, 0.079 and 0.116 mg/l (mean value 0.170 mg/l). The other group of 15 rats was exposed to 0.129, 0.099 and 0.104 mg/l (mean value 0.111 mg/l).

The concentrations of fluoride in the urine after three repeated exposures to fluorine (15 minutes each) are presented in Table III.

These data show that urinary fluoride is a reflection of the degree of exposure, but the correlation coefficient between concentration in urine and concentration in air is very low.

The ratio of the mean concentrations in air of the two groups was 1.6. The ratios of mean concentrations in urine (of the two groups) were 2.5, 3.1 and 1.5 at 24, 48 and 72 hours after exposure, respectively.

The concentration of fluoride in urine during the first 24 hours after a single 15-minute exposure to 0.173 mg/l was 46.0 mg/l. During the same interval after three exposures to a mean concentration of 0.170 mg/l, the mean urinary fluoride concentration was 58.5 mg/l. After a single exposure to 0.116 mg/l the urinary fluoride was 36.5 mg/l while after three exposures to a mean concentration in air of 0.111 mg/l the urinary fluoride was only 23.5 mg/l. These points are emphasized to illustrate the previous findings that repeated intermittent exposures to fluorine cause no more effects than a single exposure to the same concentration.

Fluoride in Urine - Dogs

Single Exposures

Dogs were exposed for 5 or 15 minutes to concentrations ranging from 0.116 to 0.310 ppm. Urine was collected for 15 or 16 days after exposure and was analyzed for fluoride. Samples of urine were taken from the urinary bladder immediately after sacrifice, on the 17th day after exposure. Due to technical difficulties samples were not obtained from all dogs on certain days. The results are presented in Table IV.

These data indicate that additional fluoride (above background) was excreted in the urine following exposures to fluorine. The greatest amount was excreted within the first 24 hours, but some was still being excreted several days later if a dog were exposed to a higher concentration and/or for a longer period of time.

The concentrations in urine were somewhat related to exposure (concentration x time) but the correlation coefficient was quite low.

Repeated Exposures

Dogs were exposed every other day for 5 or 15 minutes to concentrations ranging from 0.060 to 0.152 mg/l. The collection of urine for fluoride analysis was started immediately after the first exposure. The first samples were 24-hour samples. The dogs remained in metabolism cages except during exposure for the next 16 days. Urine from the bladder was taken immediately after sacrifice, on Day 17. The results are presented in Table V.

The "Time After Exposure in Days" refers to the first exposure. Actually exposures also were conducted on Days 3, 5 and 7. Day 8 represents a 24-hour sample after the fourth exposure.

Fluoride was excreted in the urine as noted following a single exposure, however the excretion did not increase after repeated exposures even though the dogs were exposed to additional fluorine. Apparently 48 hours between exposures was sufficient time to allow excretion of most of the excess fluoride. There was no evidence of accumulation which would have caused higher concentrations for a longer period of time.

It should be kept in mind that Day 16 in this table and "Sacrifice" correspond to the same period of time after the last exposure as Days 9 and 10 in Table IV (single exposures).

TABLE I

Fluoride Concentration in Urine of
Control Dogs and Control Rats
(Unexposed)

Dogs With Food (ppm)	Rats Without Food (ppm)	Rats With Food (ppm)
4.3	2.00	6.4
4.2	2.20	6.5
3.8	2.45	13.0
4.4	1.30	12.9
4.2	2.10	11.8
5.6	2.10	12.3
	2.10	6.9
	4.80	7.1
	1.80	7.0
	2.35	9.6
		9.8
		5.9
		7.5
		5.0
		5.3
		4.7
		4.1
		3.6
4.42 ±0.38*	2.32 ±0.45*	7.74 ±0.95*

* S.D.

TABLE II

Fluoride in Urine of Rats Exposed to Fluorine

Single Exposures

Exposure Time	Conc. mg/l	Exposure mg/l/min.	Conc. (ppm)	Urinary Fluoride (ppm) Days After Exposure: 1	2	3	4
5	0.082	0.016	53	23.0	15.5	8.7	10.8
5	0.173	0.033	111	26.5	16.5	14.8	10.5
5	0.247	0.049	158	33.5	24.5	16.0	15.5
15	0.116	0.008	75	36.5	29.5	12.8	11.3
15	0.150	0.010	96	37.0	26.5	19.5	21.0
15	0.173	0.011	111	46.0	20.0	33.3	21.0

TABLE III

Fluoride in Urine of Rats Exposed
Three Times to Fluorine

Mean Conc. mg/l		Concentration in mg/l of Urine After Last Exposure 24 hr.	48 hr.	72 hr.
.170		70.5	42.0	18.0
		59.0	48.5	22.0
		46.0	51.0	6.4
	Mean	58.5	47.2	15.5
.111		32.0	16.0	11.0
		23.5	17.5	10.0
		15.0	12.5	10.0
	Mean	23.5	15.3	10.3

TABLE IV

Fluoride Concentration in Urine of Dogs
Following Exposure to Fluorine

Single Exposures

Exposure Time	Conc. mg/l	I. D. No. of Dog	Time After Exposure in Days 1	2	3	4	8	9	10	11	15	16	Sacrifice
5	0.116	001			14.6		5.1	6.4	4.9	9.3	6.6		7.0
5	0.148	019	14.5		7.9	4.7	5.5	3.3	7.0	8.4	5.9		2.4
5	0.150	011		9.1		11.8		7.6	7.8	7.8		7.6	5.0
5	0.298	065		5.6		12.0		6.9	9.5	7.0	7.4	7.9	1.8
5	0.310	022	14.5	9.5	8.8			7.8	5.6	7.5	6.9	3.3	
15	0.128	15		12.2		14.2	7.9	10.9	11.6	10.3	4.3	8.6	5.7
15	0.147	032	52.0		1.4		8.7	4.6	5.6		10.4		7.6
15	0.256	16	18.0		10.5		6.4	8.3	2.6	7.5	8.5		8.8

TABLE V

Fluoride Concentration in Urine of Dogs
Following Exposure to Fluorine

Repeated Exposures

Exposure Time	Conc. mg/l	I. D. No. of Dog	Time After Exposure in Days 1	2	3	4	8	9	10	11	15	16	Sacrifice
5	0.085-0.104	021	14.5	*	*	19.5	13.9	9.7	8.8	11.3	5.8	3.2	5.2
5	0.106-0.120	064	*	8.3	21.0	14.5	*	7.9	2.5	6.6	1.3	5.5	6.3
15	0.060-0.152	005	18.5	*	10.9	11.7	5.7	*	7.6	10.4	12.5	10.1	9.5

* No urine was collected.

