A FIELD AND LABORATORY STUDY OF FLUORIDE UPTAKE BY OYSTERS

by

David J. Moore

Department of Zoology

North Carolina State University

at Raleigh

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John E. Hobbie was principal investigator.

ABSTRACT

The uptake and concentration of fluoride in the tissues of oysters was studied under laboratory conditions. Experimental animals were maintained for up to two months in water fluoride levels of 0.5, 2, 8, 32, and 128 ppm using fluoride free artificial sea water containing specific amounts of sodium fluoride. Tissue fluoride analyses were made at intervals throughout the experiment and the fluoride uptake rates were determined. It was found that oysters did accumulate fluoride at all of the experimental fluoride concentrations except 0.5 ppm which is within the range of natural water fluoride levels. Maximum uptake occurred during the first five days of exposure and was followed by an abrupt decrease in the accumulation rate throughout the remainder of the study period. Tissue fluoride levels as high as 100 ppm (dry weight basis) were observed in oysters from 32 ppm water; 30 ppm from 8 ppm water; and 18 ppm from 2 ppm water. Oysters with these higher tissue fluoride concentrations, e.g., 100 and 30 ppm, are believed to represent a potential public health problem if they are consumed in large amounts by the general public.

Difficulty was encountered in maintaining oysters for longer than 30 days at 32 ppm and for longer than five days at 128 ppm water fluoride. This indicates that prolonged exposure to water fluoride levels as low as 32 ppm will be disastrous to oyster populations in the affected area.

Key words: oyster*, fluoride*, uptake rate, concentration.

Objectives

This project was undertaken to investigate (1) the potential uptake and concentration of fluoride by oysters, (2) some of the effects of fluoride on oysters, and (3) the possibility that oysters from fluoridated water will pose a threat to human health due to tissue fluoride concentration.

The oyster was chosen as a test animal because of its importance as a commercial species and because of its well known capacity to concentrate heavy metals and petroleum products that are present only in trace amounts in the environment. Zinc, for example, has been detected in oysters in concentrations up to 170,000 times that of the surrounding water (Chipman et al., 1958). If a similar accumulation of fluoride occurs, a potential serious threat to the health of the oysters exists along with a health hazard to those individuals eating such contaminated oysters.

Research Procedures

Field investigations were designed to determine fluoride uptake and growth rates in oysters subjected to different water conditions at six strategic locations along the Pamlico River. These experimental areas were chosen in reference to the source of fluoride pollution, e.g., Texas Gulf Sulfur Company's effluent.

Oysters were collected, marked, measured, and put into sediment filled plastic pans which were placed in the river at the experimental sites. These efforts, however, yielded no data since the oysters could not be located after a period of three months had elapsed. The plastic pans were gone and most of the oysters were apparently covered or removed from the areas by the combination of currents, waves, and shifting sediments. An insufficient number of living oysters could be located at each site to

permit statistical analysis of the growth and fluoride uptake data. Subsequently, this phase of the project was abandoned.

Oysters were also maintained in the wet lab facilities at the Pamlico Marine Laboratory. These oysters were collected when needed from the north side of the Pamlico River at a point where they were not subjected to the effects of the mining effluent. These oysters were weighed and placed in shallow plastic pans. These pans measured 32 cm long, 27 cm wide and 13 cm deep. Each contained held four to six oysters and four liters of water. The salinity of the water was adjusted to 10 parts per thousand to approximate the conditions found in the river where the oysters were obtained. Maintenance of this salinity was made possible by using an artificial sea water mix (Aquarium Systems, Inc., Wickliffe, Ohio) and diluting it to the desired salinity. The water used for dissolving the salts and diluting the sea water was obtained from a shallow well and passed through a Barnstead mixed-bed resin column prior to use.

