REPORT OF TOXIC EFFECTS OF FLUORINE FOLLOWING SHORT-TERM INHALATION

Conducted under

NASA NGR10-007-012

Presented To

The National Aeronautics and Space Administration
Washington, D. C.

Submitted by

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University of Miami School of Medicine
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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 sublethal 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 (94OF). 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% fluorine 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 oother 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 due 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 142 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

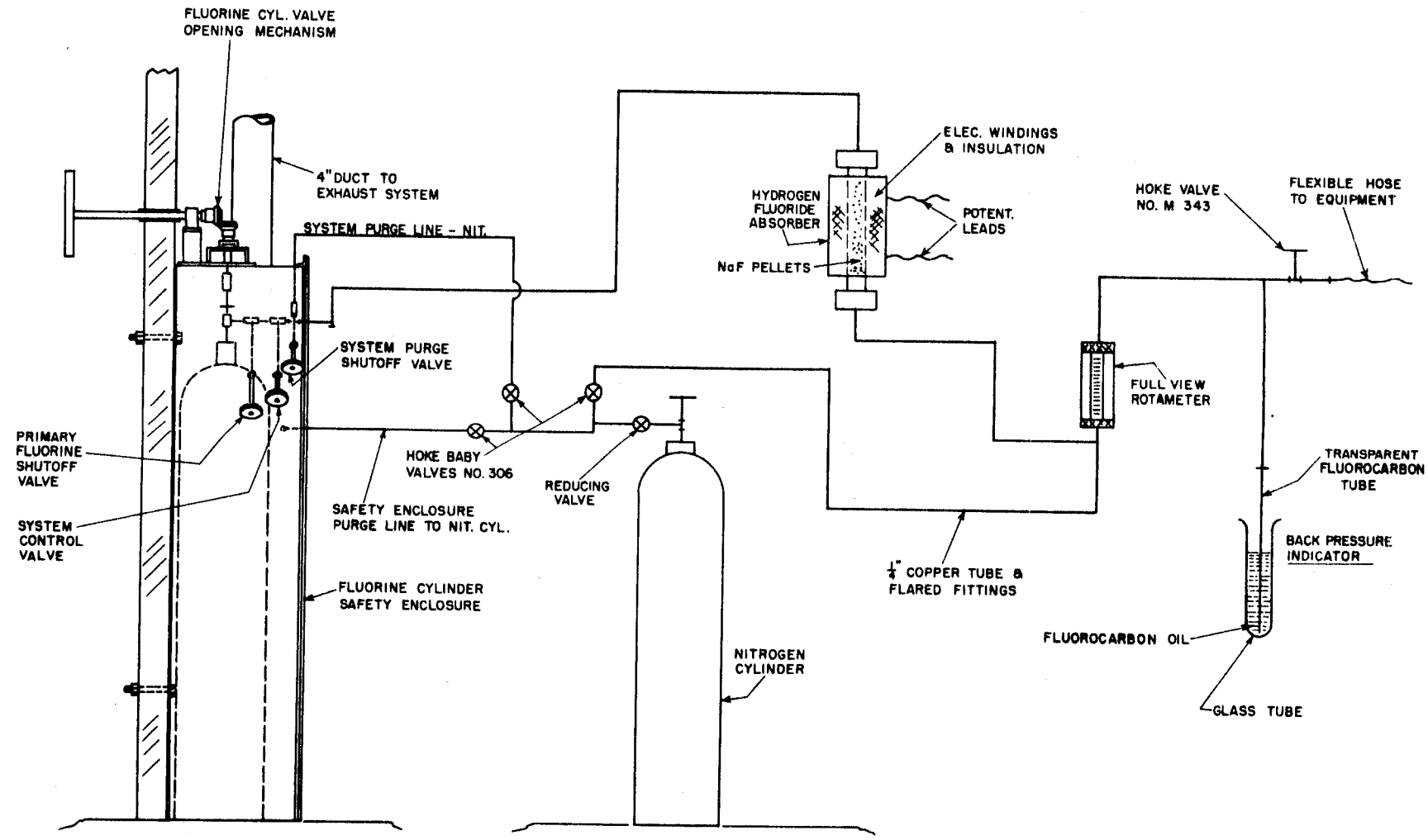
Exposure Time (min)	Rat Concentration mg/cuM	Rat ppm	Mouse Concentration mg/cuM	Mouse ppm	Guinea Pig Concentration mg/cuM	Guinea Pig ppm	Rabbit Concentration mg/cuM	Rabbit ppm
5	1085	700	935	600	----	---	1273	820
15	617	390	545	350	617	390	---	---
30	420	270	350	225	----	---	420	270
60	288	185	234	150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