Summary of
Fluoride In Urine

Urine was collected from rats and dogs after a single or after repeated exposures to fluorine. The urine was then analyzed for fluoride. These studies were designed to determine if the excretion in urine could be correlated with levels of exposure.

During the first 24 hours after a single exposure there was some correlation between the amount of fluoride excreted in the urine and the amount of fluorine in the air. The rate of excretion of fluoride decreased with time after a single exposure and was nearly normal on about the fourth day.

Repeated exposures caused slightly higher rates of excretion than a single exposure (as predicted). There was, however, no indication of a marked accumulation of fluoride.

EXPOSURES TO MIXTURES OF FLUORINE AND HYDROGEN FLUORIDE

Introduction

When fluorine is used as an oxidizer in a rocket propellant, hydrogen fluoride is formed as a result of combustion. The reaction of fluorine with many different materials following a spill also could produce hydrogen fluoride. It seems very likely, therefore, that personnel could be exposed to a mixture of fluorine and hydrogen fluoride. Such mixtures could have additive, antagonistic or synergistic actions, consequently the physiological effects should be known. Effects from exposure to known mixtures of fluorine and hydrogen fluoride have never been determined. Therefore, these experiments were started.

The toxic effects from short-term exposures to mixtures of these two compounds in different ratios were determined. Briefly this was determination of the LC_{50}, sublethal effects and no apparent effect levels in several species of animals after 15 minutes of exposure.

The first problem was to set up the mechanical equipment for handling hydrogen fluoride in the laboratory, and to be able to produce (and analyze) repeatedly the desired concentration of hydrogen fluoride in the chamber. After surmounting a number of mechanical and technical problems this has been done. Animals also were exposed to hydrogen fluoride to compare our results with those reported from other laboratories.

After being able to generate, analyze and maintain known concentrations of fluorine or hydrogen fluoride separately in the chamber, the next task was to prepare, maintain and analyze concentrations of known mixtures of the two.

Materials and Methods

The exposure chambers and fluorine admission system has previously been reported and published, M. L. Keplinger et al (Journal of Ind. Assoc. 1968). However, in the use of mixture of F_2 and HF, it was necessary to install separate mixing chambers, air flow meters and regulators prior to the gases being admitted to the chamber.

A flow meter for hydrogen fluoride modelled after that of Peterson (Analytical Chemistry 17, 54, 1945) with the modification of a stainless steel orifice being substituted for the copper was used. The orifice hole was 0.050" in diameter and 0.125" in length. This was fitted into a Swagelok Tee fitting, and a U manometer of teflon tubing fitted to the tees. Kerosene was used as the manometer fluid. The hydrogen fluoride cylinder was placed in a water bath and maintained at 80°C. The tubing from the cylinder to the surge tank to the manometer and to the air dilution bottle was heated with electrical heating tape, which was insulated with glass wool to maintain a temperature near 80°C. The surge bottle was heated with two 250 watt heat lamps. Both the surge bottle and dilution bottle were made of polyethylene.

The diluted hydrogen fluoride and fluorine gases were then introduced into a 14 liter, stainless steel mixing chamber, in which the gases could be further diluted if required before admission into the exposure chamber.

Analysis of Chamber Gases

The fluorine from the exposure chamber was pulled through two traps by means of a vacuum pump. The traps contained 150 ml of 0.0025 M potassium iodide that was made alkaline with approximately 2 g sodium bicarbonate.

If the rate of collecting the chamber gases was greater than 2 liters/min, fluorine or the oxidation of iodide to iodine was carried over into the second trap noted by the latter's yellow color. At trapping rates of 2 liters/min. and less, the amount of iodine in the second trap was very slight (corresponding to less than 0.005 mg/liter of gas).

Determination of Total Fluoride

A measure of the reaction of F_2 with water can be obtained by comparing the difference between total fluoride and fluorine concentrations. The total fluoride was determined by two methods in these exposures:

(1) A colorimetric method of Willard and Winter (Anal. Chem. 5, 7, 1933) which was modified and adapted for automation by Weinstein et al (Contributions Boyce Thompson, Ind. 22, 207, 1963). The fluoride is digested with 60% sulfuric acid and the leaching of the Lanthanium alizarin complex is measured at 624 mμ. This method was the same as that used to determine fluoride residues in tissues of the exposed animals.

(2) A second method employed the Fluoride Specific Electrode. The electrode is not absolutely specific for fluoride ions. In alkaline solutions the electrode responds to $(OH)^-$ ions as well as to fluoride ions. Nevertheless, it is still ten times more sensitive to fluoride ions than to $(OH)^-$ ions. At high acid concentration, of course, there is the tendency to form the undissociated HF molecules since the ionization constant for HF is 7.4×10^{-4}, which reduces the concentration of free fluoride ions. Therefore, the optimum pH range for using the fluoride ion electrode is between a pH of 5 and 8. Most of the measurements made in these experiments were between a pH of 5 and 7.

Since the fluoride electrode measures activity, of the fluoride ion, the concentration of the fluoride ions are dependent upon the activity coefficient which is in turn a function of the total ionic strength of the solution.

The difference between solutions containing extraneous ions and a standard made from sodium fluoride is noted in Figure I.

Tables included under "Results" give a comparison of fluoride determinations made by the Auto-Analyzer procedure and the fluoride ion electrode. In general, there was fairly good agreement between the two methods.

It was extremely difficult to regulate and to maintain the desired ratio of fluorine to hydrogen fluoride when the two gases were mixed in the chamber. In spite of many, many attempts to maintain a certain

ratio of the two, it fluctuated so that the measured ratio frequently was not the one which had been calculated previously. The analyses from a number of such trials are presented in Table I. Although the concentration of fluorine alone was easy to maintain under the conditions of operation of the chamber, the addition of HF caused variable fluctuations in both F_2 and total fluoride.

TABLE I

Concentrations of Fluorine and Fluoride In
The Air of The Exposure Chamber

Fluoride by Autoanalyzer mg/l*	mg/150cc	l of air	mg/l air	F_2 mg/l	HF mg/l	F_2:F^-	F_2:HF
57.0	8.55	20	0.428	0.083	0.345	1:5.16	1:4.15
35.0	5.25	10	0.525	0.243	0.282	1:2.16	1:1.21
17.5	2.62	10	0.262	0.072	0.190	1:3.64	1:2.64
25.0	3.75	10	0.375	0.074	0.301	1:5.07	1:4.07
38.0	5.70	10	0.570	0.169	0.401	1:3.37	1:2.38
38.5	5.80	11	0.527	0.285	0.242	1:1.85	1:0.85
35.0	5.25	10	0.525	0.069	0.456	1:7.61	1:6.62
235.0	35.25	10	3.525	0.095	3.430	1:37.2	1:36.20

* Liter of collection medium

Experimental Animals

Young adult (3 to 6 month old) Osborne-Mendel strain albino rats, Swiss white mice and New Zealand strain albino rabbits were used in these studies.