Since the artificial sea water contained no fluoride it was possible to accurately regulate the composition of this ion in the experimental tanks by adding appropriate amounts of sodium fluoride. Fluoride concentrations in this experiment ranged from 0.5 ppm to 128 ppm. There were a total of five concentrations used, each one containing four times as much fluoride as the previous one. Thus, oysters were exposed to fluoride concentrations of 0.5, 2, 8, 32, and 128 ppm. This is believed to represent a realistic range of potential fluoride concentrations in the water of the Pamlico River Estuary adjacent to the phosphate mining operations.

Oxygen was supplied by bubbling air through a single air stone in each tank. The oxygen content of the water was not determined but at no time was this believed to be much below saturation.

The oysters were fed from an algae culture maintained in fluoride-free artificial sea water. The algae were concentrated by spinning in a centrifuge and then dispensed into the individual tanks. Difficulties were encountered in maintaining the algae cultures and therefore, the oysters were undoubtedly underfed throughout most of the experiment. This made it impossible to interpret any growth changes that might have been attributed to fluoridated water.

The water temperature was not controlled and was therefore subject to some fluctuation. The temperature of the water in the tanks ranged from a June low of 25°C to approximately 30°C in August. Daily fluctuation was not more than 1°C. The water in the tanks was changed once a week and replaced by a fresh supply containing the same fluoride concentration.

Oysters to be used in the tissue fluoride analyses were removed periodically from the experimental tanks. The entire oyster was weighed; the meats were removed from the shell, blotted between paper towels until most of the free water was gone, and dried at 100°C for 48 hours in a dry heat oven. After drying, the oyster meats were ground to a fine consistency with a mortar and pestle. The ground tissues were then stored in tightly stoppered vials until further analysis.

Determination of fluoride in the oyster tissues was done using a procedure based upon the diffusion of hydrogen fluoride, developed by Singer and Armstrong (1959, 1965). The principle employed involves dissolving of tissue samples and precipitation of proteins by perchloric acid ($HClO_4$), release of fluoride as hydrogen fluoride, absorption of hydrogen fluoride with sodium hydroxide, and spectrophotometric analysis. This method is preferable to the previously employed steam distillation procedures because of its relative simplicity and economy. This method does not require ashing

of samples and involves inexpensive equipment, thus enabling a greater number of analyses to be performed in a given period of time.

A complete list of materials and a detailed description of the chemical compounds and concentrations of each required in this analysis can be found in the papers noted above. A description of the diffusion dish apparatus is also given in these references.

Procedure

1. Apply a thin layer of silicone grease to the rim of the middle partition of each diffusion dish to be used.
2. Deposit NaOH in the center well of each dish.
3. Add appropriate amount of sample (dried oyster meat) to sample ring of each dish.
4. Prepare three blank dishes by adding only $HClO_4$ to the sample ring.
5. Prepare three recovery dishes by adding appropriate amount of fluoride solution (NaF) and $HClO_4$ to the sample rings.
6. Add one drop of redistilled alcohol to the center well of each plate to spread the NaOH.
7. Cover each diffusion dish with the lid of a disposable petri dish.
8. Place the plates on ½ inch plywood boards, arrange the boards in tiers and carefully place them in a 55°-60°C oven. Place bricks or other weights on top board to insure seal.
9. Incubate the plates for 22 hours.
10. Label and weigh test tubes for all samples, blanks, recoveries and standards.
11. Just prior to removing plates from oven, prepare standard tubes by adding appropriate amounts of stock fluoride solution and NaOH to each

tube.

12. Immediately after removing plates from oven, remove petri dish covers to prevent condensation.
13. Using suction, remove and dispose of sample ring contents from all dishes.
14. Add redistilled water to center well and transfer center well contents to appropriate test tube using individual dispopipettes. Rinse center well at least five times to assure complete removal of contents.
15. Bring volume in all test tubes to approximately 10 ml with redistilled water.
16. Add one drop of phenolphthalein to each tube and mix by shaking.
17. Titrate to end point with 2.25 N and 0.125 N HCl.
18. Prepare dye from Eriochrome Cyanine R and zirconyl oxychloride solutions. These must be mixed just prior to adding to the test tubes.
19. Add one ml of dye to each tube and shake to mix.
20. Bring volume in all test tubes to exactly 20 ml by adding redistilled water while weighing on a balance.
21. Read tubes on spectrophotometer after one hour from time dye is added. Use five centimeter cells at 568 mu.