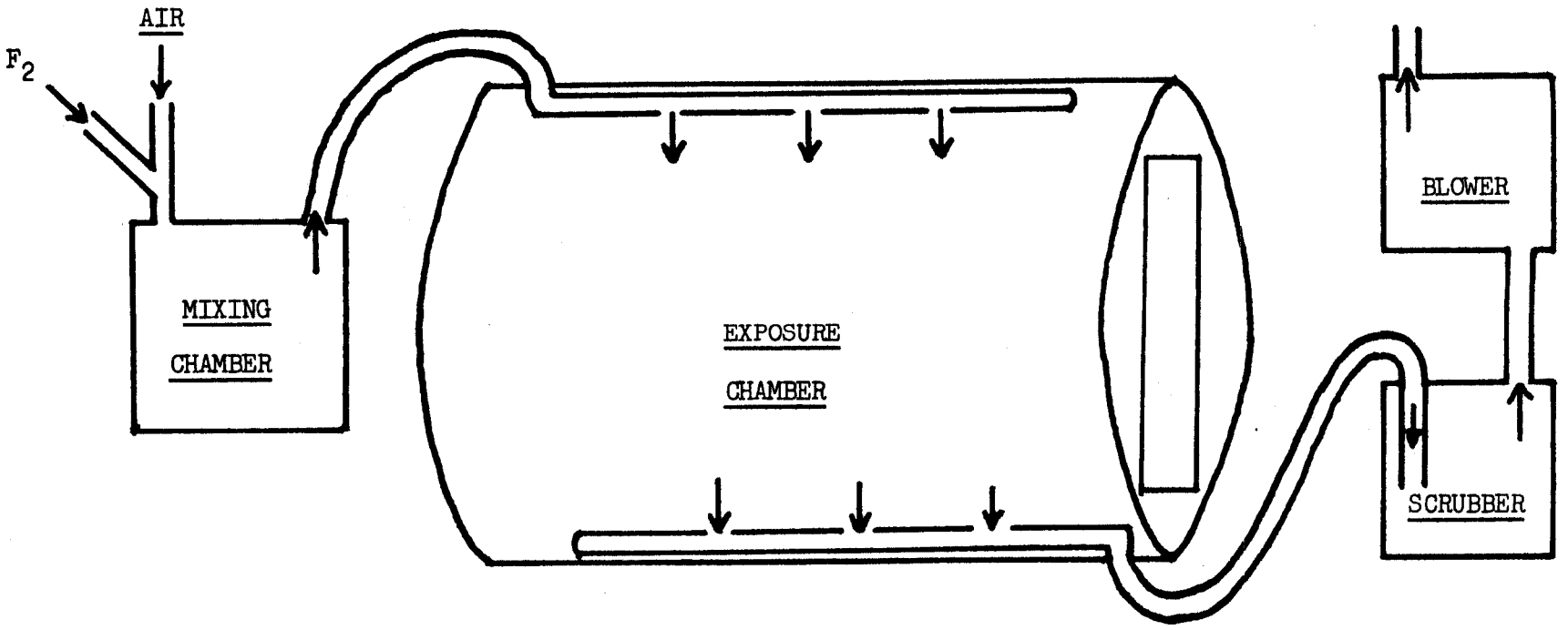
Expesure to concentrations at or below 100 ppm for 5 minutes, 70 ppm for 15 minutes, 55 ppm for 30 minutes, or 45 ppm for 60 minutes caused no apparent effects in the animals.