Results

In order to compare the toxicity of the mixture to the effects of hydrogen fluoride of fluorine alone, equitoxic ratios of F_2:HF, based on the LC_{50}'s (with concentrations expressed as mg/l) were calculated. The calculated LC_{50}'s in the rats are presented in Table II.

TABLE II

LC_{50}'s of HF & F_2 and Equitoxic Doses

Rat LC_{50}			
F_2	5 min.	1,088 mg/m^3	700 ppm
	15 min.	606 mg/m^3	390 ppm
	30 min.	420 mg/m^3	270 ppm
	60 min.	287 mg/m^3	185 ppm
HF	5 min.	4,100 mg/m^3	5000 ppm
	15 min.	2,200 mg/m^3	2700 ppm
	30 min.	1,670 mg/m^3	2050 ppm
	60 min.	1,066 mg/m^3	1300 ppm

Ratio 1:3.78 (F_2:HF)

Equitoxic = $\frac{4100}{1088} = 3.76$)
$\frac{2200}{606} = 3.66$) Mean Ratio 1:3.78
$\frac{1670}{420} = 3.98$)
$\frac{1066}{287} = 3.72$)

		mg/m^3					
5 min.	1/2 LC_{50}	544 (F_2)	+	2050 (HF)	=	2594	= Exp. LC_{50}
15	"	303	+	1100	=	1403	= "
30	"	210	+	835	=	1045	= "
60	"	144	+	533	=	677	= "

As mentioned earlier, it was extremely difficult to maintain known or desired concentrations of the mixtures in the chamber. However a number of different concentrations and ratios were run with experimental animals in the chamber.

Although a number of exposures were made where the animals either died in the chamber or soon after removal from the chamber, the ratios of F_2:HF were questionable because of analytical or technical difficulties. Consequently these exposures are not reported.

Some of the concentrations used where the ratios were known are summarized in Table III. In this table the determinations of total fluorides by the Auto-Analyzer and by the Electrode Method are presented. The concentrations listed as "ppm" refer to mg/l of collecting medium. The concentrations listed as "mg/l" refer to mg/liter of air from the chamber.

Both lethal and sublethal exposures were made to determine the LC_{50} for 15 minutes of exposure in two species of animals (rats and mice).

The LC_{50} of mice exposed to mixtures of fluorine and hydrogen fluoride for 15 minutes was 530 mg/m^3 expressed as total fluoride (.274 mg F^- as F_2/m^3 plus 256 mg F^- as HF/m^3) which was very close to the LC_{50} of fluorine alone (583 mg F^-/m^3).

The LC_{50} of rats exposed for 15 minutes was 620 mg F^-/m^3 which was also very close to the LC_{50} of fluorine alone (606 mg F^-/m^3).

Signs of intoxication were similar to those seen from fluorine alone. These included marked irritation to the eyes, nose and respiratory tract; forced expiration, stiffened, sticky fur; erratic movements and convulsions just prior to death, apparently from anoxia. There were no unusual or unexpected signs of intoxication.

A number of exposures of rats, mice or rabbits for 5, 10, 15, 30 or 60 minutes to varying ratios of fluorine to hydrogen fluoride as well as varying concentrations of total fluoride were made to determine sublethal effects. One of the main purposes of this phase of the study was to determine any synergistic or potentiating effects.

Some of these results are summarized in Figures 2 to 5. For a guide line or comparison the LC_{50} is plotted on each graph. The equation and constant were derived from the LC_{50} data. Total fluoride x t 1/2 = k. For rats k is equal to 2.4. Concentrations of the mixtures which were lethal also are included for comparison.

In Figure 2 the exposures of rats for 15 minutes are presented. In exposures numbered 4 and 5 the total fluoride concentrations were four to ten times the LC_{50}. All the rats did not survive the 15 minute exposure in number 5. In number 4 all rats survived while in the chamber but died within a few hours. The amount of fluorine in these two exposures was very low compared to total fluoride. During exposures numbered 1, 2 or 3 the ratio of fluorine to HF and to total fluoride was higher but the animals survived, undoubtedly because the total fluoride concentration was below the LC_{50}.

In Figure 3, exposures of rats and mice for 15, 20 or 30 minutes are presented. During these exposures the gases were mixed in an attempt to keep the concentrations of fluorine and hydrogen fluoride equal or as near to a 1:1 ratio as possible. Some of the rats in group number 1 died (6/10) and 4 of 10 mice (group number 4) died. The animals in groups number 2 and 3 all survived. These data indicate that the lethal effects were directly related to the total concentration of fluoride and that the effects of the combination of the two gases were essentially additive.

The concentrations of total fluoride during the exposures in Figure 4 were below the LC_{50}. The ratios of fluorine to hydrogen fluoride were approximately 1:2 while the ratios of fluorine to total fluoride were approximately 1:3. All animals (rats and mice were exposed in the same group) survived; indicating again that there is no synergistic or potentiation by mixtures of F_2 and HF, and that the effects depend upon concentration of total fluoride.

In Figure 5 the concentrations of fluorine, hydrogen fluoride and total fluoride during exposures of rabbits are presented. The concentration of total fluoride was about the same in all three exposures. The ratio of fluorine to HF or total fluoride was varied considerably. The ratio of fluorine was very low in number 1, very high in number 3 and about equal to the concentration of HF in number 2. None of the animals died and there was no difference in signs of intoxication.

This experiment was designed to determine more than additive effects. Again there were none, which indicated that the effects depended upon the total fluoride concentration.