Blank preparations - provide information on amount of fluoride in the various chemicals used.

Recovery preparations - provide information on amount of fluoride recovered after incubation from known fluoride concentrations.

Standard tubes - provide spectrophotometric reading on known fluoride concentrations not subjected to incubation.

Results and Conclusions

The total wet weight of each oyster was obtained both prior to placing

them in the experimental tanks and again when they were removed for tissue fluoride analysis. These data were not obtained to yield quantitative information on growth, but were designed to give a rough idea of the general health of the oyster. In the 0.5, 2, 8, and 32 ppm fluoride water the oysters usually maintained a constant weight or changed just slightly. In most instances the change involved a minor loss in weight but 10 of the 66 oysters involved at these fluoride levels showed a weight gain. In no case was the gain or loss of weight more than 0.4 gram, which indicates that many of the oysters were ingesting sufficient food for maintenance energy. Those that did lose weight, lost such a small percentage of their total body weight that it is considered insignificant as far as body health is concerned.

Those oysters maintained at 128 ppm fluoride lost between one and two grams over a period of five days and up to four grams after exposure for ten days. Most of the oysters died in 128 ppm fluoride water if they were exposed for more than five days. Those few that did not die lost weight steadily. Therefore, further work at this fluoride concentration was not attempted since this is believed to be well above the lethal concentration of fluoride for oysters under these laboratory conditions.

The data obtained at all fluoride levels were plotted as scatter diagrams to give a visual impression of the relationship existing between the fluoride accumulation and the time factor involved. Analyses consisted of determining both linear and quadratic equations for each data set. The F test was used to test the significance of linearity at the .025 level. In all but the 0.5 ppm fluoride level, significant curvilinearity in the regression was noted. Therefore, the quadratic fit was assumed to be most appropriate and was used to express the relationship between fluoride uptake and the duration of exposure to the fluoridated water. At the 0.5 ppm

fluoride level, linear regression provided the best fit and was thus used to describe the data.

Uptake data for oysters subjected to 0.5 and 2 ppm fluoride water over a 60 day interval are shown in Figure 1. All data are represented in parts per million fluoride in oyster meats on a dry weight basis. Since oysters loss approximately 80 percent of their original body weight when dried, these figures can be reduced by 80 percent to obtain wet weight data.

The regression curve at 0.5 ppm fluoride indicates a steady loss of fluoride from the oysters over the time interval involved. Although there is not a significant difference (0.5 level) between the fluoride concentration in the control oysters (fresh from the river) and the readings after 60 days in 0.5 ppm fluoride water, additional data in each category might well reveal a significant difference. These tissue fluoride levels are not unexpected since the fluoride content of the river water in the area where the oysters were obtained varies from approximately 0.2 to 1.5 ppm throughout the year.

Fluoride uptake in oysters subjected to 2 ppm water fluoride (Fig. 1) shows an initial rapid accumulation followed by an abrupt decrease in the uptake rate which indicates that an asymptote may be reached. This apparent asymptotic maximum is reached between 15 and 18 ppm tissue fluoride. This perhaps represents the maximum amount of fluoride that can be accumulated at this water fluoride level since this point is reached at approximately 20 days of exposure and does not change significantly throughout the 60 day exposure period.