NOTE -- All valves were Matheson 940 F

FLUORINE CYLINDER LABORATORY SET UP

FIGURE 1

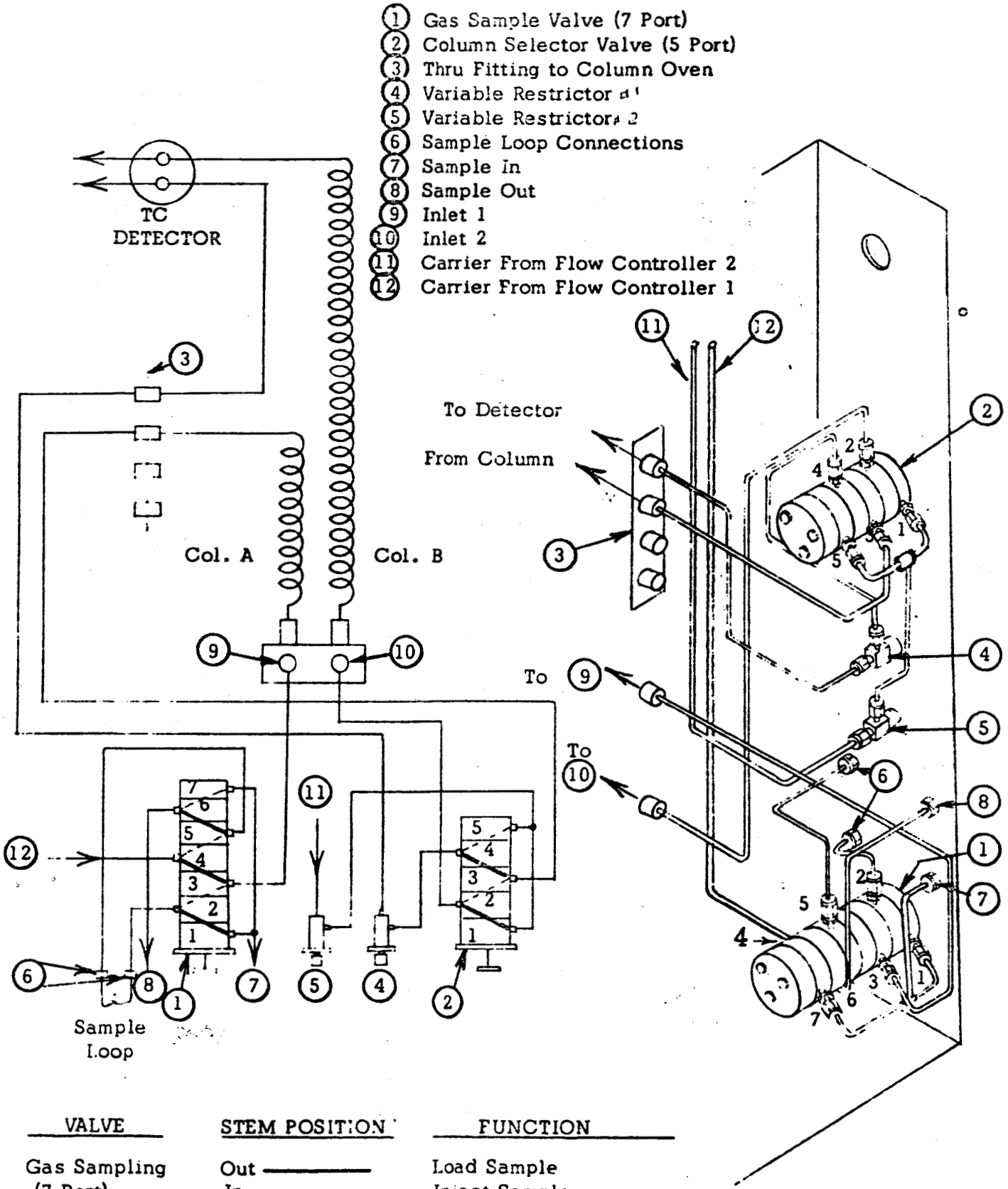


SCHEMATIC OF CHAMBER AND EQUIPMENT

FIGURE 2

INSTALLATION OF GAS SAMPLING AND COLUMN SELECTOR VALVE

For Series or Parallel Operation of Two Columns



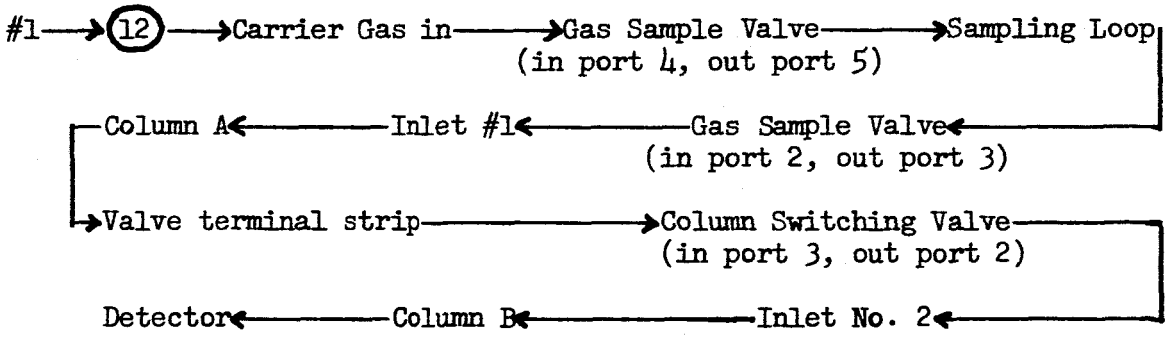
VALVE	STEM POSITION	FUNCTION
Gas Sampling (7 Port)	Out ———	Load Sample
	In - - - - -	Inject Sample
Column Selector (5 Port)	Out ———	Columns in Parallel
	In - - - - -	Columns in Series

FIGURE 3

FLOW PATTERN USING PLUMBING ARRANGEMENT

Condition A.

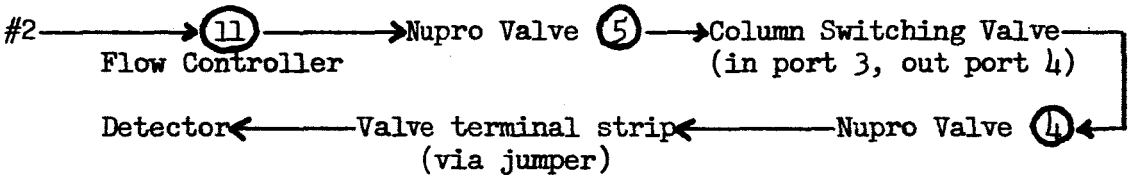
Gas Sampling Valve in, Column Switching Valve in