Figure 1

Concentration of Fluoride Ion
vs. Millivolts

A -- no interferring ions

B -- a working curve,
i.e. neutralized KOH

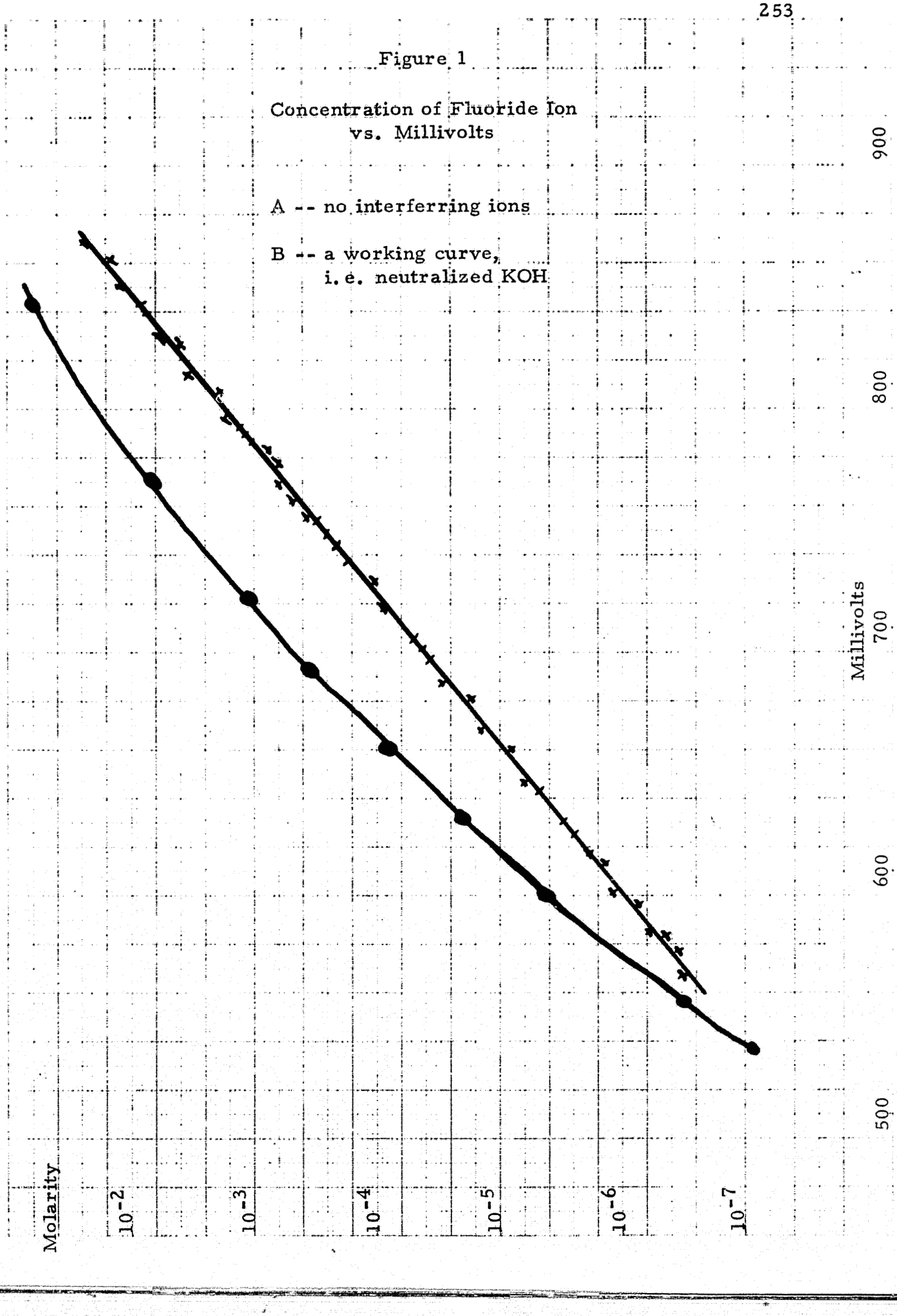

Figure 2

Exposures of Rats
to Fluorine and Hydrogen Fluoride Mixtures

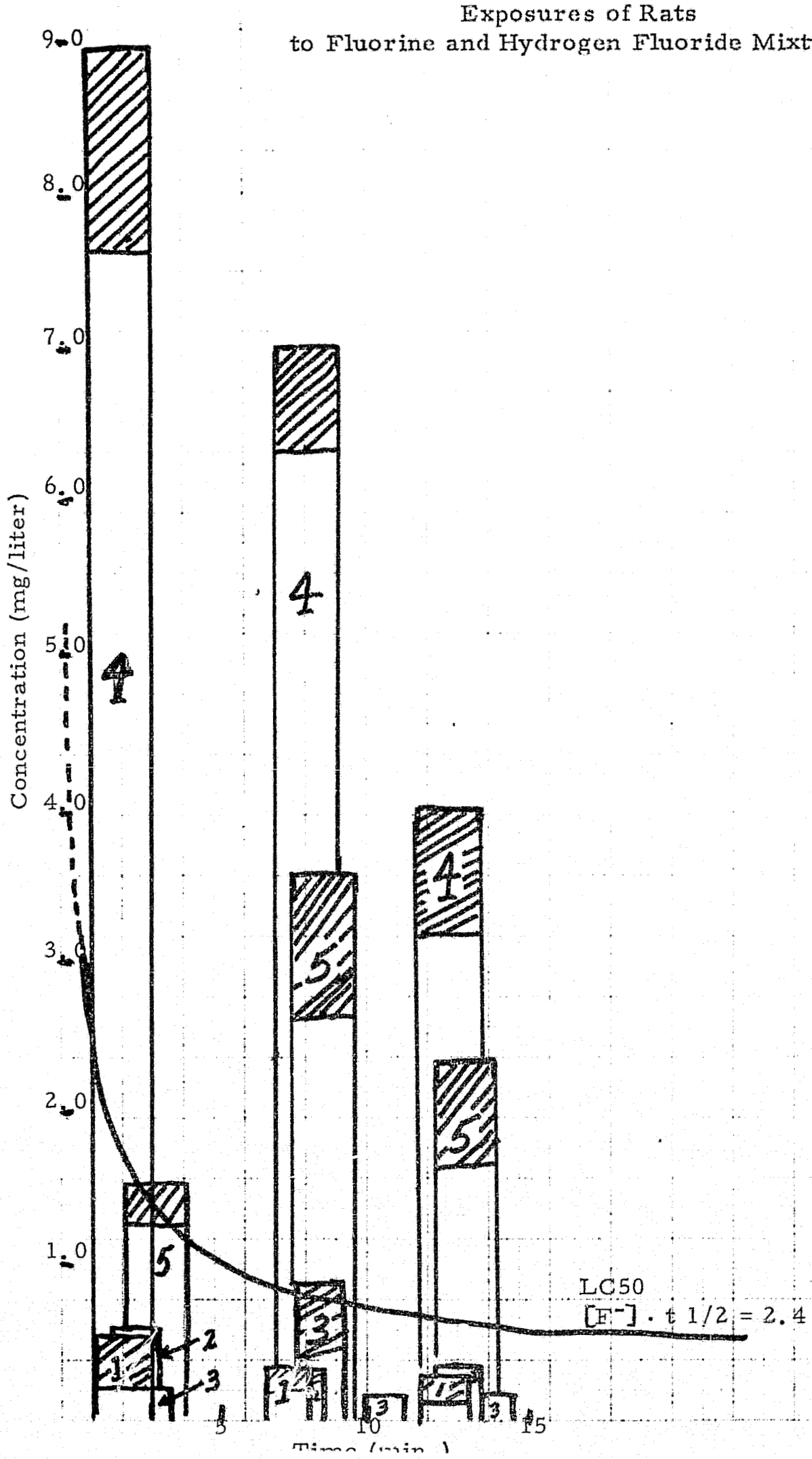

Figure 3

Exposures of Rats and Mice
to Fluorine and Hydrogen Fluoride Mixtures

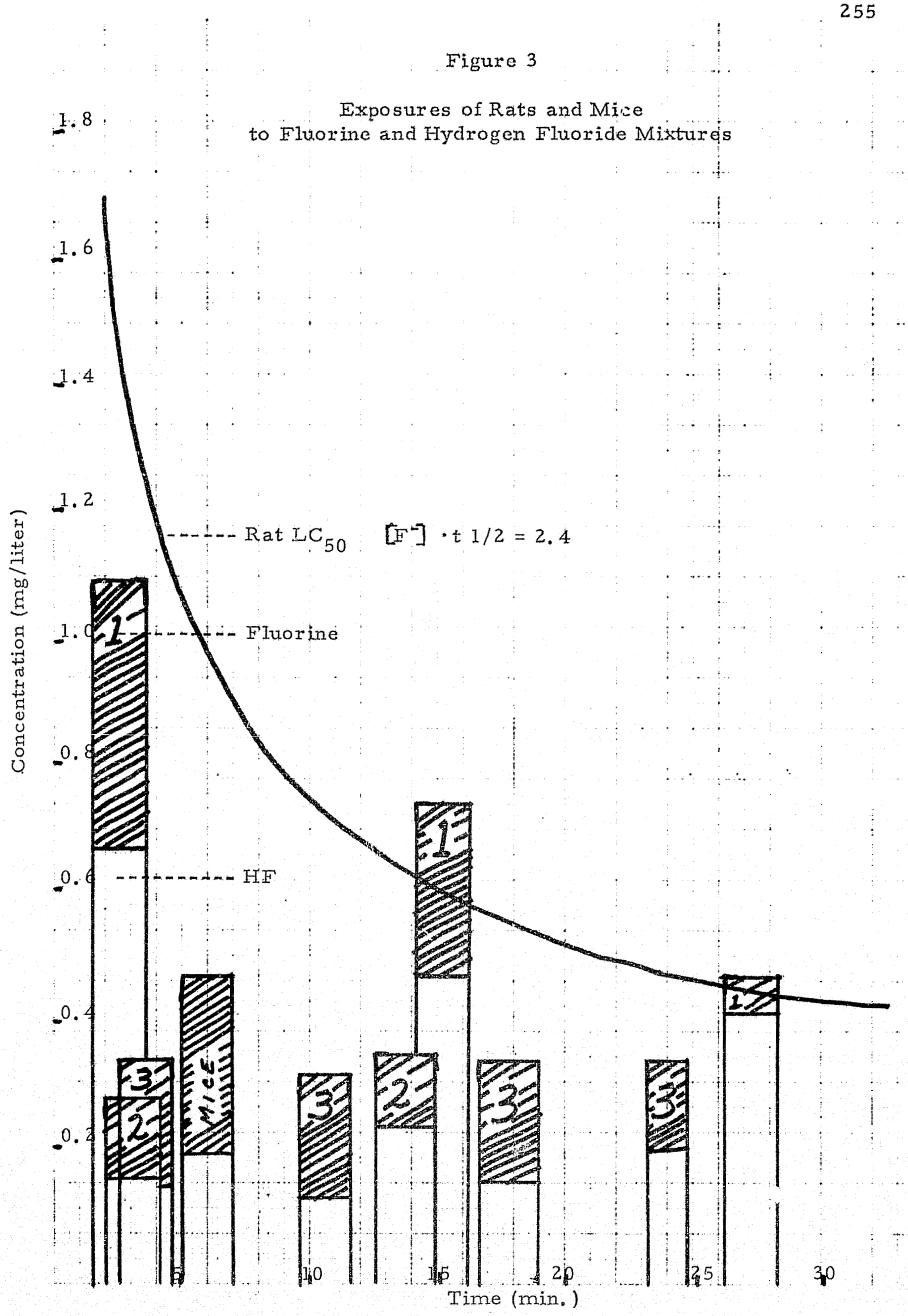

Figure 4