The curve representing the uptake rate at 2 ppm water fluoride is typical of a quadratic expression. If this type of curve is extrapolated, it takes the shape of a parabola. There is no reason to believe that the

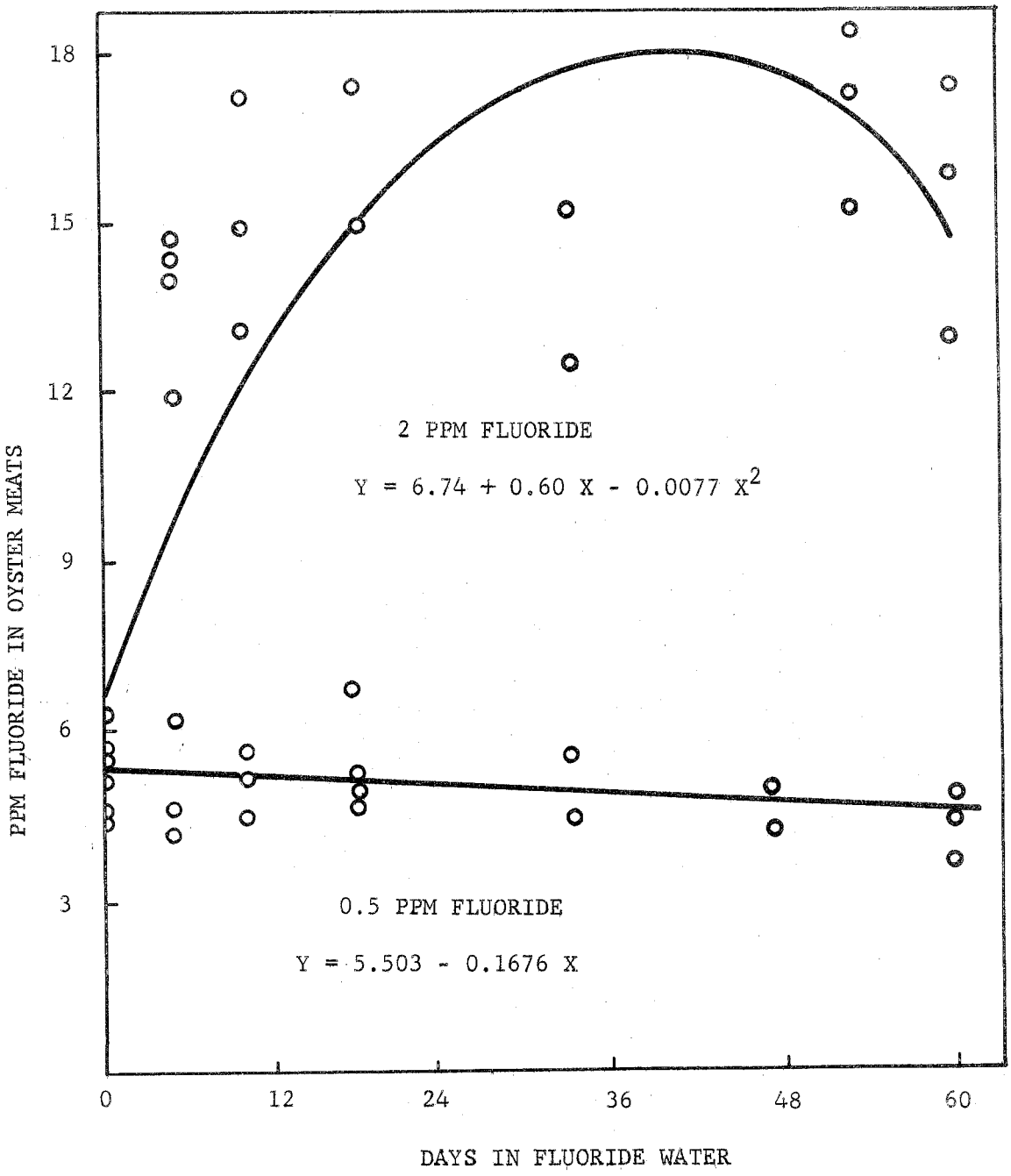

Figure 1. Fluoride uptake in oysters subjected to 0.5 and 2 ppm water fluoride.

actual fluoride level in the oyster tissues will decrease after reaching a maximum point as is indicated by the curve. A more realistic representation of the uptake rate could perhaps be illustrated by a hand-drawn curve showing a nearly linear uptake for the first 8-10 days of exposure. This curve would then level off at approximately 66 ppm fluoride and remain at this point for the duration of the 60 days of exposure.