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

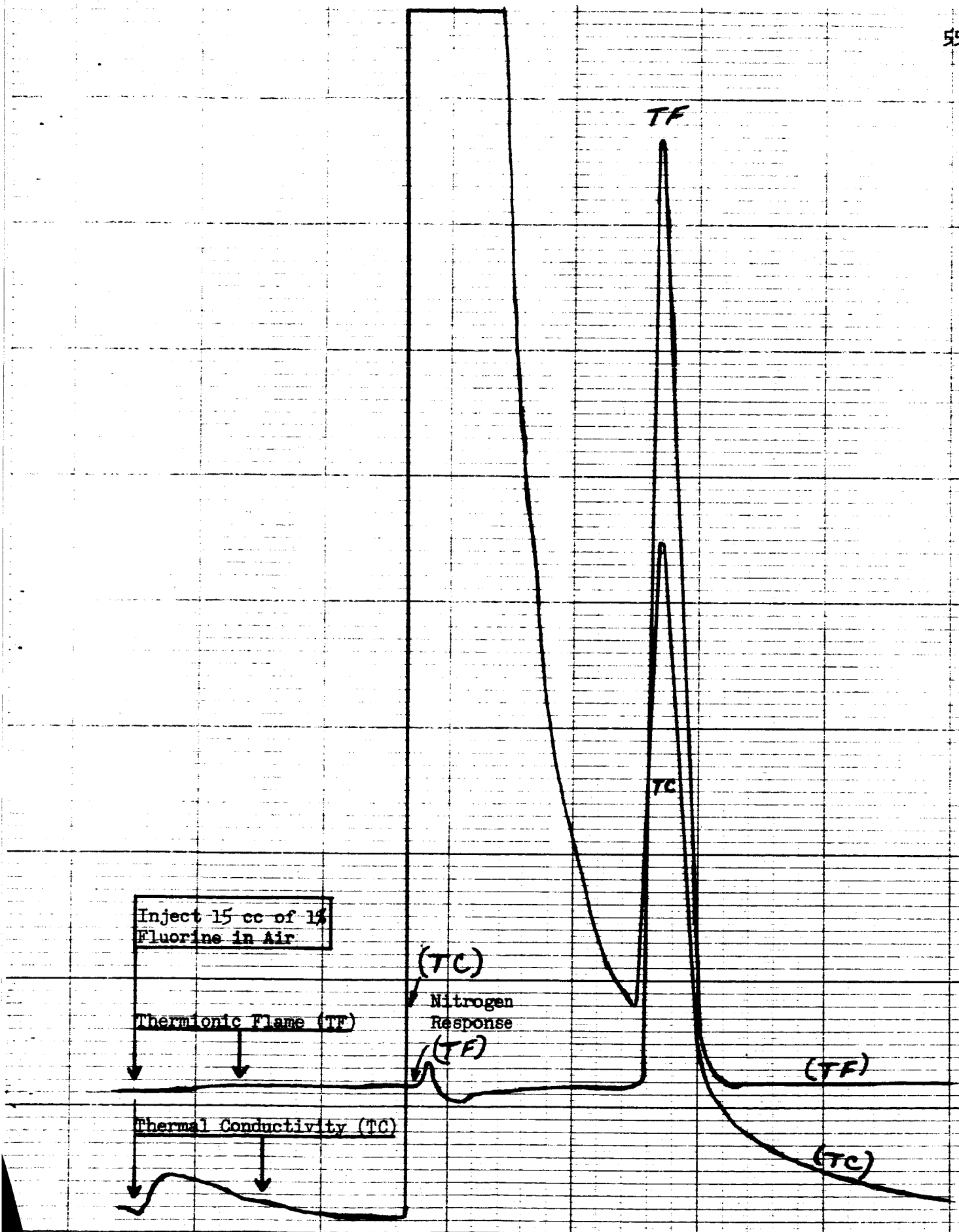

TF
TC
Inject 15 cc of 1% Fluorine in Air
(TC)
Nitrogen Response
(TF)
Thermionic Flame (TF)
(TF)
Thermal Conductivity (TC)
(TC)