Exposures of Rats and Mice
to Fluorine and Hydrogen Fluoride Mixtures

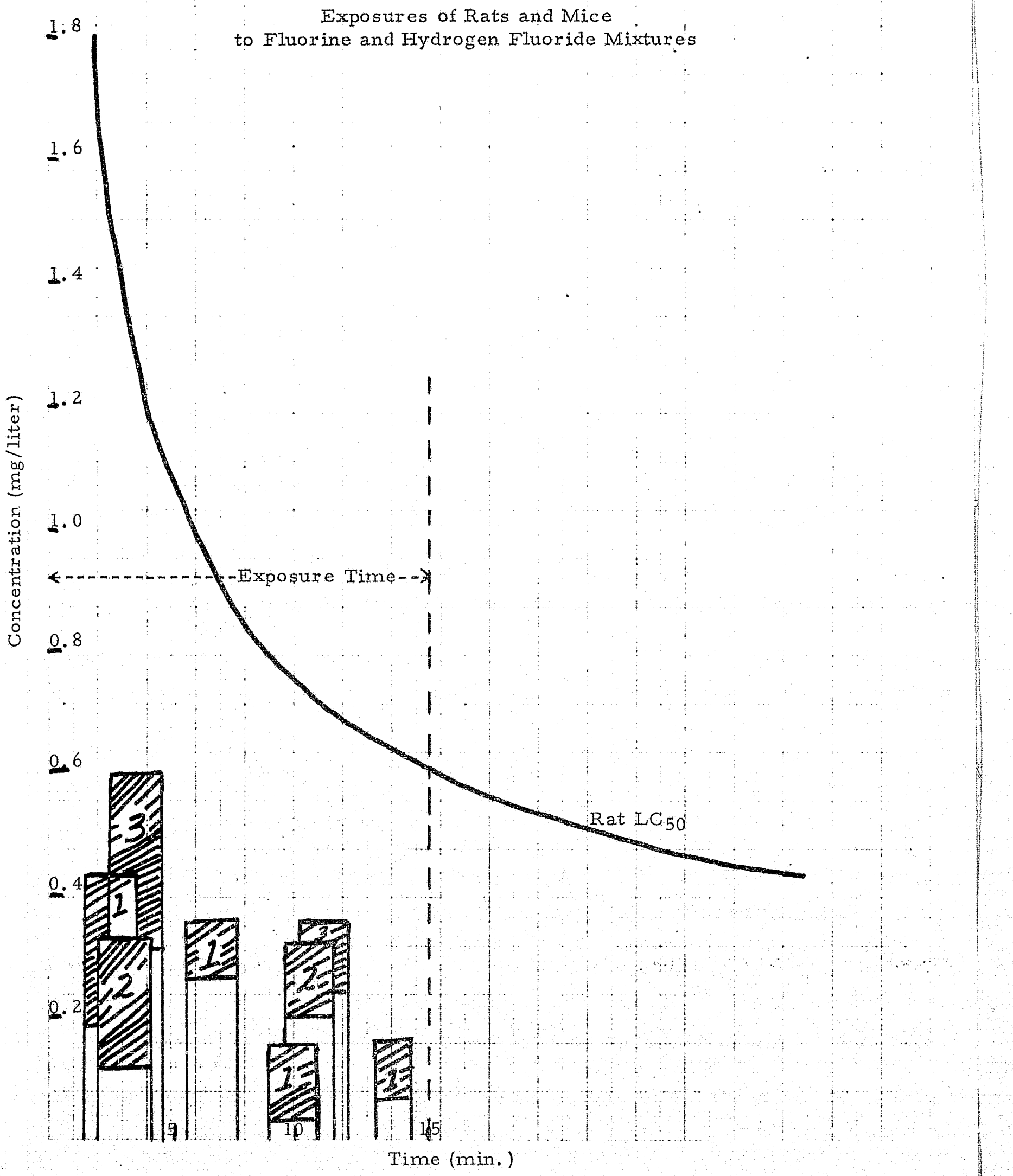

Figure 5

Exposures of Rabbits
to Fluorine, Hydrogen Fluoride
and Total Fluoride Mixtures

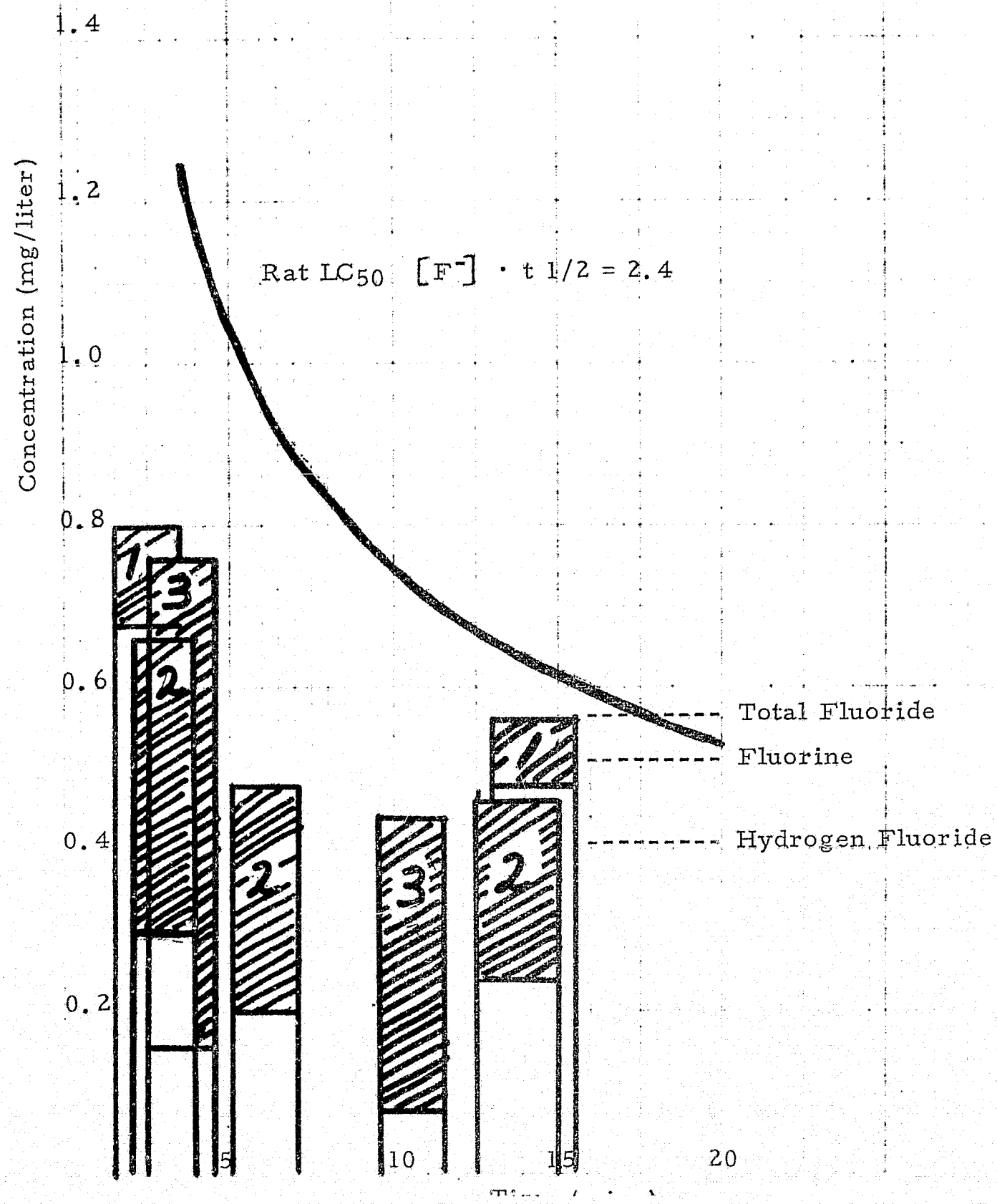

TABLE III

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	mg/l A-A	mg/l Elect.	HF Conc* mg/l
5	11.9	0.908	53.0	-	1.590	-	0.602
10	-	-	53.0	34.0	0.795	0.510	-
5	12.5	0.955	53.0	-	1.590	-	0.635
5	11.1	0.840	50.0	-	1.290	-	0.450
10	6.3	0.238	-	34.0	-	0.510	0.272
10	7.8	0.294	23.0	23.0	0.690	0.690	0.396
5	7.2	0.547	15.0	15.0	0.450	0.450	-
5	-	-	11.5	12.0	0.344	0.360	-
5	1.7	0.129	10.0	-	0.300	-	0.171
6	-	-	13.5	4.25	0.338	0.106	-
10	7.3	0.275	19.5	-	0.292	-	-
10	6.3	0.238	8.2	-	0.122	-	-
10	11.0	0.418	30.0	-	0.450	-	0.032
6	9.2	0.583	18.0	-	0.450	-	-
5	-	-	11.5	12.5	0.344	0.360	-
6	5.7	0.361	10.5	-	0.262	-	-