Figure 2 shows the uptake of fluoride in oysters subjected to water fluoride levels of 8 and 32 ppm. Quadratic curves fit both sets of data better than linear expressions. In both cases there appears to be an initial rapid accumulation of fluoride in the tissues. In the 8 ppm fluoride data there appears to be a leveling off of the uptake rate at about 30 ppm. These data might also be represented more realistically by a hand-drawn curve showing a linear uptake for the first 6-8 days followed by a sharp decline in the rate of uptake for the remainder of the study period.

The data obtained at 32 ppm water fluoride are more difficult to interpret primarily because no observations were available beyond 33 days of exposure. Oysters kept beyond this time died before they could be sampled. It appears from this information that 32 ppm water fluoride is high enough to virtually eliminate any oyster populations living within the affected area.

The observations made at the 32 ppm water fluoride concentration show an initial accumulation of fluoride similar to, but much more pronounced than that observed at the 2 and 8 ppm fluoride levels. The data collected over the first 18 days of exposure indicate an abrupt decline in the rate of fluoride uptake after reaching approximately 60 ppm in the first five days. The three observations at 33 days of exposure disagree with this

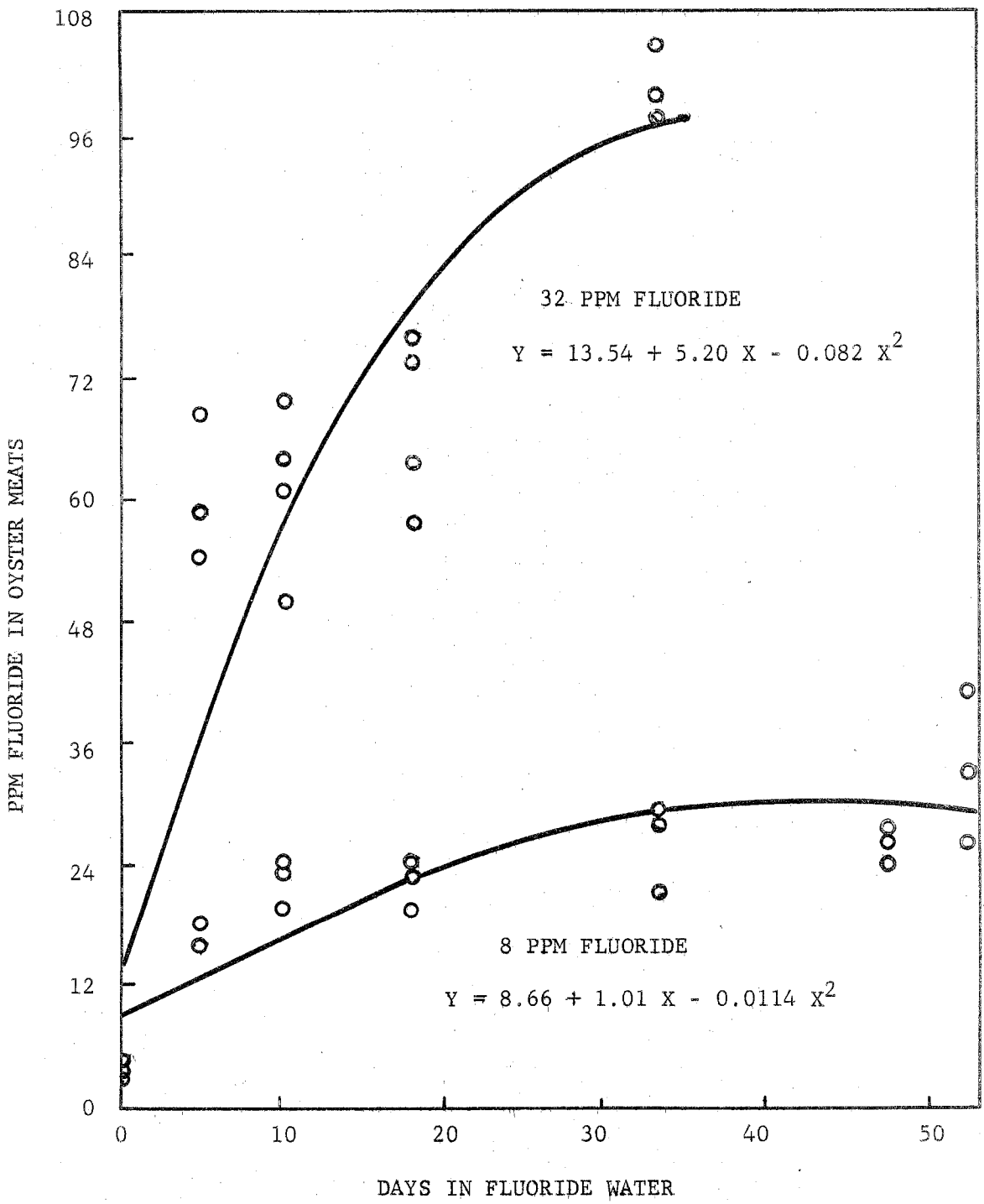

Figure 2. Fluoride uptake in oysters subjected to 8 and 32 fluoride water.

indicated rate of decline since they show a much higher concentration of tissue fluoride than would be expected. There is a possibility that these three observations are abnormally high and thus misleading. If these three oysters were near death, it is possible that the fluoride exchange mechanism within their tissues was no longer functioning, thus resulting in exceptionally high fluoride levels. Further research will be necessary to determine more precisely the tissue fluoride levels under these circumstances.

It is apparent that tissue fluoride levels of at least 70 ppm can be attained when oysters are subjected to 32 ppm fluoride water. This amounts to approximately 14 ppm on a wet weight basis which is 6.3 mg of fluoride per pound of oyster meats. This could substantially increase the daily food-borne fluoride intake of an individual. It has already been estimated (Marier and Rose, 1966) that the total fluoride intake per adult individual in