FIGURE 6

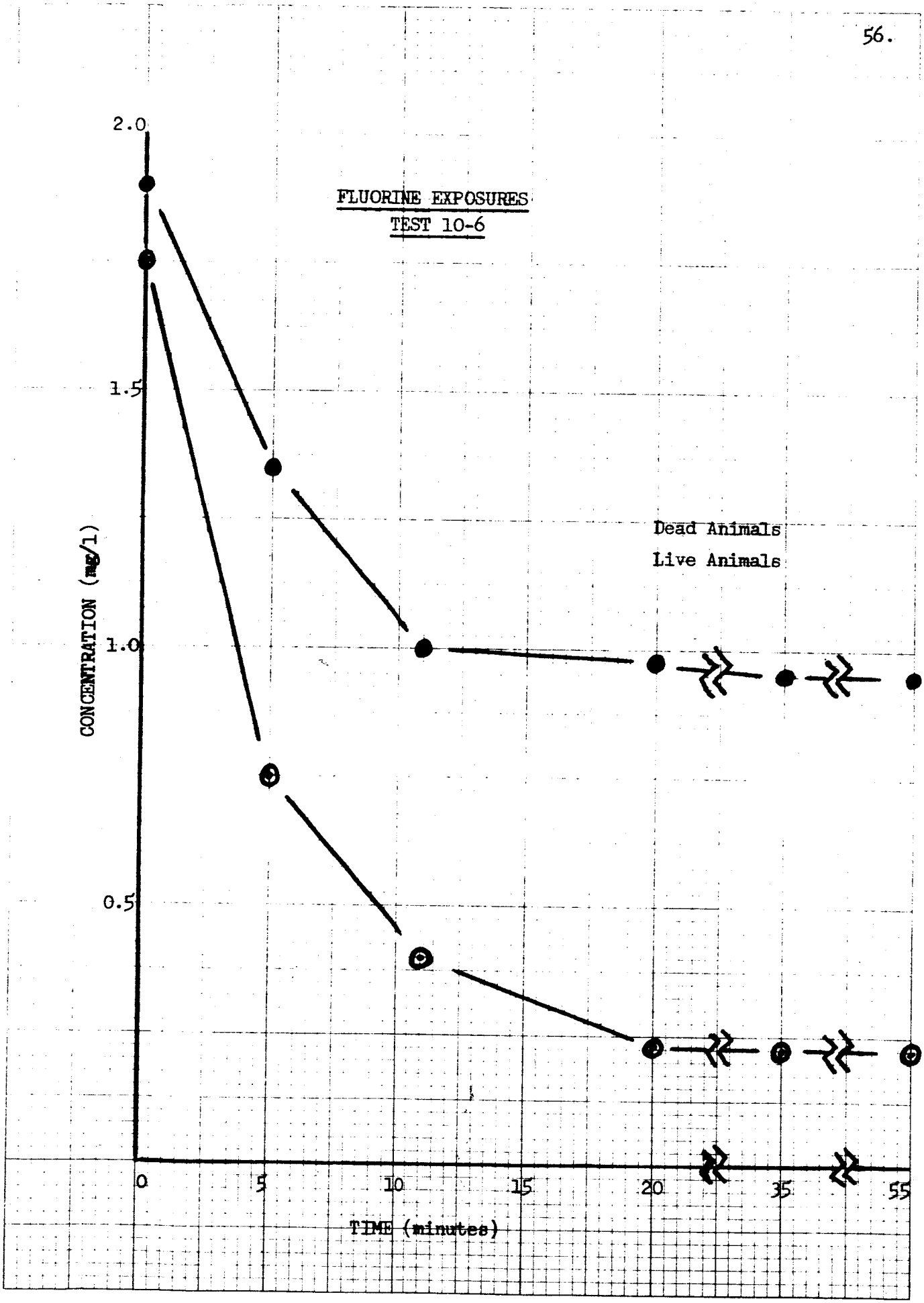

FLUORINE EXPOSURES
TEST 10-6
2.0
1.5
1.0
0.5
CONCENTRATION (mg/l)
Dead Animals
Live Animals
0
5
10
15
20
35
55
TIME (minutes)