TABLE III continued

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	mg/l A-A	mg/l Elect.	HF Conc* mg/l
5	9.3	0.709	18.0	-	0.540	-	-
5	4.3	0.328	54.5	-	1.630	-	1.302
7	-	-	66.0	76.0	1.410	1.630	-
5	2.6	0.198	51.5	-	1.550	-	1.350
5	-	-	26.0	30.4	0.780	0.915	-
13	3.7	0.108	14.5	-	0.168	-	0.060
10	3.1	0.118	11.5	-	0.172	-	0.054
10	-	-	20.0	19.0	0.300	0.285	-
10	7.1	0.270	30.5	-	0.457	-	0.187
12	-	-	44.0	42.5	0.550	0.532	-
13	10.5	0.307	19.5	-	0.224	-	-
11.5	-	-	51.5	42.5	0.672	0.532	-
10	4.3	0.163	17.0	-	0.255	-	0.092
10	-	-	61.0	42.5	0.915	0.532	-
10	9.7	0.368	183.0	-	2.740	-	2.372
7	11.1	0.604	172.0	-	3.680	-	3.076
5	-	-	100.0	475.0	3.000	14.200	-
11	-	-	530.0	475.0	7.220	6.500	-

TABLE III continued

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	Total Fluoride mg/l A-A	Total Fluoride mg/l Elect.	HF Conc* mg/l
10	3.4	0.127	595.0	-	8.930	-	8.803
10	-	-	465.0	475.0	6.980	7.120	-
8	1.1	0.052	215.0	-	4.020	-	3.968
10	3.9	0.142	64.0	-	0.960	-	0.818
10	-	-	64.0	60.0	0.960	0.900	-
10	4.2	0.158	111.0	-	1.660	-	1.502
10	6.3	0.249	315.0	-	4.720	-	4.471
10	0.7	0.027	129.0	-	1.930	-	1.903
6	-	-	141.0	95.0	3.530	2.380	-
10	2.3	0.088	136.0	-	2.360	-	2.272
10	1.4	0.053	9.4	-	0.141	-	0.088
10	3.4	0.127	12.7	-	0.191	-	0.064
10	5.7	0.216	18.0	-	0.270	-	0.054
10	5.4	0.235	22.0	-	0.330	-	0.095
10	10.5	0.400	26.0	-	0.490	-	0.010
10	-	-	43.0	42.3	0.645	0.523	-
10	-	-	58.0	49.6	0.870	0.750	-
5	9.9	0.753	22.0	-	0.760	-	0.007

TABLE III continued

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	Total Fluoride mg/l A-A	Total Fluoride mg/l Elect.	HF Conc* mg/l
10	3.5	0.133	29.0	-	0.435	-	0.302
10	-	-	24.0	15.1	0.360	0.226	-
10	4.2	0.160	10.0	-	0.450	-	0.290
10	-	-	11.0	7.2	0.165	0.108	-
10	4.5	0.171	18.0	-	0.270	-	0.099
10	3.2	0.121	22.0	-	0.330	-	0.209
10	-	-	21.5	19.0	0.323	0.285	-
12	3.5	0.113	42.0	-	0.525	-	0.412
10	1.7	0.064	40.0	-	0.600	-	0.532
10	-	-	24.0	27.8	0.360	0.417	-
10	3.9	0.149	33.5	-	0.503	-	0.354
5	10.0	0.760	19.0	-	0.930	-	0.170
10	6.3	0.240	20.5	-	0.308	-	0.068
10	-	-	19.0	28.7	0.285	0.430	-

TABLE III continued

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	mg/l A-A	mg/l Elect.	HF Conc* mg/l
10	5.4	0.205	14.5	-	0.217	-	0.012
20	-	-	24.0	75.5	0.180	0.566	-
11	10.7	0.367	25.0	-	0.375	-	0.008
14	-	-	39.0	31.0	0.418	0.360	-
10	3.2	0.121	18.0	-	0.270	-	0.149
10	7.4	0.281	37.5	-	0.563	-	0.282
10	10.2	0.387	33.0	-	0.495	-	0.108
10	-	-	51.0	47.5	0.765	0.713	-
10	3.2	0.122	38.0	-	0.570	-	0.448
10	7.4	0.281	30.5	-	0.458	-	0.177
10	-	-	38.0	-	0.570	-	-
5	5.2	0.396	18.5	-	0.556	-	0.160
20	10.2	0.194	71.0	-	0.533	-	0.339
10	11.0	0.417	73.0	-	1.095	-	0.678
10	7.0	0.264	50.0	-	0.750	-	0.492
10	1.5	0.051	32.0	-	0.480	-	0.423

TABLE III continued

Concentrations of Fluorine and Hydrogen Fluoride
In The Exposure Chamber

Gas Sample Vol. (liters)	$Na_2S_2O_3$ N/100 Vol. (ml)	F_2 mg/l	Total Fluoride A-A ppm	Total Fluoride Elect. ppm	mg/l A-A	mg/l Elect.	HF Conc* mg/l
10	3.2	0.121	19.0	-	0.285	-	0.164
12	3.0	0.114	24.0	-	0.360	-	0.246
10	7.2	0.274	32.0	-	0.480	-	0.206
10	5.0	0.190	23.0	-	0.344	-	0.154
10	5.0	0.190	21.5	-	0.322	-	0.132
10	4.5	0.171	23.0	-	0.344	-	0.173
10	4.4	1.650	23.0	23.9	0.344	0.359	0.178

* Calculated from the difference between Total F and F_2

Key:

A-A - Auto Analyzer Method
Elect. - Fluoride Ion Electrode

Gross Pathology

Lethal exposures to a mixture of fluorine and hydrogen fluoride caused changes similar to those observed in animals exposed to fluorine or HF alone.

The trachea and bronchi were congested and contained frothy, bloody fluid. The lungs were voluminous, congested, hemorrhagic and emphysematous. The cut sections of the lungs showed marked congestion and exuded lots of hemorrhagic fluid when pressed.

The liver was slightly enlarged, congested, friable and very dark brown in color. The kidneys were somewhat congested and discolored.