fluoridated (1 ppm) communities is from 2 to 5 mg per day. Krepkogorsky (1963) recommends that total ingestion of fluoride by adults should not exceed 3.2 mg per day. In view of these facts, it seems likely that the addition of oysters containing 14 ppm fluoride to the diet will increase the daily fluoride intake nearer to, if not above, the maximum safe limit for an individual.

An attempt was also made to determine fluoride uptake rates in oysters living in 128 ppm fluoride water. The mortality rate after five days at this fluoride level was so great that only two additional observations were possible. These were made on oysters maintained for eight days under these conditions. Four oysters analyzed after five days of exposure showed 250, 265, 273, and 275 ppm fluoride in their tissues. The two measurements obtained at eight days showed 290 and 320 ppm fluoride. It is apparent that oysters could not survive extended periods of exposure to water fluoridated

to this level.

Experiments were also undertaken to determine how long the oysters could retain fluoride after being removed from fluoridated water. Only seven observations were made but the consistency of the results indicates that fluoride is released from oysters at least as fast as it is accumulated.

Oysters were maintained at 2, 8, and 32 ppm water fluoride for a period of 20 days and then placed in fluoride free water for four days. Tissue fluoride analyses showed fluoride levels of 4.3 and 4.8 ppm in oysters from 2 ppm fluoride water; 4.2 and 4.7 ppm in those from 8 ppm water; and 4.0, 5.0, and 5.5 ppm in oysters from 32 ppm fluoride water. In all cases the tissue fluoride level had returned to a level found in untreated oysters within four days after removal from the fluoridated environment. This flushing ability might prove to be helpful in ridding contaminated oysters of undesirable or potentially harmful concentrations of fluoride prior to using them as food for man.

Achievement of Objectives

The original objectives of this project were not all achieved since the field aspect was unsuccessful. Information on the uptake rates of fluoride in oysters and data on the concentrations of fluoride lethal to oysters were obtained.

Publications from this Project

At the present time no publications have resulted from this project. However, it is hoped that a publication will be forthcoming at a later date.

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