FIGURE 7

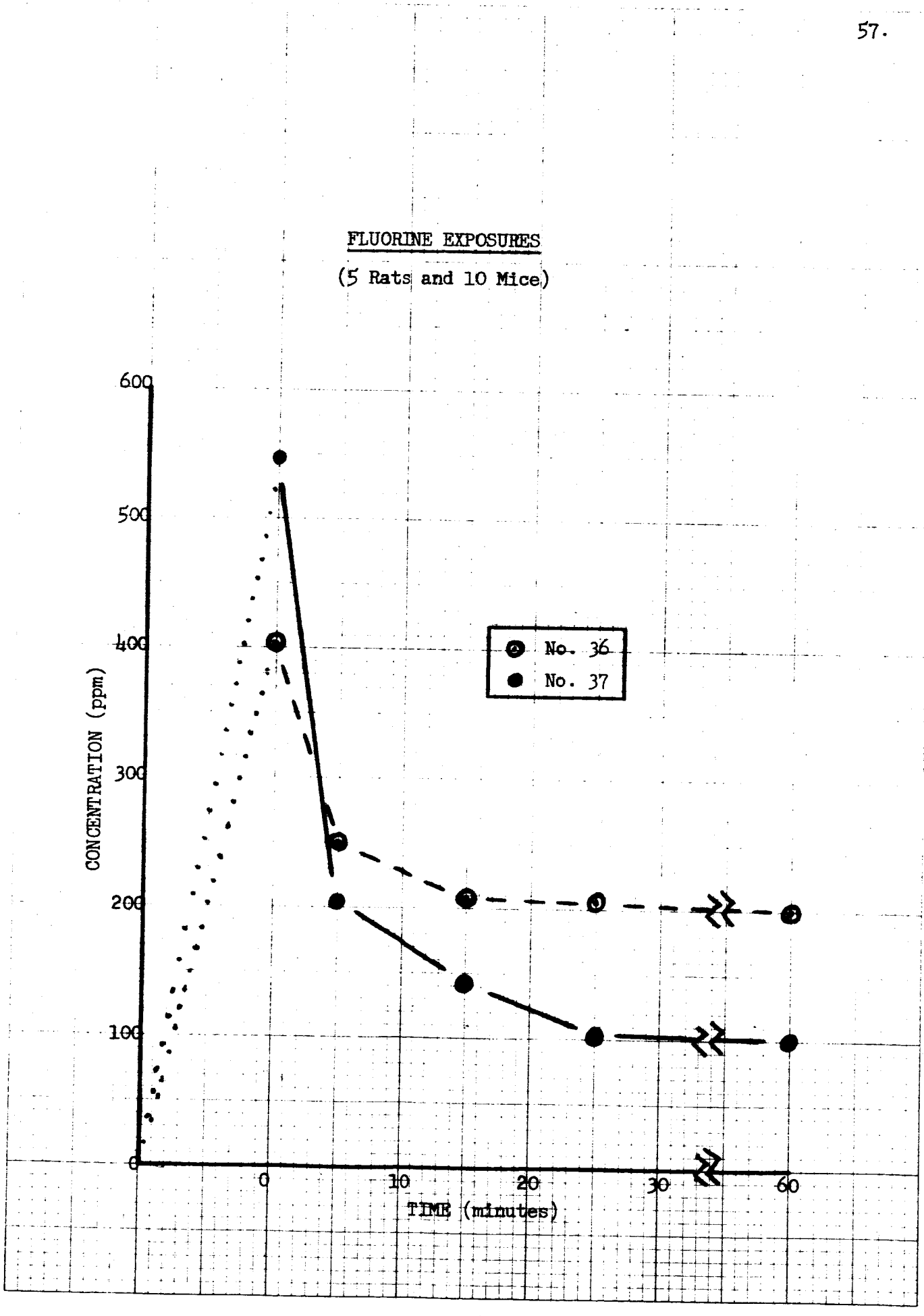

FLUORINE EXPOSURES
(5 Rats and 10 Mice)
600
500
400
300
200
100
0
CONCENTRATION (ppm)
No. 36
No. 37
0
10
20
30
60
TIME (minutes)