Micropathology

The lungs showed moderate to marked hemorrhagic congestion, edematous exudate in the alveoli, edema of the loose peribronchiolar and perivascular connective tissue; and swelling and desquamation of bronchial epithelial cells at lethal concentrations. At lower levels of F2:HF there was congestion and edema; the degree of change depending upon the concentration.

At lethal or near lethal concentrations the liver showed severe acute congestion, marked sinusoidal dilatation, slight hydropic degeneration, some portal infiltration by round cells, acidophilic cytoplasm and minimal necrosis of the liver cells. At lower concentrations there was slight to moderate congestion and swelling of the cells.

The kidney showed parenchymatous degeneration, some necrosis in the renal cortical tubules, some glomerular necrosis and cloudy swelling of the convoluted tubules in the boundary zone at lethal or near lethal concentrations. After exposure to lower concentrations there was some cloudy swelling of the tubular epithelium in the convoluted tubules, slight enlargement of the glomerulus and congestion in the capillaries.

Fluoride In Tissues After Exposures To Mixtures of Fluorine and Hydrogen Fluoride

The amount of fluoride in the lungs, liver and kidneys of rats was determined after exposures to mixtures of fluorine and hydrogen fluoride for 5 to 30 minutes. The method of analysis was the same as described previously.

Tissues from some rats were analyzed following exposure to lethal concentrations of the mixture. Rats were exposed for 5, 15 or 30 minutes. The results are presented in Table IV. The CT values for the exposures (from top to bottom) were 17.6, 18.9, 8.6 and 12.8 mg/l/min. It should be emphasized that, although the animals died, the periods of survival were quite different. Those exposed to 3.525 mg/l for 5 minutes or 1.260 mg/l for 15 minutes died within 30 minutes after being taken from the chamber. Those exposed to 0.570 mg/l for 15 minutes died within 8 to 18 hours after being taken from the chamber, while those exposed to 0.428 ppm for 30 minutes died within one to six hours after being taken from the chamber.

The mean concentrations in the lungs and kidneys were about the same, with concentrations in lungs higher in some animals and concentrations in kidneys higher in others. The levels of fluoride in the liver were lower than those in the lungs or kidneys of most animals.

The levels in the tissues were closely correlated with the CT values of animals exposed for 5 or 15 minutes. For example the CT, expressed as mg/l/min., was related to the mean concentration in lungs, expressed as ppm, as 17.6 to 37, 18.9 to 32, and 8.6 to 17. The levels in tissues of

the rats exposed for 30 minutes, however, were not correlated with CT to the same degree (CT 12.8 mg/l/min. to 57 ppm in lungs).

Tissues from other rats were analyzed for fluoride after sub-lethal exposures to mixtures of fluorine and hydrogen fluoride. The animals were sacrificed 48 hours after exposure. These results are presented in Table V. The mean fluoride levels of these groups, as well as two other groups of five animals each, are summarized in Table VI.

Concentrations of fluoride in the lungs of all groups were significantly higher than the levels in control rats. These levels were quite well correlated with the levels of total fluoride in the air.

The concentrations in the liver or kidney were higher than levels in control rats in some instances, but not in others. The levels in these tissues were not well correlated with the level of total fluoride in air.

TABLE IV

Fluoride Concentrations In Tissues of Rats
Exposed to Lethal Concentrations of
Mixtures of Fluorine and Hydrogen Fluoride

Exposure Time (minutes)	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue		
		Lung (ppm)	Liver (ppm)	Kidney (ppm)
5	3.525	47	37	33
		35	21	30
		44	36	41
		33	32	38
		24	37	26
	Mean	37	33	34
15	1.260	45	25	30
		24	27	40
		21	24	33
		44	40	32
		24	21	21
	Mean	32	27	31
15	0.570	15	12	12
		30	12	24
		12	8	14
		16	10	17
		13	9	15
	Mean	17	10	16
30	0.428	63	46	51
		62	44	48
		50	43	57
		62	53	47
		47	60	64
	Mean	57	49	53

TABLE V

Fluoride Concentration in Tissues of
Rats Exposed to Mixtures of
Fluorine and Hydrogen Fluoride

(Sacrificed 48 hours after exposure)

Exposure Time (minutes)	Concentration of Fluoride in Air (mg/l)	Concentration of Fluoride in Tissue		
		Lung (ppm)	Liver (ppm)	Kidney (ppm)
15	0.262	7.6	2.0	4.5
		8.5	4.5	8.5
		2.0	2.0	2.5
		2.5	2.5	5.2
		10.5	3.0	3.0
	Mean	5.2	2.8	5.2
15	0.300	10.0	5.5	3.8
		7.0	7.0	4.4
		5.5	5.5	4.4
		6.2	7.0	3.8
		6.2	5.0	3.8
	Mean	7.0	6.0	4.0
15	0.280	5.0	4.4	5.5
		5.5	4.4	6.2
		7.2	3.8	4.4
		6.5	5.5	11.2
		5.0	4.4	3.8
	Mean	5.8	4.5	6.2
20	0.326	10.0	3.5	6.8
		8.0	3.5	3.5
		6.0	3.5	4.0
		5.5	4.0	4.0
		3.5	3.0	3.5
	Mean	6.6	3.5	4.4
30	0.344	6.8	2.2	6.0
		5.5	4.0	4.0
		8.0	3.5	6.5
		3.5	3.0	-
		6.0	5.0	-
	Mean	6.0	3.5	5.5

TABLE VI

Concentrations of Fluoride in Tissues of Rats
After Exposures to Mixtures of Hydrogen Fluoride
plus Fluorine

Time of Each Exposure (minutes)	Total Fluoride Concentration (mg/l)	Fluoride in Tissues Mean Concentration		
		Lung (ppm)	Liver (ppm)	Kidney (ppm)
15	0.450	10.5*	3.0	3.0
15	0.262	5.2*	2.8	5.2*
15	0.300	7.0*	6.0*	4.0
15	0.280	5.8*	4.5*	6.2*
30	0.480	8.6*	3.0	14.7*
20	0.326	6.6*	3.5	4.4
30	0.344	6.0*	3.5	5.5*

* Significantly greater than concentrations in control rats.

Summary

These exposures were made to determine if additive, less than additive or more than additive effects occur from the mixture of F_2 and HF. The LC50's and/or effects in rats, mice and rabbits indicated that the lethal effects were closely related to the total concentration of fluoride. Therefore, the effects were essentially additive.

Pathology (gross and microscopic) in the tissues of these animals and signs of intoxication were very similar to those found in tissues of animals exposed to fluorine or to hydrogen fluoride alone.

Tissue concentrations (lung, liver and kidney) were closely related to total fluoride concentration in air, regardless of whether the fluoride was from fluorine or from hydrogen fluoride.