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Fluorine Toxicity Studies

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In our last report toxic effects from a single inhalation of fluorine for 5, 15, 30 and 60 minutes were described. The effects caused by fluorine raised a number of questions and attempts are now being made to answer these questions. The experiments being conducted and summaries of information collected are as follows:

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 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.

Concentration (ppm)	Time (min.)	Effects
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

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.

Repeated Intermittent Exposures

Same Concentration

From the previous data, effects in man following a single exposure could be predicted fairly accurately. The possibility exists, however, of more than one short term exposure, with varying intervals of time between the exposures. Will several such exposures have cumulative effects? If a man is exposed once should re-exposure be avoided completely? Therefore, experimental animals were exposed several times to the same concentrations of fluorine.

Mice, rats and rabbits were exposed to fluorine four times at weekly intervals. Two levels of exposure were used, one which caused slight effects after a single exposure and one which caused marked effects following a single exposure. The animals were sacrificed at 7, 14, 21 and 45 days after the last exposure.

Mice were exposed for five minutes to 103, 109, 116 and 158 ppm, levels which should cause 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 should cause Grade 3 gross damage in the lungs and gross damage to the liver and kidneys.

At the lower concentrations the gross pathology in the lungs was Grade 1. Four exposures, one week apart, caused no more damage than a single exposure. The livers were essentially normal. The kidneys had questionable or slight gross changes at 7, 14, 21 and 45 days after the last exposure.

At the higher concentrations, Grade 3 gross damage in the lungs and

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 gross changes. The livers were normal. The kidney had slight gross 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 to the lungs. The livers and kidneys were essentially normal, with a few cases of questionable gross pathology.

The concentrations for sixty-minute exposures were 43, 50, 56 and 65 ppm. The lungs had Grade 1 gross lung 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. 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 gross pathology). A single exposure to 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 71 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 range from 88 to 170 ppm. The livers and kidneys were normal. The lungs had gross 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 (Grade 4 and 5).

Rats also were exposed for 30 minutes to 46, 64, 68 and 59 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 wefe 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.

Microscopic examination of the tissues is being done, but results are not available.

Different Concentrations

This experiment was conducted to determine if previous exposures to fluorine would change the susceptability of the animal to a lethal exposure.

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 this 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 indicated that effects from four exposures to fluorine repeated at weekly intervals caused no cumulative effects. In fact, it appeared that the effects of all four exposures might be less than the effects from a single exposure. A decreased effect would indicate some type of tolerance. Therefore, an experiment was designed to check for tolerance from a single exposure. If a tolerance to fluorine can be developed, the range of concentrations which will produce it, the time for onset of tolerance and the duration of the tolerance should be determined. Mice were exposed to 30 ppm fluorine for 60 minutes (a level which does not cause any apparent effects). 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 was exposed.

The animals which died were autopsied immediately. Those which survived were sacrificed and autopsied 14 days after the fifteen minute exposure. Microscopic examination of the tissues is being done but it is not completed. The lungs, liver and kidney were weighed.

The LC_{50}'s of the mice determined at 4 and 24 hours following the first exposure were 310 and 350 ppm respectively. These were higher but not significantly different from 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 (245 ppm) was lower than the LC_{50} of control mice exposed at the same time (315 ppm) but the difference was not significant.

When the survivors were sacrificed and autopsied 14 days after the exposures for determination of the LC_{50}'s, the pre-exposed mice did not show nearly as much damage in the lungs as the control mice (which had not been subjected to fluorine previously).

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. The difference in lung weights between treated and untreated mice was not as apparent at the 96-hour interval.

The weights of the lungs of all mice were normal when sacrificed 14 days after the exposures to fluorine for determination of the LC_{50}'s.

The weights of the kidneys were increased in animals which succumbed to fluorine (and were autopsied immediately). They were normal, however, from the animals sacrificed 14 days after exposure. Like the lungs, the weights of the kidneys of untreated mice were heavier than the kidneys of the treated or pre-exposed mice.

These preliminary data indicate that there may be a certain amount of tolerance to the edemagenic effects of fluorine, even after a single exposure to a low concentration. This experiment will be continued to

check for the possible development of tolerance.

Edema Formation

Since the previous studies had shown 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 and 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, briefly, is that fresh lung is weighed, placed in a freezer (-20°C) overnight, transferred to an evacuated desiccator and reweighed 48 hours after the animal is sacrificed.

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. Data were collected using the whole lungs and using only the right lung. 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 worked out 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 higher 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 experiments are being continued to define more clearly the formation of edema and the types of exposure to fluorine which cause it.

Succinic Dehydrogenase

Sulfhydryl and disulfide compounds protect against the effect 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. Since fluorine is an irritant, the first biochemical test to measure its effect was, most logically, succinic dehydrogenase.

The succinic dehydrogenase content of fresh lung tissue was determined by the method of Kun and Abood, Briefly, tissue homogenates, in the presence of succinic dehydrogenase, reduce tetrazolium (colorless) to a red formozan. The amount of color produced is proportional to the amount of succinic dehydrogenase. This 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.

The results to date, are very, very preliminary. The indication however, is that exposure to fluorine causes 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 suggest that at least certain exposures to fluorine cause an increase in succinic dehydrogenase content of the lungs of rats and that the increase is still apparent

5 days after exposure.

Obviously, these experiments are being continued to confirm the preliminary findings, to determine effects of fluorine on succinic dehydrogenase in other organs and to evaluate the mechanisms behind this apparent effect.

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 should be determined.

Mice--To date the lethal effects of fluorine have been measured in w 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. The differences are hardly significant. The data indicate, however, 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.

Additional groups of different ages of mice and additional species of animals will be used in these studies.

Analytical Chemistry

Since our last report, additional work has been done to improve the analytical methods for fluorine in air using gas liquid chromatography. Work has also been done to improve or develop methods for the determination of fluorides in urine and other animal tissues.

Summary

Studies to determine the toxic effects of fluorine are being continued. The results summarized in this report generally are preliminary. The concentrations which cause irritation in man have been determined. When rats and mice survive for six months after exposures which damage the kidneys, regeneration of the tissue is essentially complete. Repeated, intermittent exposures to fluorine appeared to cause less effects than a single exposure at the same concentration. Animals exposed to low concentrations of fluorine appear to be less susceptible to the effects caused by exposure to high concentrations of fluorine than animals which have not been exposed previously. The formation of edema following exposure to fluorine has been shown. Succinic dehydrogenase may be increased in the lung after inhalation of fluorine. Very young and rather old mice apparently are no more susceptible to fluorine than the young adult animal.