FIGURE 8

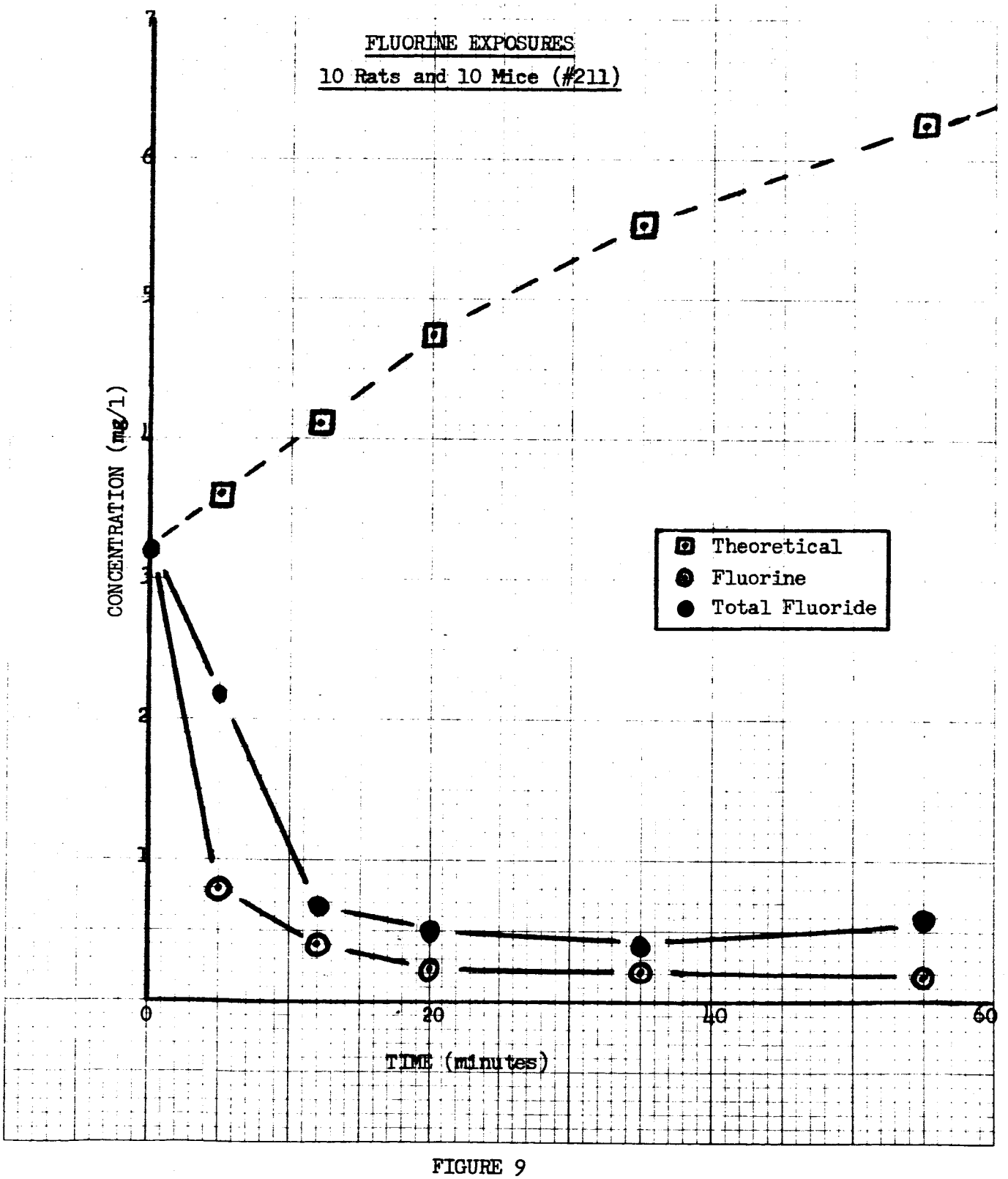

FLUORINE EXPOSURES
10 Rats and 10 Mice (#211)
CONCENTRATION (mg/l)
TIME (minutes)
0
20
40
60
Theoretical
Fluorine
Total Fluoride

FIGURE 9

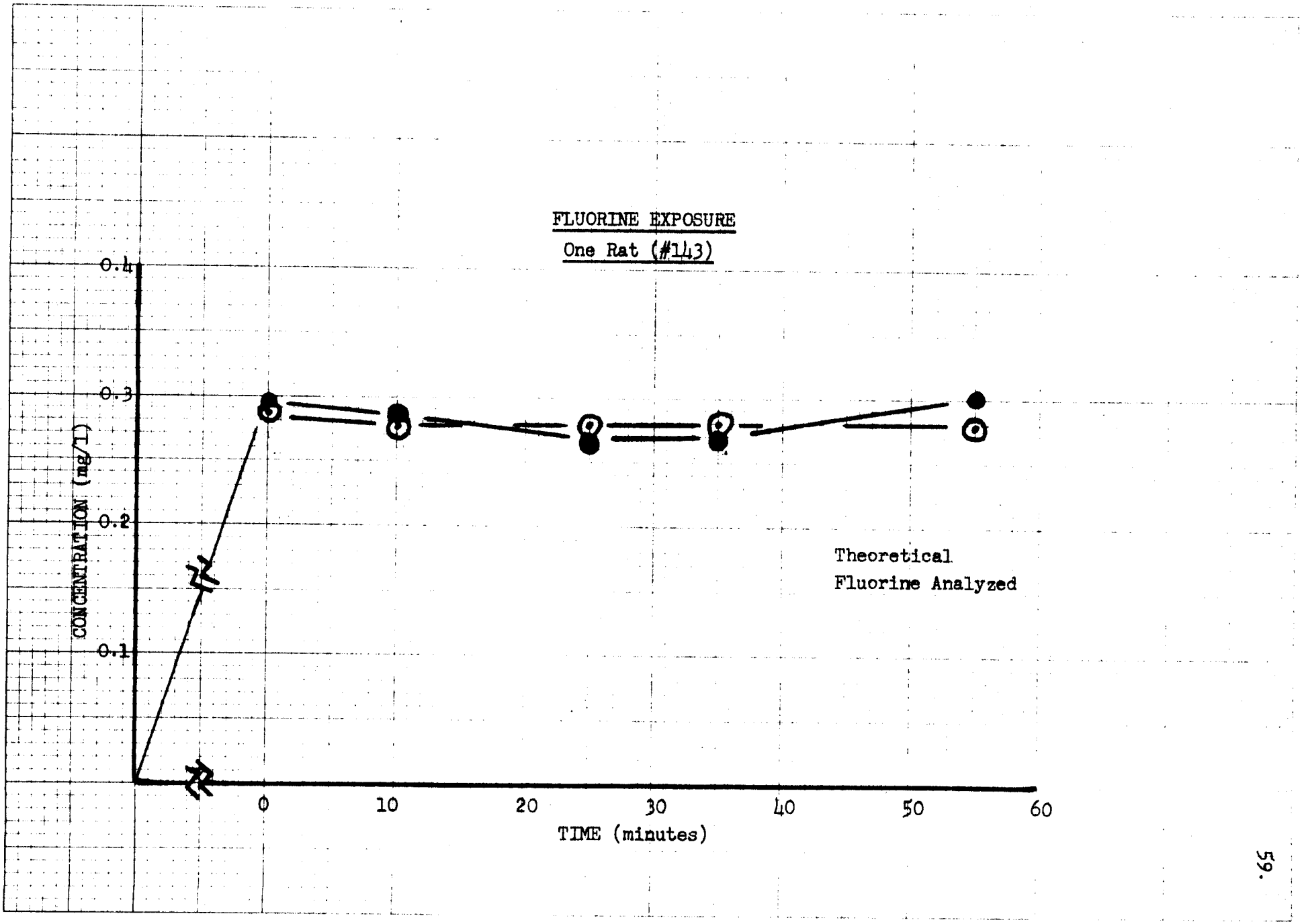

FLUORINE EXPOSURE
One Rat (#143)
0.4
0.3
0.2
0.1
CONCENTRATION (mg/L)
0
10
20
30
40
50
60
TIME (minutes)
Theoretical
Fluorine Analyzed

FIGURE 10

SUBLETHAL EFFECTS IN RATS AND MICE EXPOSED TO FLUORINE

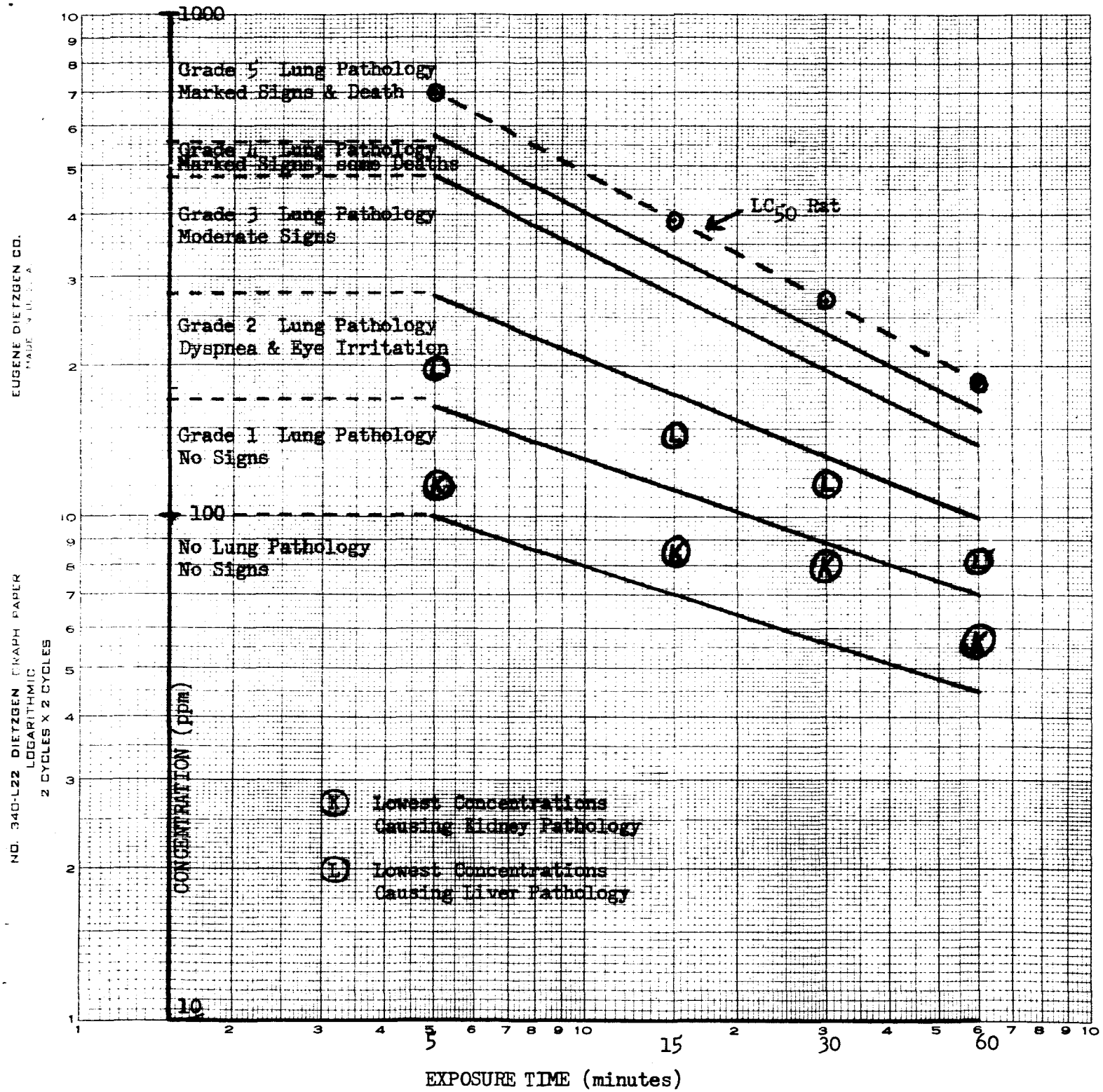


FIGURE 11

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 $\pm$ Standard Deviation 99 $\pm$ 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}		19/20 Confidence Limits (ppm)
		mg/M^3	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